Novel Potential Antimigraine Compounds:

Carotid and Systemic Haemodynamic Effects in a Porcine Model of Migraine

Kapil Kapoor

Novel Potential Antimigraine Compounds:

Carotid and Systemic Haemodynamic Effects in a Porcine Model of Migraine

Thesis, Erasmus University, Rotterdam. With summary in Dutch

Printed by: Optima, Rotterdam

Novel Potential Antimigraine Compounds:

Carotid and Systemic Haemodynamic Effects in a Porcine Model of Migraine

Nieuwe Mogelijke Antimigraine stoffen:

Carotid en Systemisch Haemodynamische Effecten in een varken model voor Migraine

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
Prof.dr.ir. J.H. van Bemmel
en volgens besluit van het College voor Promoties

De openbare verdediging zal plaatsvinden
op woensdag 26 november 2003 om 09.45 uur

door

Kapil Kapoor

geboren te Lucknow, India

Promotiecommissie

Promotor: Prof. dr. P.R. Saxena

Overige leden: Prof. dr. M.D. Ferrari
Prof. dr. P.J. Koudstaal
Prof. dr. J. Verweij

Financial support of the following is gratefully acknowledged:
Anglo-Dutch Migraine association

TABLE OF CONTENTS

CHAPTER 1

Migraine overview

1. Migraine overview

1.1. Definition, classification and diagnostic criteria

The term migraine stems from *hemicrania*, describing a periodic disorder consisting of paroxysmal unilateral headache, accompanied by nausea, vomiting, photophobia and/or phonophobia. *Hemicrania* was later changed to Latin words - *hemigranea* and *migranea*; eventually the French cognate, migraine, gained acceptance in the eighteenth century and has prevailed ever since. A working definition of migraine is benign recurring headache and/or neurological dysfunction usually attended by pain-free interludes and often provoked by stereotyped stimuli (1). Migraine may be triggered by certain factors (red wine, menses, hunger, lack of sleep, glare, perfumes, periods of let down) and relieved by others (sleep, pregnancy). Premonitory symptoms occur hours to a day or two before a migraine attack with or without aura. Migraine is more common in females, with a hereditary predisposition towards attacks and the cranial circulatory phenomenon appears to be secondary to a primary central nervous system (CNS) disorder.

The Headache Classification Committee of the International Headache Society (IHS) published the classification and diagnostic criteria for headache disorders in 1988 (Table 1.1) (2). The terms “common migraine” and “classical migraine” have been replaced by “migraine without aura” and “migraine with aura”, respectively. These operational criteria have been validated by different approaches and have enabled us to distinguish different headache entities in a reliable manner (3-6). In recent years, the IHS criteria have been used world-wide in several multicentre double-blind drug trials, which have shown a reasonably consistent response rate to triptans (7), reflecting a consensus in the defined migraine group. In a recent MAZE survey, the Migraine Disability Assessment Scale (MIDAS) questionnaire has been used to assess the impact of migraine on work, home and social lives. MIDAS scores confirmed the debilitating effect of migraine; >50% of respondents had a MIDAS grade of III or IV, indicating moderate or severe disability. Less than one-third of patients reported that their current medication was consistently effective and only 36% were 'very satisfied' with their current therapy (8). These results show that migraine patients world-wide are still not receiving adequate treatment and a significant unmet need in migraine care still remains.

1.2. Pathophysiology of migraine

Although the pathophysiology of migraine is still far from clear, three phases mark the characteristic clinical features of migraine: initiating trigger phase, aura and finally the headache phase. Little is known about the initial triggering phase, but, certainly over the years, there is a better understanding regarding its pathogenesis of migraine headache (9-12). Theories, which have been propounded, are briefly described below:

1.2.1. Vasodilator theory of migraine

It was Heyck in 1969 (9), who proposed that opening of the cranial arteriovenous anastomoses, which is reflected with abrupt reduction in the difference between arterial and jugular venous blood oxygen saturation, heralds the onset of a migraine attack. In addition, it was found that the

Table 1.1. Classification and diagnostic criteria for migraine according to the Headache Classification Committee of The International Headache Society.

Migraine without aura

A. At least 5 attacks fulfilling B-D.

B. Headache attacks, lasting 4-72 hours (untreated or unsuccessfully treated).

C. Headache has at least two of the following characteristics:
   1. Unilateral localisation
   2. Pulsating quality
   3. Moderate or severe intensity
   4. Aggravation by walking stairs or similar routine physical activity

D. During headache at least one of the following:
   1. Nausea and/or vomiting
   2. Photophobia and Phonophobia

E. At least one of the following:
   1. History, physical- and neurological examinations do not suggest association with head trauma, vascular or non-vascular disorders, use of or withdrawal from noxious substances, non-cephalic infections, metabolic disorders or disorder of cranial or facial structures.
   2. History and/or physical- and/or neurological examinations do suggest such disorder, but it is ruled out by appropriate investigations.
   3. Such disorder is present, but migraine attacks do not occur for the first time in close temporal relation with the disorder.

Migraine with aura

A. At least 2 attacks fulfilling B

B. At least 3 of the following 4 characteristics
   1. One or more fully reversible aura symptoms indicating focal cerebral cortical- and/or brain stem dysfunction.
   2. At least one aura symptom develops gradually over more than 4 minutes or, 2 or more symptoms occur in succession
   3. No aura symptom lasts more than 60 minutes. If more than one aura symptom is present, accepted duration is proportionally increased.
   4. Headache follows aura with a free interval of less than 60 minutes. (It may also begin before or simultaneously with the aura).

C. At least one of the following: See above under E. for migraine without aura

diameter of temporal arteries is increased in patients suffering from migraine; treatment with ergotamine normalised the vessel diameter (13). Mechanical distension of the cerebral arteries does cause pain (14). Further, intravenous administration of serotonin, a known vasoconstrictor,

alleviates pain (15). This theory has been put to question by studies, which demonstrate the lack of correlation between pain and vasodilatation in migraineurs (16-18).

1.2.2. Neurological theory of migraine

This theory explains the premonitory symptoms associated with migraine, which is difficult to explain by a vascular hypothesis. Thus, it suggests that there is abnormal firing and neurotransmitter release at certain brain neurones (19). This expanding cortical spreading depression (CSD) manifests as aura symptoms associated with the initiation of the migraine attack. CSD probably starts with a cellular efflux of K^+, leading to depolarisation and a period of relative electrical silence (20). Evidence is accumulating that CSD might be associated with migraine and other neurological disorders. New imaging techniques will provide a greater understanding of these phenomena and could lead to newer methods of treatment (20). Several observations, like the absence of pain sensing fibres in the brain or the prevalence of headache, after rather than during a stressful insult, point to some inconsistencies in this theory.

1.2.3. Neurogenic dural inflammation theory

Neurogenic inflammation within cephalic tissues, involving vasodilatation and plasma protein extravasation, has been proposed as a mechanism in migraine pathogenesis. There is a release of pro-inflammatory vasoactive peptides, including substance P, neurokinin A and calcitonin gene-related peptide (CGRP) (21, 22). While substance P and neurokinin A increase blood vessel permeability (with transient vasodilatation), CGRP causes a profound and a long lasting vasodilatation without increasing blood vessel permeability (23). During electrical stimulation of the trigeminal ganglion or the intravenous administration of capsaicin, neurogenic plasma protein extravasation develops within the dura mater (24). Studies have shown that neurogenic plasma protein extravasation from blood vessels in the dura mater can be reduced by sumatriptan, 5-carboxytryptamine, dihydroergotamine, ergotamine as well as methysergide (25). The therapeutic efficacy of triptans is believed to be due to their ability to block the stimulated secretion of vasoactive neuropeptides from trigeminal nerves to break the vicious nociceptive cycle in migraine (26). A component of this nociceptive cycle involves activation of mitogen-activated protein kinase signalling pathways. Indeed, activation of mitogen-activated protein kinase pathways can increase CGRP synthesis and secretion (27). More recently, another neurotransmitter and vasodilator, nitric oxide (NO) has been proposed to play a role in the development of migraine headache and inhibition of its synthesis by nitric oxide synthase inhibitors results in reduced frequency and intensity of migraine attacks (28). The initial headache is now believed to be via a direct action of the NO-cGMP pathway that causes vasodilatation by vascular smooth muscle relaxation, while the delayed headache is likely to be a result of triggering trigeminovascular activation. In fact, delayed headache response triggered by NO donors in humans may be, in part, due to increased nNOS (neuronal nitric oxide synthase) activity in the trigeminal system that causes CGRP release and dural vessel dilation. Further, eNOS (endothelial nitric oxide synthase) activity in the endothelium causes NO production and smooth muscle relaxation by direct activation of the NO-cGMP pathway, and may be involved in the initial headache response (21).

To summarise (Figure 1.1), an unknown triggering event results in cranial vasodilatation leading to enhanced blood volume in each cardiac cycle. This increases pulsations within affected blood vessels. The increased pulsations are sensed by ‘stretch’ receptors in the vessel wall. The resultant enhancement in the perivascular (trigeminal) sensory nerve activity provokes

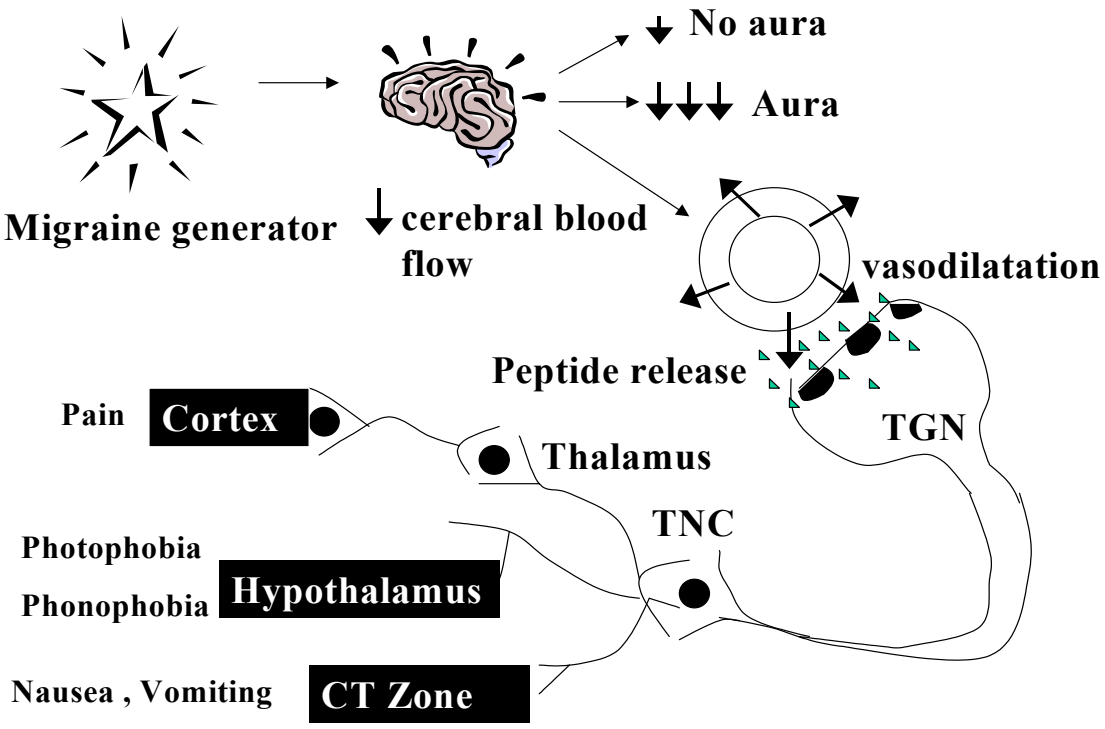


Figure 1.1. Schematic diagram illustrating the putative pathogenesis of migraine and the possible sites of action of antimigraine compounds.

headache and other associated symptoms. This stimulation of the trigeminal nerve may release other neuropeptides, thus reinforcing vasodilatation and perivascular nerve activity (29).

1.3. Experimental models for acutely acting antimigraine drugs

The choice of the models used to investigate pharmacological actions of newly synthesised compounds largely determines whether or not useful medications will be discovered. Detection of compounds with a pharmacological profile similar to a drug with known clinical activity may lead to compounds superior to existing drugs. The novelty of the compound may however be minor. On the other hand, compounds acting as antagonists at the newly discovered receptors or against the newly described naturally occurring mediators, or the agonists mediating their action, may lead to potentially useful compounds, which are likely to be novel.

1.3.1. Animal models

The migraine models described are based on the view that intracranial extracerebral vasodilatation is an integral part of pathophysiology of migraine and that ergot alkaloids and sumatriptan, which do not readily cross the blood-brain barrier owe their therapeutic efficacy primarily to the vasoconstriction of the dilated blood vessels (11, 30, 31).

1.3.1.1. Constriction of dilated carotid arteriovenous anastomoses

The involvement of arteriovenous anastomoses (Figure 1.2) is based on the findings that (i) during migraine, the oxygen saturation difference between arterial and jugular venous blood

decreases and this is normalised after treatment or spontaneous alleviation of the attack (9, 32) and (ii) antimigraine agents decrease carotid blood flow by a vasoconstrictor action exclusively on the arteriovenous anastomoses (33, 34).

Using selective serotonin (5-hydroxytryptamine; 5-HT) 5-HT_{1B} (SB224289) and 5-HT_{1D} (BRL15572) receptor antagonists, it was demonstrated that the constriction of porcine carotid arteriovenous anastomoses as well as canine external carotid vasculature by sumatriptan is mediated via 5-HT_{1B} receptors and not 5-HT_{1D} or 5-HT_{1F} receptors (35, 36). Over the years, this vascular model, which focuses on the measurement of arteriovenous anastomotic blood flow, has proven its worth for screening of compounds with therapeutic activity in migraine.

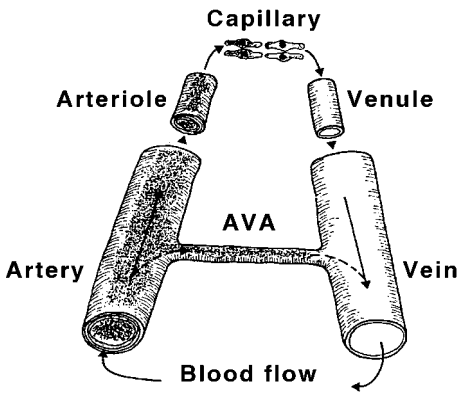


Figure 1.2. Schematic depiction of arteriovenous anastomoses.

1.3.1.2. Suppression of 'neurogenic' inflammation

Activation of the trigeminal nerves during a migraine attack has been suggested to produce headache by provoking a painful, sustained neurogenic inflammation (vasodilatation and plasma protein extravasation) within the meningeal vasculature (17). Trigeminal sensory afferent fibres are known to contain pro-inflammatory neuropeptides, like CGRP, substance P and neurokinin A. Electrical or chemical stimulation of the trigeminal ganglion in animals induces plasma extravasation in the dura mater (37-40). Increases in the cerebral and meningeal blood flow are mainly attributed to the actions of CGRP (41), whereas extravasation seems to be mediated predominantly by substance P release and NK_1 receptor activation (42). It is important to mention that activity of a compound in this model does not necessarily mean effectiveness in migraine, as has been observed with compounds like CP122288 (43), $ET_{A/B}$ receptor antagonist bosentan (44) and tachykinin NK_1 receptor antagonists, lanepitant (45) and RPR100893-20 (46).

1.3.1.3. Inhibition of cranial vasodilatation

Electrical stimulation of the trigeminal ganglion causes dilatation in the dural vessels, as observed in anaesthetised rat (37) and decreases carotid vascular resistance in cat (47), monkey (48) and guinea pig (49). Recent evidence suggests a role for neuropeptides, like CGRP, released from the trigeminal sensory nerve endings in the development of migraine. On stimulation of trigeminal ganglion in cats, there is an increase in the cerebral blood flow, which can be blocked by CGRP antagonist $CGRP_{8-37}$ and by sumatriptan (18, 41). In a recent study on marmosets, stimulation of the trigeminal ganglion resulted in an ipsilateral increase in the facial blood flow due to the release of CGRP. Administration of BIBN4096BS significantly and dose-dependently inhibited this response evoked by trigeminal ganglion stimulation (50). These newly developed compounds are small molecules with high selectivity for human CGRP receptors. Hypothetically, these agents should be efficacious in the relief of migraine headaches via blockade of the effects of CGRP (51).

1.3.1.4. Central trigeminal neuronal inhibition

Noxious electrical, mechanical, or chemical stimulation of cerebral blood vessels in anaesthetised cats elicits nociceptive firing in trigeminal nerves, which can be detected electrophysiologically in central nuclei, such as the trigeminal nucleus caudalis (the first central synapse of the trigeminal fibres) or in higher centres, e.g. thalamus (52). Known antimigraine

drugs, like zolmitriptan and naratriptan, have shown their ability to inhibit the action potentials generated in trigeminal nucleus caudalis after superior sagittal sinus stimulation in the cat (53, 54). Similarly, the efficacy of sumatriptan in inhibiting evoked potentials and c-*fos* expression in the nucleus caudalis was observed, but only after disruption of the blood brain barrier. CP-99,994, a NK_1-receptor antagonist, readily gains access into the CNS and blocks c-*fos* expression suggesting a central action on the trigeminal nerves, in addition to blockade of the NK_1 receptors located on cranial vasculature.

1.3.2. Human models for migraine

1.3.2.1. Contraction of isolated cranial vessels (for therapeutic efficacy)
Triptans have the ability to potently contract isolated human cranial vessels, including the middle meningeal, temporal, middle cerebral and basilar arteries (55-58). This model has been useful for screening acutely acting antimigraine drug, like sumatriptan and other triptans, due to the fact that cranial vasculature is rich in 5-$HT_{1B/1D}$ receptors as compared to 5-HT_2 receptors in peripheral vessels (59). Concentration response curves to agonists are made and, subsequently, the E_{max} (maximal contractile responses) and pEC_{50} (agonist potency) and pA_2 (antagonist potency) used in the study are calculated (60).

1.3.2.2. Contraction of isolated coronary artery and saphenous vein (for potential side effects)
All current acutely acting antimigraine drugs have a potential to contract the human coronary arteries (61). Although 5-HT_2 receptors predominate over 5-HT_1 receptors, the contraction of human coronary arteries by triptans is a class effect and is mediated mainly by activation of 5-HT_1 receptors (61-63). *In vitro* studies have demonstrated that the coronary vasoconstrictor effect of ergot alkaloids but not of the triptans, lasts longer and is resistant to repeated wash (29, 61). Therefore, one can predict the possible side effects associated with known acutely acting antimigraine compounds and their structural analogues or novel compounds with potential antimigraine activity using this model.

1.3.2.3. Headache induced by glyceryl trinitrate or histamine
Human models, using glyceryl trinitrate (64) and histamine provocation, have been validated as potential diagnostic tests for migraine (65, 66). Intravenous infusion of glyceryl trinitrate or histamine is given in healthy subjects, followed by scoring of the intensity of the headache on a 0-10 point rating scale. With histamine, the development of the headache is rapid, reaching peak levels and then declines (67). Potential antimigraine drugs may be evaluated in these models.

1.4. Drug discovery in migraine

Target and lead discoveries remain the main component of today's early pharmaceutical research for the management of human diseases. The aim of target discovery is the identification and validation of suitable drug targets or receptors for therapeutic intervention, whereas lead discovery identifies novel chemical molecules that act on those receptors. When lead molecules have been identified, they have to be optimised in terms of potency, selectivity, pharmacokinetics and toxicology. For compounds that are targeted to the central nervous system, another important aspect is blood-brain barrier penetration.

Over the years, the drug discovery in migraine is aimed to abolish acute attacks of headache and for prophylactic use, with minimal side effects. Both groups have drugs that are specific for migraine (ergot alkaloids and triptans to abort attacks) and those that are used on

account of other properties (non-specific action) (68). Analgesics, anxiolytics or sedatives and antidepressants are mainly used in the amelioration of symptoms, like pain, nausea, vomiting, anxiety, fear and occasionally depression. During a migraine attack there is gastric stasis, which lead to failure of some patients to respond to treatment. This prompted the development of compounds having antiemetic and prokinetic activity, like metoclopramide (69).

It was in 1926 that ergotamine was introduced for the treatment of migraine on the assumption that heightened sympathetic activity is the main cause for this disease, but the vasoconstrictor effect of ergotamine on extracranial vasculature was soon apparent (13). Recently, it is thought that the therapeutic efficacy of the ergots could be related to inhibition of the neurogenic inflammation (70) or central inhibition of the trigeminal neurones by binding to the receptors in the trigeminal nucleus caudalis (71). In 1945, dihydroergotamine was introduced in migraine therapy as a more potent sympatholytic agent than ergotamine (72). This therapeutic action of ergotamine and dihydroergotamine is believed to be through the activation of α-adrenoceptors (particularly α_2) and 5-HT (particularly 5-$HT_{1B/1D}$) receptors and, perhaps, D_2 receptor blockade (36, 73). In addition, activation of certain novel receptors may also be involved (36). Perhaps due to the involvement of multiple receptors, the ergot alkaloids are associated with a number of side effects, including nausea and vomiting, gangrene and myocardial infarction (74, 75). Thus, the selectivity of 5-$HT_{1B/1D}$ receptors associated with sumatriptan and the second-generation triptans represented an important step forward in migraine therapy, by providing better-tolerated relief.

1.4.1. The triptans

1.4.1.1. Sumatriptan

The development and clinical use of sumatriptan, the first serotonin 5-$HT_{1B/1D}$ receptor agonist, ushered a new era for the clinicians treating patients for migraine. The fact that compounds mimicking 5-HT at the craniovascular receptors should abort migraine attacks stems from the observations that (i) urinary excretion of 5-hydroxyindoleacetic acid increases, while platelet 5-HT decreases during migraine; (ii) migraine-like symptoms can be precipitated by reserpine and alleviated by 5-HT, which causes carotid vasoconstriction; and (iii) ergotamine and methysergide elicit selective vasoconstriction, partly via 5-HT receptors located on cephalic arteriovenous anastomoses (76). This resulted in the synthesis of the tryptamine derivatives having selectivity for the craniovascular 5-HT_1 receptors and the subsequent identification and introduction of sumatriptan by Humphrey and colleagues (77). Although triptans have some variable 5-HT_{1A}, 5-HT_{1E} or 5-HT_{1F} agonist actions, it seems that the commonality of their action is at the 5-$HT_{1B/1D}$ receptor sites (78). The distribution of 5-HT_{1B} and 5-HT_{1D} receptors and their potential involvement in the antimigraine activity, has been demonstrated by pharmacological, immunocytochemical and molecular techniques (59, 79-81). In the spinal trigeminal tract of human brain stem, a greater proportion of fibres (>1 μm diameter) expressed CGRP immunoreactivity, compared with substance P or 5-HT_{1D} receptor immunoreactivity. The 5-HT_{1D} receptor immunoreactivity was localised on some CGRP and substance P immunoreactive fibres. This suggests that 5-HT_{1D} receptors can regulate the release of CGRP and substance P and may be relevant to the clinical effectiveness of 5-$HT_{1B/1D}$ receptor agonists in the treatment of migraine and other cranial pain syndromes (82).

The effects of sumatriptan and other triptans include carotid vasoconstriction, inhibition of trigeminal nerves innervating intracranial blood vessels and dura mater and inhibition of trigeminal neurones in trigeminocervical complex in the brain stem and the upper cervical spinal

cord. It appears that the antimigraine activity of triptans is the combined result of cranial vasoconstriction by activation of 5-HT_{1B} receptors and inhibition of neuropeptide release. Although it is a significant achievement in migraine treatment, sumatriptan is not without some limitations, for e.g. low oral bioavailability, short half-life, metabolism via monoamine oxidase in the liver and its inability to cross the blood brain barrier (83-85). Moreover, approximately 30% of patients report inadequate relief or do not respond to oral sumatriptan or are prone to headache recurrence ranging from 33 to 38% (86, 87). The mechanism of headache recurrence is unclear, but it reflects that the migraine attack outlasts the duration of treatment effect (88). The subcutaneous route of administration of sumatriptan is most efficacious and fast acting as compared to the oral route, but the adverse effect profile is also more when compared to other delivery methods (88). The adverse effects associated with the use of sumatriptan use are observed in about 40% of the patients and they include most notably tightness or pressure in the chest, neck and/or throat, shortness of breath, palpitations and anxiety (89). Although the risk of coronary ischaemia with sumatriptan treatment is commonly stated, in a recent study on the Japanese population, chest symptoms following sumatriptan injection are not strongly associated with coronary ischaemia (90). Therefore, the mechanism of chest symptoms following sumatriptan administration should be further elucidated.

Alternative formulations of sumatriptan appear promising in improving the tolerability and efficacy profile of sumatriptan. In addition, investigators have turned to the development of new drugs, the so called "second-generation triptans": zolmitriptan (AstraZeneca), naratriptan (GlaxoSmithKline), rizatriptan (Merck Sharp & Dohme), eletriptan (Pfizer), almotriptan (Almirall Prodesfarma); frovatriptan (Vernalis/Elan), donitriptan (Pierre Fabre). In general, the second-generation triptans exhibit a superior pharmacokinetic profile, like higher bioavailability, increased plasma half-life and shorter t_{max} compared to sumatriptan (Table 1.2) (76). In addition, their higher lipophilicity can facilitate penetration into the brain.

1.4.1.2. Zolmitriptan

Zolmitriptan was the first of the second-generation triptans that was approved after sumatriptan. Apart from its better oral bioavailability and brain penetration as compared to sumatriptan, it also yields active metabolites, one of which (*N*-desmethyl metabolite 183C91) retains 5-$HT_{1B/1D}$ receptor agonist activity (at least twice as potent as the parent molecule) (91). Patients with hepatic impairment also seem to tolerate zolmitriptan well, requiring no dosage adjustment.

1.4.1.3. Naratriptan

Naratriptan has the highest oral bioavailability amongst the triptans. The affinity at the 5-HT receptor subtypes (5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F}) is also high and is more lipophilic (92) with easy brain penetrability. Because of the fact that the recommended dose has been set low, the clinical adverse effect profile of naratriptan is more favourable, but the clinical efficacy is inferior, as compared to other triptans. Thus, naratriptan may be the drug of choice in patients who have a mild migraine attack and are sensitive to the adverse effect profile of other triptans. The incidence of headache recurrence seems lower after treatment with naratriptan 2.5 mg (17 to 28 %) than that observed with other triptans. However, this may be partly related to its lower clinical efficacy.

1.4.1.4. Rizatriptan

Rizatriptan has a high affinity for 5-$HT_{1B/1D}$ receptors, but has limited interaction with other 5-HT receptors, including 5-$HT_{1A,}$ 5-HT_{1E} and 5-HT_{1F} (93). It undergoes a moderate first-pass

Table 1.2. Pharmacokinetic profile of triptans.

Compound	Formu-lation	Dose (mg)	T_{max} (h)		Protein binding (%)	V_d (L/kg)	$T_{1/2\beta}$ (h)	Bioavai-lability (%)
			Outside attack	During attack				
Sumatriptan	Oral	100	2.5		14-21	2.4	2-2.5	14
	sc	6	0.25				2	96
	in	20					2	
Zolmitriptan	Oral	2.5	2		25	7	3	40
Naratriptan	Oral	2.5	1.5-2		28-31	170	5-6	63-74
Rizatriptan	Oral	10	1	1	14	110-140	2-2.5	47
Eletriptan	Oral	80	1.5	2.8			4-5	50
Almotriptan	Oral	12.5	2.5				3.6	70
Frovatriptan	Oral	2.5	2-4	2-4			25	24-30

sc, subcutaneous; in, intranasal; $T_{max,}$ time to reach peak plasma concentration; $T_{1/2\beta}$, terminal elimination half-life; V_d, volume of distribution.

metabolism, which limits its oral bioavailability (94). Of special note is the fact that rizatriptan has been observed to accumulate in the breast milk of rats at concentrations 5-fold higher than the plasma concentration. Consequently, caution should be exercised for its use in nursing women. Nonetheless, rizatriptan is quite effective in treating acute migraine, with a dose-related increase in efficacy (95).

1.4.1.5. Eletriptan

Eletriptan is a conformationally restricted analogue of sumatriptan and it displays increased affinity for 5-$HT_{1B/1D}$ receptors (96). Drugs inhibiting Cyp3A4 isoenzyme may affect the metabolism of eletriptan. Eletriptan 20 mg, 40 mg, and 80 mg are effective for the treatment of an acute migraine attack. This effectiveness is dose-related, with statistically significant differences between doses for pain-free response and 24-hour outcomes. Eletriptan also compares well with other triptans available for outcomes measured up to 2 hours and provides meaningful relief for 24 hours. Taken as a single dose, it is well tolerated. The incidence of minor adverse effects is dose-dependent, with 80 mg giving significantly more adverse effects than 40 mg (97).

1.4.1.6. Almotriptan

Almotriptan binds equally well to both 5-HT_{1B} and 5-HT_{1D} receptors (98). It displays superior vasoconstrictor profile in human meningeal artery as compared to sumatriptan and has also been demonstrated to inhibit plasma protein extravasation in guinea pigs (99). It has a favourable adverse effect profile and is not associated with any increase in the heart rate or blood pressure and does not cross blood brain barrier (100). Importantly, however, it is as efficacious in migraine as sumatriptan (101, 102).

1.4.1.7. Frovatriptan

Frovatriptan has recently been marketed. Like other triptans, frovatriptan demonstrates functional cerebrovascular selectivity compared with coronary circulation (103, 104). The drawback associated with frovatriptan is that it does not have a fast onset of action (105). A comparative efficacy and pharmacokinetic profile of triptans is given in Table 1.2.

1.4.1.8. Donitriptan

Donitriptan (F11356; hydrochloride salt or F12640; mesylate salt, Pierre Fabre, Castres, France) has been selected from a series of compounds because of its exceptional high efficacy at 5-$HT_{1B/1D}$ receptors *in vitro* and in pharmacological models of migraine *in vivo* (106, 107). In isolated guinea pig trigeminal ganglion neurons, F11356 was more potent (pD_2: 7.3 versus 6.7) than sumatriptan in inducing outward hyperpolarizing Ca^{2+}-dependent K^+ current. In anaesthetised pigs, it elicited a cranioselective and potent carotid vasoconstriction (108). *In vitro* studies have confirmed its cranioselectivity, with similar coronary side-effect profile as sumatriptan (109). Therefore, due to its long duration of action and excellent tolerability in animals, donitriptan may provide an attractive alternative to currently available treatments. The drug is currently undergoing clinical evaluation.

1.4.2. Role of α-adrenoceptors in migraine

As described above, carotid arteriovenous anastomoses are dilated and play an important role in the pathogenesis of migraine. Therefore, it is reasonable to believe that α-adrenoceptors could be involved in maintaining the vascular tone of the carotid circulation, which may provide a potential avenue for the development of newer antimigraine drugs (110). It has been shown that several acutely acting antimigraine agents, including the ergots and triptans, produce potent vasoconstriction in the canine and porcine carotid vasculature mediated by 5-$HT_{1B/1D}$ receptors (33, 73, 111). Interestingly, the canine carotid vasoconstrictor responses of the ergot alkaloids are mediated by 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors (73) as well as an unidentified receptors/mechanism (36). On the other hand, the vasoconstriction of porcine carotid arteriovenous anastomoses by sumatriptan is mediated exclusively by 5-HT_{1B} receptors (111). The above lines of evidence, combined with the high affinity of ergotamine and dihydroergotamine at α-adrenoceptors (112), suggest their therapeutic efficacy (113) may be partly explained by an action mediated via α-adrenoceptors. However, the possible involvement of α-adrenoceptors in the porcine carotid vasculature has been hampered, mainly on the basis that porcine carotid arteriovenous anastomoses were described to be insensitive to sympathetic nerve stimulation or intracarotid infusions of noradrenaline (114). This discrepancy is striking since in conscious pigs arteriovenous anastomoses are under a vasoconstrictor sympathetic tone, which involves α_1-adrenoceptors (115). In fact, sympathetic nerve stimulation as well as exogenous noradrenaline causes α-adrenoceptor-mediated vasoconstriction of arteriovenous anastomoses in the hind limb of several species (116-120). Finally, *in vitro* experiments have shown that stimulation of α-adrenoceptors results in contraction of the isolated carotid artery of several species, including the dog (121, 122), rabbit (123) and pig (124).

A series of compounds have been described with α-adrenoceptor agonistic activity (125). One such compound, S19014 (spiro[(1,3-diazacyclopent-1-ene)-5:2'-(4',5'-dimethylindane)]), shows a high affinity at both α_1- and α_2-adrenoceptor subtypes. Interestingly, S19014 shows a wide variation in its efficacy (maximum effect, E_{max}) and potency (EC_{50}, concentration needed to

elicit 50% of E_{max}) in contracting rabbit, dog and human saphenous vein (EC_{50}: 18, 79 and 8500 nM, respectively; E_{max}: 92, 49 and 36% of K^+-induced contraction, respectively), rabbit aorta (EC_{50}: 816 nM; E_{max}: 36% of K^+-induced contraction) and dog femoral artery (practically inactive) (126).

1.4.3. Role of CGRP in migraine

Figure 1.3. Aminoacid sequences of human and rat CGRP showing high degree of homology.

CGRP exists in two forms, designated α-CGRP and β-CGRP or CGRP-I and CGRP-II (127). The main feature of this peptide is a disulphide linkage between Cys2 and Cys7, a probable region of α-helix between residues 12 and 19 and a C-terminal amide; Figure 1.3 (23, 128). CGRP-α was cloned in the early 1980s from the alternative splicing of the calcitonin gene (129, 130). Subsequently, characterisation of CGRP-β bearing high sequence homologies with the α-form was done (131). Other members of this family include calcitonin, amylin and adrenomedullin. Due to their structural similarities, the peptides share some biological activities, suggesting a possible interaction with similar G-protein coupled receptors. The receptors have been characterised on the basis of pharmacological responses and radioligand binding studies (132). These peptides are widely distributed in the peripheral tissues as well as the central nervous system and induce multiple biological effects, including potent vasodilatation (CGRP and adrenomedullin), reduction in nutrient intake (amylin) and decreased bone resorption (calcitonin). CGRP inhibitors have therapeutic potential in conditions where excessive CGRP-mediated vasodilatation is present, i.e. neurogenic inflammation, migraine and other headaches, thermal injury, circulatory shock and flushing in menopause (133). Exciting progress has been made in this field with the development of a selective dipeptide CGRP receptor antagonist (BIBN4096BS) (50) and cloning of **R**eceptor **A**ssociated **M**embrane **P**roteins (RAMPs) and **R**eceptor **C**omponent **P**roteins (RCPs), both of which are key chaperones required for functional activation of G-protein-coupled receptors.

1.4.3.1. CGRP receptor subtypes

CGRP acts via two types of CGRP receptors: $CGRP_1$ and $CGRP_2$. $CGRP_1$ receptor subtype is particularly sensitive to the antagonistic properties of the fragment human $CGRP_{8-37}$, but mostly insensitive to the weak agonist effects of the linear analogue [$Cys(ACM)^{2,7}$]hCGRPα (134). By contrast, the $CGRP_2$ receptor subtype is less sensitive to $CGRP_{8-37}$, while the linear analogue demonstrated agonist potency in the 10^{-8}-10^{-7} M range for this receptor (134). However, the final demonstration of the existence of these receptor subtypes must come from their respective cloning and the development of fully selective agonists and antagonists, preferably of non-peptide nature. Progress on these fronts has been slow and difficult. This is probably because of the unique molecular features of the $CGRP_1$ receptor. However, potent small molecule peptides as well as non-peptide antagonists of these receptors are finally becoming available (50, 135). The proposed classification of CGRP receptors is described in Table 1.3 (23, 128, 132, 135).

Table 1.3. Pharmacological characteristics of CGRP receptor subtypes.

Parameter	Receptor subtype	
	$CGRP_1$	**$CGRP_2$**
Potency	CGRPα, CGRPβ>ADM>amylin	CGRPα, CGRPβ>ADM>amylin
Selective agonist	None	$[Cys(ACM)^{2,7}]$hCGRPα
Selective antagonist	$hCGRP_{8-37}$ (pA_2: 7-8) BIBN4096BS (pA_2: 8 and up to 11 in human SK-N-MC cells) SB-273779 (K_i: 250±15 nM) for cloned $hCGRP_1$	BIBN4096BS (pA_2: 6.5-7) $CGRP_{8-37}$ (pA_2: 5.5-6.5)
Second messenger	G_s (cAMP production)	G_s
Prototypical bioassays	Atrium, pulmonary artery, spleen, SK-N-MC cells.	Vas deferens, urinary bladder, liver, HCA-7 cells

ADM, adrenomedullin

1.4.3.2. Molecular features of CGRP receptors

A G-protein coupled receptor (GPCR) clone was isolated from rat pulmonary blood vessels and termed calcitonin receptor like receptor (CRLR) (136). The relevance of CRLR as a CGRP receptor remained elusive till the discovery of the RAMPs. RAMPs are a novel family of transmembrane proteins (137). These proteins facilitate intracellular translocation of the CRLR-maturing protein and its insertion into the plasma membrane. Moreover, the various RAMPs (RAMP1, RAMP2, RAMP3) dramatically alter the pharmacological profile of CRLR (137). RAMP1 (148 amino acid protein) is a single domain and its co-transfection with CRLR in a variety of cells, like HEK293, COS-7 and oocytes, confers a $CGRP_1$ receptor like profile to CRLR with CGRPα and $CGRP_{8-37}$ exhibiting high affinities for this receptor complex. $CGRP_{8-37}$ inhibited the effects of CGRP on cAMP production in these co-transfected cells. On the other hand, CRLR-RAMP2 receptor complex behaves as an adrenomedullin receptor with adrenomedullin-like peptides having much greater affinity than CGRP derivatives (137). Recently, the respective expression of CRLR, RAMP1 and RAMP2 was investigated in rat osteoblast-like UMR106 cells. A dominant functional interaction of CRLR with RAMP1 was observed (138). Although the simultaneous expression of RAMP2 did not effect CRLR-RAMP1 association of the functional CGRP receptor, RAMP1 inhibited the association of RAMP2 with CRLR, hence reducing the likelihood of generating an adrenomedullin-like receptor. Thus, RAMPs

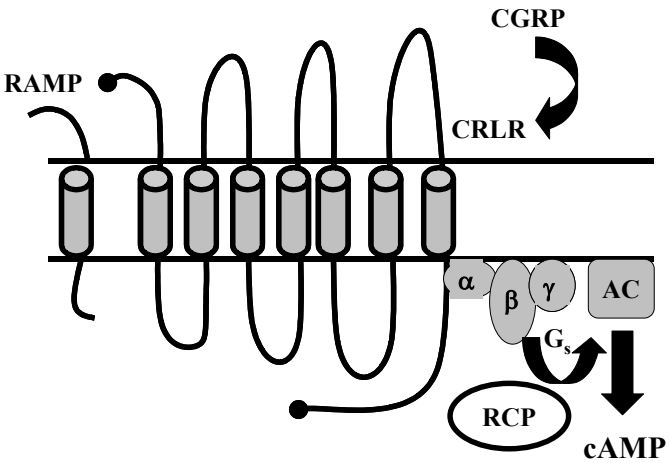


Figure 1.4. Schematic representation of $CGRP_1$ receptor showing interactions of RAMP1 protein with CRLR and a receptor component protein (RCP). This complex is tightly coupled to G_s to promote cAMP production.

might also have a role in receptor desensitisation. The respective expression of various RAMPs is differentially regulated. In human endothelial and vascular smooth muscle cells, mRNAs for CRLR and RAMP2 are more abundant than those of RAMP1 and RAMP3, while the expression of RAMP1, but not RAMP2, is dependent on the plasma concentration of corticosterone (139). Thus, the physiological response of a given tissue to CGRP and related peptides can be markedly changed depending on the level of expression of RAMPs. It is thus of interest to establish the regulation of CRLR and the various RAMPs precisely in a given tissue or organ because such regulation could have a major impact in a variety of pathological conditions. In addition to the RAMPs, the CGRP-receptor complex might require another protein known as RCP to function optimally. RCPs (146 amino acid long) do not belong to any specific class of proteins; Figure 1.4 (140).

1.4.3.3. Development of non-peptide CGRP receptor antagonists

Various structure-activity studies have been conducted to develop smaller molecules that behave as antagonists. Among these peptides, [Asp31, Pro34, Phe35]$CGRP_{27-37}$ and [Pro34, Phe35]$CGRP_{27-37}$ were recently reported as potent antagonists at the $CGRP_1$ receptor expressed in SK-N-MC cells (141). A major breakthrough in the field was reported recently following the development of a potent dipeptide CGRP antagonist, BIBN4096BS (50, 142) (Figure 1.5).

BIBN4096BS demonstrates a very high affinity for the human CGRP receptor expressed in SK-N-MC cells (K_i: 14.4±6.3 pM; pA_2: 11; compared to pA_2: 7.5 for $CGRP_{8-37}$), to block CGRPα-induced cAMP production in these cells (50). Interestingly, BIBN4096BS demonstrates species specificity with a 100-fold lower affinity for the rat (3.4±0.5 nM) versus the human (32±1.7 pM) $CGRP_1$ receptor (50). Interestingly, BIBN4096BS was shown to competitively antagonise the effects of hCGRPα with pA_2 values in the nanomolar range in the rat atria, such a potency being 10-fold higher than that of $CGRP_{8-37}$ (Table 1.3). However, in the rat vas deferens, a $CGRP_2$ receptor bioassay showed BIBN4096BS less potent at inhibiting the effects of these various CGRP homologues. This selectivity profile discriminates between the $CGRP_1$ and $CGRP_2$ receptors. Thus, BIBN4096BS should prove useful to provide new information on CGRP receptor subtypes. Comparative pA_2 values of $hCGRP_{8-37}$ and BIBN4096BS against CGRP in various prototypical assays are shown in Table 1.3.

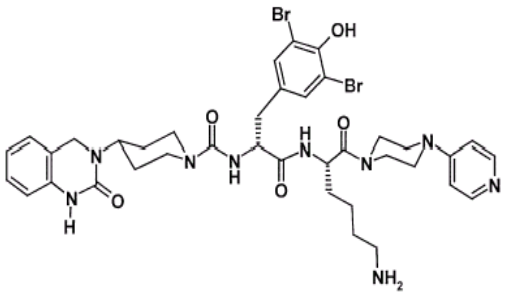


Figure 1.5. Chemical structure of BIBN4096BS.

Another compound SB-273779 [N-methyl-N-(2-methylphenyl)-3-nitro-4-(2-thiazolyl sulfinyl)nitrobenzanilimide], a selective non-peptide antagonist at the $CGRP_1$ receptor has been described. SB-273779 inhibits [^{125}I]CGRP binding to SK-N-MC (human neuroblastoma cells) and human cloned $CGRP_1$ receptor with K_i values of 310 and 250 nM, respectively (135). SB-273779 also inhibits CGRP-activated adenyl cyclase in these systems with IC_{50} values of 390 nM and 210 nM. In addition, SB-273779 antagonised CGRP-mediated stimulation of intracellular Ca^{2+} in recombinant CGRP receptors, inhibition of insulin-stimulated [^{14}C]deoxyglucose uptake, vasodilation in rat pulmonary artery, and decrease in blood pressure in anaesthetised rats (135). These results suggest that SB-273779 is a valuable tool for studying CGRP-mediated functional responses in complex biological systems.

1.4.3.4. Neuroanatomical localisation and physiological aspects of CGRP in relation to migraine

Most sensory fibres to cranial structures are derived from the trigeminal ganglion. CGRP, substance P and neurokinin A are the dominant neuropeptides found in the sensory nerves among which CGRP seems to be the major constituent (143). The role of CGRP in the pathogenesis of migraine appears to be due to its potent vasodilator action (18, 22, 144). Electrical stimulation of trigeminal ganglion induces release of CGRP and substance P (40, 145). In the trigeminal ganglion, CGRP-immunoreactive neurones occur in high numbers (40% of all neuronal cells), whereas substance P-immunoreactive neurones are fewer (18%), as has also been observed in cats, with CGRP: substance P ratio being 3:1 (143). Both CGRP and substance P are described as potent vasodilators of cranial vessels, with the former being around 10-1000 times more potent (143). Interestingly, neurokinin receptor antagonists do not have any beneficial effects in aborting acute migraine attack (45). In addition, there are indications that CGRP is released during migraine headache and not substance P (22). A model has been proposed to explain the role of mitogen-activated protein kinase (MAPK) activity and 5-HT_1 receptor activation on CGRP gene expression in neuronal cells (Figure 1.6) (27). Activation of MAPK pathways, following depolarisation or by inflammatory mediators, initiates a cascade involving extracellular signal regulated kinases (ERK), Jun amino terminal kinases (JNK) and P38 proteins that ultimately lead to an increase in CGRP gene expression and release (27). 5-HT_1 receptor agonists, such as sumatriptan, elicit a prolonged elevation of intracellular Ca^{2+} that mediates repression of stimulated CGRP transcription and secretion via induction of specific phosphatases (27). The regulation of CGRP gene expression by MAPK signal transduction cascades may be particularly relevant to migraine pathology, since many inflammatory mediators implicated in migraine pathophysiology are known activators of MAPK pathways.

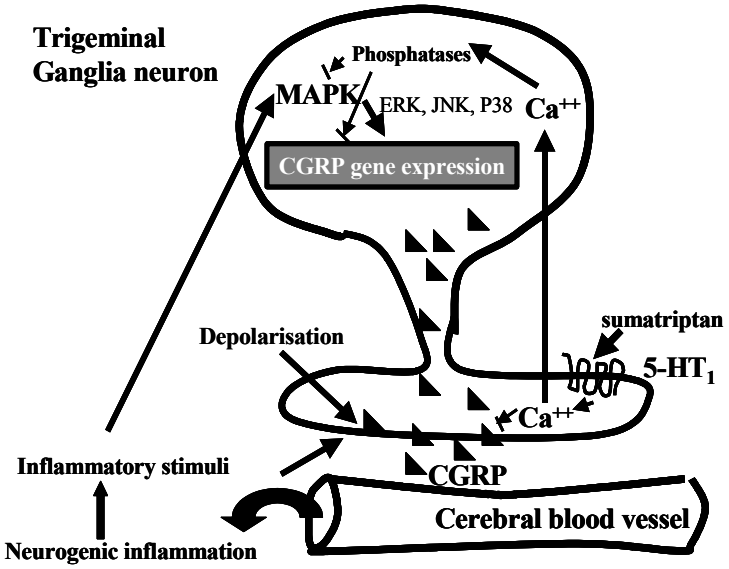


Figure 1.6. Model of CGRP regulation in migraine and effect of serotonergic antimigraine drugs.

Experiments on guinea pig basilar artery have demonstrated that the CGRP-mediated relaxation is due to $CGRP_1$ receptors and are not dependent on intact endothelium (146). This is supported with pharmacological studies on human intracranial arteries, which have shown that they have mainly $CGRP_1$ receptors that mediate relaxation (147, 148). Additionally, CGRP-induced vasodilatation has been observed in human lenticulostriate arteries of various diameters and this is mediated via $CGRP_1$ receptors (149, 150).

1.5. Aims of the thesis

Taking an account of the present chapter, the aims of the present thesis were:

- To investigate the possible role of α-adrenoceptors as well as CGRP and 5-HT receptors with respect to antimigraine activity.
- To evaluate therapeutic potential of certain novel compounds (donitriptan, S19014, A61604 and BIBN4096BS) in porcine model of migraine and their possible mechanism of action using selective agonists and antagonists.

References

1. Raskin NH. Diseases of the central nervous system. Harrison's principles of internal medicine. USA: The McGrawHill companies Inc., 1998:2307-2311.
2. Olesen J. Headache Classification Committee of the International Headache Society. Classification and Diagnostic Criteria for Headcahe disorders, Cranial Neuralgias and Facial Pain. Cephalalgia 1988;8:1-28.
3. Merikangas KR, Whitaker AE, Angst J. Validation of diagnostic criteria for migraine in the Zürich longitudinal cohort study. Cephalalgia 1993;13:47-53.
4. Michel P, Dartigues JF, Henry P, Yacoub MH. Validity of the International Headache Society Criteria for Migraine. Neuroepidemiology 1993;12:51-57.
5. Henry P, Michel P, Brochet B, Dartiques JF, Tison S, Salamon R. A nationwide survey of migraine in France: prevalence and clinical features in adult. Cephalalgia 1992;12:229-237.
6. Rasmussen BK, Jensen R, Olesen J. A population-based analysis of the diagnostic criteria of the Internatinal Headache Society. Cephalalgia 1991;11:129-134.
7. Stewart WF, Lipton R, Celentano DD, Reed ML. Prevalence of migraine headcahe in the United States. JAMA 1992;267:64-69.
8. Brandes JL. Global Trends in Migraine Care: Results from the MAZE Survey. CNS Drugs 2002;16:13-18.
9. Heyck H. Pathogenesis of migraine. Res Clin Stud Headache 1969;2:1-28.
10. Moskowitz MA. The neurobiology of vascular head pain. Ann Neurol 1984;16:157-168.
11. Saxena PR, Ferrari MD. 5-HT_1-like receptor agonists and the pathophysiology of migraine. Trends Pharmacol Sci 1989;10:200-204.
12. Welch KM, Barkley GL, Tepley N, Ramadan NM. Central neurogenic mechanisms of migraine. Neurology 1993;43:S21-25.
13. Graham JR, Wolff HG. Mechanism of migraine headache and action of ergotamine tartrate. Arch Neurol Psychia 1938;39:737-763.
14. Nichols FT, Mawad M, Mohr JP, Stein B, Hilal S, Michelsen WJ. Focal headache during balloon inflation in the internal carotid and middle cerebral arteries. Stroke 1990;21:555-559.
15. Kimball RW, Friedman AP, Vallejo E. Effect of serotonin in migraine patients. Neurology 1960;10:107-111.
16. Andersen AR, Friberg L, Olsen TS, Olesen J. Delayed hyperemia following hypoperfusion in classic migraine. Arch Neurol 1988;45:154-159.

17. Moskowitz MA. Neurogenic versus vascular mechanisms of sumatriptan and ergot alkaloids in migraine. Trends Pharmacol Sci 1992;13:307-311.
18. Goadsby PJ, Edvinsson L. The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats. Ann Neurol 1993;33:48-56.
19. Johnson KW, Phebus LA, Cohen ML. Serotonin in migraine: Theories animal models and emerging therapies. Prog Drug Res 1998;51::221-224.
20. James MF, Smith JM, Boniface SJ, Huang CL-H, Leslie RA. Cortical spreading depression and migraine:new insights from imaging? Trends Neurosci 2001;24:266-271.
21. Akerman S, Williamson DJ, Kaube H, Goadsby PJ. Nitric oxide synthase inhibitors can antagonize neurogenic and calcitonin gene-related peptide induced dilation of dural meningeal vessels. Br J Pharmacol 2002;137:62-68.
22. Goadsby PJ, Edvinsson L, Ekman R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. Ann Neurol 1990;28:183-187.
23. Van Rossum D, Hanisch UK, Quirion R. Neuroanatomical localization, pharmacological characterization and functions of CGRP, related peptides and their receptors. Neurosci Biobehav Rev 1997;21:649-678.
24. Markowitz S, Saito K, Moskowitz MA. Neurogenically mediated leakage of plasma protein occurs from blood vessels in dura mater but not brain. J Neurosci 1987;7:4129-4136.
25. Moskowitz MA. Neurogenic inflammation in the pathophysiology and treatment of migraine. Neurology 1993;43:S16-20.
26. Tepper SJ, Rapoport AM, Sheftell FD. Mechanisms of action of the 5-$HT_{1B/1D}$ receptor agonists. Arch Neurol 2002;59:1084-1088.
27. Durham P, Russo A. New insights into the molecular actions of serotonergic antimigraine drugs. Pharmacol Ther 2002;94:77-92.
28. Lassen LH, Ashina M, Christiansen I, Ulrich V, R. G, Donaldson J, Olesen J. Nitric oxide synthase inhibition: a new principle in the treatment of migraine attacks. Cephalalgia 1998;18:27-32.
29. De Vries P, Villalón CM, Saxena PR. Pharmacological aspects of experimental headache models in relation to acute antimigraine therapy. Eur J Pharmacol 1999;375:61-74.
30. Humphrey PPA, Feniuk W. Mode of action of the anti-migraine drug sumatriptan. Trends Pharmacol Sci 1991;12:444-446.
31. Ferrari MD, Saxena PR. Clinical and experimental effects of sumatriptan in humans. Trends Pharmacol Sci 1993;14:129-133.
32. Saxena PR. Cranial arteriovenous shunting, an *in vivo* animal model for migraine. In: Olesen J, Moskowitz MA, eds. Experimental headache models. Philadelphia, USA: Lippincott-Raven Publishers, 1995:189-198.
33. Willems EW, De Vries P, Heiligers JPC, Saxena PR. Porcine carotid vascular effects of eletriptan (UK-116,044): a new 5-$HT_{1B/1D}$ receptor agonist with anti-migraine activity. Naunyn-Schmiedebergs Arch Pharmacol 1998;358:212-219.
34. De Vries P, Heiligers JPC, Villalón CM, Saxena PR. Blockade of porcine carotid vascular response to sumatriptan by GR127935, a selective 5-HT_{1D} receptor antagonist. Br J Pharmacol 1996;118:85-92.

35. De Vries P, Sanchez-Lopez A, Centurion D, Heiligers JPC, Saxena PR, Villalón CM. The canine external carotid vasoconstrictor 5-HT_1 receptor: blockade by 5-HT_{1B} (SB224289), but not by 5-HT_{1D} (BRL15572) receptor antagonists. Eur J Pharmacol 1998;362:69-72.
36. De Vries P, Villalón CM, Heiligers JPC, Saxena PR. Characterization of 5-HT receptors mediating constriction of porcine carotid arteriovenous anastomoses; involvement of 5-$HT_{1B/1D}$ and novel receptors. Br J Pharmacol 1998;123:1561-1570.
37. Shepheard SL, Williamson DJ, Beer MS, Hill RG, Hargreaves RJ. Differential effects of 5-$HT_{1B/1D}$ receptor agonists on neurogenic dural plasma extravasation and vasodilation in anaesthetized rats. Neuropharmacology 1997;36:525-533.
38. Lee WS, Moskowitz MA. Conformationally restricted sumatriptan analogues, CP-122,288 and CP-122,638 exhibit enhanced potency against neurogenic inflammation in dura mater. Brain Res 1993;626:303-305.
39. Goadsby PJ, Duckworth JW. Effect of stimulation of trigeminal ganglion on regional cerebral blood flow in cats. Am J Physiol 1987;253:R270-274.
40. Goadsby PJ, Edvinsson L, Ekman R. Release of vasoactive peptides in the extracerebral circulation of humans and the cat during activation of the trigeminovascular system. Ann Neurol 1988;23:193-196.
41. Goadsby PJ. Inhibition of calcitonin gene-related peptide by h-$CGRP_{(8-37)}$ antagonizes the cerebral dilator response from nasociliary nerve stimulation in the cat. Neurosci Lett 1993;151:13-16.
42. Hargreaves RJ, Williamson DJ, Shepheard SL. Neurogenic inflammation: relation to novel antimigraine drugs. In: Edvinsson L, ed. Migraine and headache pathophysiology. London: Martin Dunitz, 1999:93-101.
43. Roon KI, Olesen J, Diener HC, Ellis P, Hettiarachchi J, Poole PH, Christianssen I, Kleinermans D, Kok JG, Ferrari MD. No acute antimigraine efficacy of CP-122,288, a highly potent inhibitor of neurogenic inflammation: results of two randomized, double-blind, placebo-controlled clinical trials. Ann Neurol. 2000;47:238-241.
44. May A, Gijsman HJ, Wallnöfer A, Jones R, Diener HC, Ferrari MD. Endothelin antagonist bosentan blocks neurogenic inflammation, but is not effective in aborting migraine attacks. Pain 1996;67:375-378.
45. Goldstein DJ, Wang O, Saper JR, Stoltz R, Silberstein SD, Mathew NT. Ineffectiveness of neurokinin-1 antagonist in acute migraine: a crossover study. Cephalalgia 1997;17:785-790.
46. Diener HC. RPR100893, a substance-P antagonist, is not effective in the treatment of migraine attacks. Cephalalgia 2003;23:183-185.
47. Lambert GA, Bogduk N, Goadsby PJ, Duckworth JW, Lance JW. Decreased carotid arterial resistance in cats in response to trigeminal stimulation. J Neurosurg 1984;61:307-315.
48. Goadsby PJ, Lambert GA, Lance JW. Stimulation of the trigeminal ganglion increases flow in the extracerebral but not the cerebral circulation of the monkey. Brain Res 1986;381:63-67.
49. Beattie DT, Connor HE. The influence of the trigeminal gamglion on carotid blood flow in anaesthetized guinea-pigs. Br J Pharmacol 1994;112:262-266.
50. Doods H, Hallermayer G, Wu D, Entzeroth M, Rudolf K, Engel W, Eberlein W. Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist. Br J Pharmacol 2000;129:420-423.

51. Edvinsson L. Calcitonin gene-related peptide (CGRP) and the pathophysiology of headache: therapeutic implications. CNS Drugs 2001;15:745-753.
52. Zagami AS, Lambert GA. Stimulation of cranial vessels excites nociceptive neurons in several thalamic nuclei of the cat. Exp Brain Res 1990;81:552-566.
53. Goadsby PJ, Knight YE. Direct evidence for central sites of action of zolmitriptan (311C90): an autoradiographic study in cat. Cephalalgia 1997;17:153-158.
54. Goadsby PJ, Knight YE. Inhibition of trigeminal neurones after intravenous administration of naratriptan through an action at 5-hydroxytryptamine ($5\text{-}HT_{1B/1D}$) receptors. Br J Pharmacol 1997;122:918-922.
55. Razzaque Z, Heald MA, Pickard JD, Maskell L, Beer MS, Hill RG, Longmore J. Vasoconstriction in human isolated middle meningeal arteries: determining the contribution of $5\text{-}HT_{1B}$- and $5\text{-}HT_{1F}$-receptor activation. Br J Clin Pharmacol 1999;47:75-82.
56. Verheggen R, Hundeshagen AG, Brown AM, Schindler M, Kaumann AJ. $5\text{-}HT_{1B}$ receptor-mediated contractions in human temporal artery: evidence from selective antagonists and 5-HT receptor mRNA expression. Br J Pharmacol 1998;124:1345-1354.
57. Hamel E, Bouchard D. Contractile $5\text{-}HT_1$ receptors in human isolated pial arterioles: correlation with $5\text{-}HT_{1D}$ binding sites. Br J Pharmacol 1991;102:227-233.
58. Olivar T, Razzaque Z, Nwagwu M, Longmore J. Neurogenic vasodilation in rabbit basilar isolated artery: involvement of calcitonin-gene related peptide. Eur J Pharmacol 2000;395:61-68.
59. Longmore J, Shaw D, Smith D, Hopkins R, McAllister G, Pickard JD, Sirinathsinghji DJ, Butler AJ, Hill RG. Differential distribution of $5HT_{1D}$- and $5HT_{1B}$-immunoreactivity within the human trigemino-cerebrovascular system: implications for the discovery of new antimigraine drugs. Cephalalgia 1997;17:833-842.
60. Van den Broek RW, MaassenVanDenBrink A, De Vries R, Bogers AJ, Stegmann AP, Avezaat CJ, Saxena PR. Pharmacological analysis of contractile effects of eletriptan and sumatriptan on human isolated blood vessels. Eur J Pharmacol 2000;407:165-173.
61. MaassenVanDenBrink A, Reekers M, Bax WA, Ferrari MD, Saxena PR. Coronary side-effect potential of current and prospective antimigraine drugs. Circulation 1998;98:25-30.
62. Connor HE, Feniuk W, Humphrey PPA. 5-Hydroxytryptamine contracts human coronary arteries predominantly via $5\text{-}HT_2$ receptor activation. Eur J Pharmacol 1989;161:91-94.
63. Bax WA, Renzenbrink GJ, Van Heuven-Nolsen D, Thijssen EJ, Bos E, Saxena PR. 5-HT receptors mediating contractions of the isolated human coronary artery. Eur J Pharmacol 1993;239:203-210.
64. Iversen HK. Human migraine models. Cephalalgia 2001;21:781-785.
65. Schumacher GA, Wolff HG. Experimental studies on headache. Arch Neurol Psychiat 1941;45:199-213.
66. Iversen HK, Olesen J. Headache induced by a nitric oxide donor (nitroglycerin) responds to sumatriptan. A human model for development of migraine drugs [see comments]. Cephalalgia 1996;16:412-418.
67. Thomsen LL, Olesen J. Human Models of Headache. In: Olesen J, Tfelt-Hansen P, Welch KMA, eds. The Headaches. Philadelphia: Lippincott Williams & Wilkins, 2000:203-209.
68. Saxena PR, Den Boer MO. Pharmacology of antimigraine drugs. J Neurol 1991;238:S28-35.

69. Mitchelson F. Pharmacological agents affecting emesis. A review (part I). Drugs 1992;43:295-315.
70. Markowitz S, Saito K, Moskowitz MA. Neurogenically mediated plasma extravasation in dura mater: effect of ergot alkaloids. A possible mechanism of action in vascular headache. Cephalalgia 1988;8:83-91.
71. Hoskin KL, Kaube H, Goadsby PJ. Central activation of the trigeminovascular pathway in the cat is inhibited by dihydroergotamine. A c-Fos and electrophysiological study. Brain 1996;119:249-256.
72. Horton BT, Peters GA, Blumenthal LS. A new product in the treatment of migraine: A preliminary report. Mayo Clin Proc 1945;20:241-248.
73. Villalón CM, De Vries P, Rabelo G, Centurión D, Sánchez-López A, Saxena PR. Canine external carotid vasoconstriction to methysergide, ergotamine and dihydroergotamine: a role of 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors. Br J Pharmacol 1999;126:385-394.
74. Peroutka SJ. Drugs effective in the therapy of migraine. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, eds. Goodman and Gilman's the pharmacological basis of therapeutics. New York: McGraw-Hill, 1996:487-502.
75. Roithinger FX, Punzengruber C, Gremmel F, Hinterreiter M, Holzner F, Pachinger O. Myocardial infarction after chronic ergotamine abuse. Eur Heart J 1993;14:1579-1581.
76. Saxena PR, Tfelt-Hansen P. Triptan, 5-$HT_{1B/1D}$ receptor agonists in the acute treatment of migraine. In: Olesen J, Tfelt-Hansen P, Welch KMA, eds. The headaches. Philadelphia: Lippincott Williams & Wilkins, 2000:411-438.
77. Humphrey PP, Feniuk W, Perren MJ, Connor HE, Oxford AW, Coates LH, Butina D. GR43175, a selective agonist for the 5-HT_1-like receptor in dog isolated saphenous vein. Br J Pharmacol 1988;94:1123-1132.
78. Goadsby PJ. Serotonin receptors and the acute attack of migraine. Clin Neurosci 1998;5:18-23.
79. Hamel E, Fan E, Linville D, Ting V, Villemure JG, Chia LS. Expression of mRNA for the serotonin 5-hydroxytryptamine$_{1D\beta}$ receptor subtype in human and bovine cerebral arteries. Mol Pharmacol 1993;44:242-246.
80. Nilsson T, Longmore J, Shaw D, Jansen-Olesen I, Edvinsson L. Contractile 5-HT_{1B} receptors in human cerebral arteries: pharmacological characterization and localization with immunocytochemistry. Br J Pharmacol 1999;128:1133-1140.
81. Bouchelet I, Case B, Olivier A, Hamel E. No contractile effect for 5-HT_{1D} and 5-HT_{1F} receptor agonists in human and bovine cerebral arteries: similarity with human coronary artery. Br J Pharmacol 2000;129:501-508.
82. Smith D, Hill RG, Edvinsson L, Longmore J. An immunocytochemical investigation of human trigeminal nucleus caudalis: CGRP, substance P and 5-HT_{1D}-receptor immunoreactivities are expressed by trigeminal sensory fibres. Cephalalgia 2002;22:424-431.
83. Fowler P, Fuseau E, Chilton J, Hussey EK, Moore KP. The clinical pharmacology of sumatriptan nasal spray. Cephalalgia 1995;15 (suppl 14):238.
84. Dixon CM, Park GR, Tarbit MH. Characterization of the enzyme responsible for the metabolism of sumatriptan in human liver. Biochem Pharmacol 1994;47:1253-1257.
85. Blier P, Bergeron R. The safety of concomitant use of sumatriptan and antidepressant treatments. J Clin Psychopharmacol 1995;15:106-109.

86. Mathew NT, Salonen R. Defining optimal dosing for sumatriptan tablets in the acute treatment of migraine. Int J Clin Pract Suppl 1999;105:2-6.
87. Salonen R, Ashford EA, Gibbs M, Hassani H. Patient preference for oral sumatriptan 25 mg, 50 mg, or 100 mg in the acute treatment of migraine: a double-blind, randomized, crossover study. Sumatriptan Tablets S2CM11 Study Group. Int J Clin Pract Suppl 1999;105:16-24.
88. Tfelt-Hansen P. Efficacy and adverse events of subcutaneous, oral and intranasal sumatriptan used for migraine treatment: a systematic review based on number needed to treat. Cephalalgia 1998;18:532-538.
89. Visser WH, de Vriend RH, Jaspers MW, Ferrari MD. Sumatriptan in clinical practice: a 2-year review of 453 migraine patients. Neurology 1996;47:46-51.
90. Tomita M, Suzuki N, Igarashi H, Endo M, Sakai F. Evidence against strong correlation between chest symptoms and ischemic coronary changes after subcutaneous sumatriptan injection. Intern Med 2002;41:622-625.
91. Martin GR, Dixon R. Pre-clinical and clinical pharmacology of the novel anti-migraine compound 311C90. Headache 1995;53:291.
92. Connor HE, Feniuk W, Beattie DT, North PC, Oxford AW, Saynor DA, Humphrey PP. Naratriptan: biological profile in animal models relevant to migraine. Cephalalgia 1997;17:145-152.
93. Beer M, Middlemiss D, Stanton J, Lomgmore J, Hargreaves R, Noble A, Scholey K, Bevan Y, Hill R, Baker R, Street L, Matassa V, Iversen L. In vitro pharmacological profile of the novel 5-HT_{1D} receptor agonist MK-462. Cephalalgia 1995;15 (Suppl.14):203.
94. Vyas KP, Halpin RA, Geer LA, Ellis JD, Liu L, Cheng H, Chavez-Eng C, Matuszewski BK, Varga SL, Guiblin AR, Rogers JD. Disposition and pharmacokinetics of the antimigraine drug, rizatriptan, in humans. Drug Metab Dispos 2000;28:89-95.
95. Oldman AD, Smith LA, McQuay HJ, Moore RA. Rizatriptan for acute migraine. Cochrane Database Syst Rev 2001:CD003221.
96. McHarg AD, Napier CM, Stewart M, Melrose HL, Wallis RM. The functional activity of eletriptan and other 5-$HT_{1B/1D}$ agonists at the human recombinant 5-HT_{1B} and 5-HT_{1D} receptors. Headache 1999;39:369.
97. Smith LA, Oldman AD, McQuay HJ, Moore RA. Eletriptan for acute migraine. Cochrane Database Syst Rev 2001:CD003224.
98. Bou J, Domenech T, Gras J, al. e. Pharmacological profile of almotriptan, a novel antimigraine agent. Cephalalgia 1997;19:421-422.
99. Bou J, Cardelus I, Llenas J, al. e. Antimigraine potential of almotriptan in animal models. Methods Find Exp Clin Pharmacol 1997;19 (Suppl):A107.
100. Gras J, Llupia J, Llenas J, Palacios JM. Safety profile of almotriptan, a new antimigraine agent. Effects on central nervous system, renal function and respiratory dynamics. Arzneimittelforschung 2001;51:726-732.
101. Cabarrocas X, Zayas JM, Suris M. Equivalent efficacy of oral almotriptan, a new 5-$HT_{1B/1D}$ agonist, compared with sumatriptan 100 mg. Headache 1998;38:377.
102. Spierings EL, Gomez-Mancilla B, Grosz DE, al. e. Oral almotriptan vs. oral sumatriptan in a double-blind, randomized, parallel-group study in migraine patients. Headache 2000;40:433.

103. Parsons AA, Raval P, Smith S, Tilford N, King FD, Kaumann AJ, Hunter J. Effects of the novel high-affinity 5-$HT_{1B/1D}$-receptor ligand frovatriptan in human isolated basilar and coronary arteries. J Cardiovasc Pharmacol 1998;32:220-224.
104. Elkind AH, Satin L, Keywood C. Frovatriptan cardiovascular safety in patients at high risk or with overt coronary artery disease during an acute migraine attack. Headache 2000;40:407.
105. Rapoport AM, Keywood C. Frovatriptan-dose response studies. Headache 1999;39:375.
106. Dukat M. Donitriptan (Pierre Fabre). Curr Opin Investig Drugs 2001;2:415-418.
107. Perez M, Halazy S, Pauwels PJ, Colpaert FC, John GW. F-11356 5-$HT_{1B/1D}$ receptor agonist (Antimigraine). Drugs Future 1999;24:605-612.
108. John GW, Pauwels PJ, Perez M, Halazy S, Le Grand B, Verscheure Y, Valentin JP, Palmier C, Wurch T, Chopin P, Marien M, Kleven MS, Koek W, Assi MB, Carilla-Durand E, Tarayre JP, Colpaert FC. F 11356, a novel 5-hydroxytryptamine (5-HT) derivative with potent, selective, and unique high intrinsic activity at 5-$HT_{1B/1D}$ receptors in models relevant to migraine. J Pharmacol Exp Ther 1999;290:83-95.
109. Van den Broek RW, MaassenVanDenBrink A, Mulder PG, Bogers AJ, Avezaat CJ, John GW, Saxena PR. Comparison of contractile responses to donitriptan and sumatriptan in the human middle meningeal and coronary arteries. Eur J Pharmacol 2002;443:125-132.
110. Willems EW, Valdivia LF, Villalón CM, Saxena PR. α−Adrenoceptors and acute migraine therapy. Drug News Perspect 2002;15:140-146.
111. De Vries P, Willems EW, Heiligers JPC, Villalón CM, Saxena PR. Investigations of the role of 5-HT_{1B} and 5-HT_{1D} receptors in the sumatriptan-induced constriction of porcine carotid arteriovenous anastomoses. Br J Pharmacol 1999;127:405-412.
112. Leysen JE. Serotonergic binding sites. In: Vanhoutte PM, ed. Serotonin and the cardiovascular system. New York: Raven Press, 1985:43-62.
113. Tfelt-Hansen P, Saxena PR, Dahlof C, Pascual J, Lainez M, Henry P, Diener H, Schoenen J, Ferrari MD, Goadsby PJ. Ergotamine in the acute treatment of migraine: A review and european consensus. Brain 2000;123:9-18.
114. Verdouw PD, Duncker DJ, Saxena PR. Poor vasoconstrictor response to adrenergic stimulation in the arteriovenous anastomoses present in the carotid vascular bed of young Yorkshire pigs. Arch Int Pharmacodyn Ther 1984;272:56-70.
115. Den Boer MO, Van Woerkens LJ, Somers JA, Duncker DJ, Lachmann B, Saxena PR, Verdouw PD. On the preservation and regulation of vascular tone in arteriovenous anastomoses during anesthesia. J Appl Physiol 1993;75:782-789.
116. Folkow B, Sivertsson R. Aspects of the difference in vascular reactivity between cutaneous resistance vessels and A-V anastomoses (cats). Angiologica 1964;1:338-345.
117. Spence RJ, Rhodes BA, Wagner HJ. Regulation of arteriovenous anastomotic and capillary blood flow in the dog leg. Am J Physiol 1972;222:326-332.
118. Baker CH, Davis DL, Sutton ET. Neural control of nutritional and nonnutritional circuits in the dog hindpaw. Am J Physiol 1978;234:H384-391.
119. Hales JRS. Radioactive microsphere techniques for studies of the circulation. Clin exp Pharmacol Physiol 1974;1:31-46.
120. Hales JR, Foldes A, Fawcett AA, King RB. The role of adrenergic mechanisms in thermoregulatory control of blood flow through capillaries and arteriovenous anastomoses in the sheep hind limb. Pflugers Arch 1982;395:93-98.

121. Kawai Y, Kobayashi S, Ohhashi T. Existence of two types of postjunctional α-adrenoceptors in the isolated canine internal carotid artery. Can J Physiol Pharmacol 1988;66:655-659.
122. Kohno Y, Saito H, Takita M, Kigoshi S, Muramatsu I. Heterogeneity of α_1-adrenoceptor subtypes involved in adrenergic contractions of dog blood vessels. Br J Pharmacol 1994;112:1167-1173.
123. Muramatsu I. Relation between adrenergic neurogenic contraction and alpha$_1$-adrenoceptor subtypes in dog mesenteric and carotid arteries and rabbit carotid arteries. Br J Pharmacol 1991;102:210-214.
124. Ohgushi M, Yasue H, Kugiyama K, Murohara T, Sakaino N. Contraction and endothelium dependent relaxation via alpha adrenoceptors are variable in various pig arteries. Cardiovasc Res 1993;27:779-784.
125. Cordi AA, Lacoste JM, Descombes JJ, Courchay C, Vanhoutte PM, Laubie M, Verbeuren TJ. Design, synthesis, and structure-activity relationships of a new series of α-adrenergic agonists: spiro [(1,3-diazacyclopent-1-ene)-5,2'-(1',2',3',4'- tetrahydronaphthalene)]. J Med Chem 1995;38:4056-4069.
126. Descombes J-J, Menant Y, Barou A, Cordi A, Verbeuren TJ. S19014 is a partial agonist at alpha-adrenoceptors that selectively contracts the veins. Pharmacol Toxicol 1998;83 (Suppl. 1):92.
127. Poyner DR. Calcitonin gene-related peptide: multiple actions, multiple receptors. Pharmacol Ther 1992;56:23-51.
128. Poyner D. Pharmacology of receptors for calcitonin gene-related peptide and amylin. Trends Pharmacol Sci 1995;16:424-428.
129. Amara SG, Jonas V, Rosenfeld MG, Ong ES, Evans RM. Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. Nature 1982;298:240-244.
130. Rosenfeld MG, Lin CR, Amara SG, Stolarsky L, Roos BA, Ong ES, Evans RM. Calcitonin mRNA polymorphism: peptide switching associated with alternative RNA splicing events. Proc Natl Acad Sci USA 1982;79:1717-1721.
131. Amara SG, Arriza JL, Leff SE, Swanson LW, Evans RM, Rosenfeld MG. Expression in brain of a messenger RNA encoding a novel neuropeptide homologous to calcitonin gene-related peptide. Science 1985;229:1094-1097.
132. Poyner DR, Sexton PM, Smith DM, Quiron R, Born W, Muff R, Fischer JA, Foord SM. International Union of Pharmacology. XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin and calcitonin receptors. Pharmacol Rev 2002;54:233-246.
133. Doggrell SA. Migraine and beyond: cardiovascular therapeutic potential for CGRP modulators. Expert Opin Investig Drugs 2001;10:1131-1138.
134. Quirion R, Van Rossum D, Dumont Y, St-Pierre S, Fournier A. Characterization of $CGRP_1$ and $CGRP_2$ receptor subtypes. Ann N Y Acad Sci 1992;657:88-105.
135. Aiyar N, Daines RA, Disa J, Chambers PA, Sauermelch CF, Quiniou M, Khandoudi N, Gout B, Douglas SA, Willette RN. Pharmacology of SB-273779, a nonpeptide calcitonin gene-related peptide 1 receptor antagonist. J Pharmacol Exp Ther 2001;296:768-775.
136. Juaneda C, Dumont Y, Quirion R. The molecular pharmacology of CGRP and related peptide receptor subtypes. Trends Pharmacol Sci 2000;21:432-438.

137. McLatchie LM, al e. RAMPs regulate the transport and ligand specificity of the calcitonin-receptor like receptor. Nature 1998;393:333-339.
138. Buhlmann N, Leuthauser K, Muff R, Fischer JA, Born W. A receptor activity modifying protein (RAMP2)-dependent adrenomedullin receptor is a calcitonin gene-related peptide receptor when coexpressed with human RAMP1. Endocrinology 1999;140:2883-2890.
139. Frayon S, Cueille C, Gnidehou S, de Vernejoul MC, Garel JM. Dexamethasone increases RAMP1 and CRLR mRNA expressions in human vascular smooth muscle cells. Biochem Biophys Res Commun 2000;270:1063-1067.
140. Luebke AE, Dahl GP, Roos BA, Dickerson IM. Identification of a protein that confers calcitonin gene-related peptide responsiveness to oocytes by using a cystic fibrosis transmembrane conductance regulator assay. Proc Natl Acad Sci USA 1996;93:3455-3460.
141. Rist B, Lacroix JS, Entzeroth M, Doods HN, Beck-Sickinger AG. $CGRP_{27-37}$ analogues with high affinity to the $CGRP_1$ receptor show antagonistic properties in a rat blood flow assay. Regul Pept 1999;79:153-158.
142. Doods H. Development of CGRP antagonists for the treatment of migraine. Curr Opin Investig Drugs 2001;2:1261-1268.
143. Uddman R, Tajti J, Edvinsson L. Neuronal messengers and peptide receptors in human cranial ganglia. In: Edvinsson L, ed. Migraine and headache pathophysiology. London: Martin Dunitz Ltd, 1999:31-41.
144. Goadsby PJ, Edvinsson L. Human in vivo evidence for trigeminovascular activation in cluster headache. Neuropeptide changes and effects of acute attacks therapies. Brain 1994;117:427-434.
145. Buzzi MG, Carter WB, Shimizu T, Heath H, 3rd, Moskowitz MA. Dihydroergotamine and sumatriptan attenuate levels of CGRP in plasma in rat superior sagittal sinus during electrical stimulation of the trigeminal ganglion. Neuropharmacology 1991;30:1193-1200.
146. Jansen-Olesen I, Kaarill L, Edvinsson L. Characterization of $CGRP_1$ receptors in the guinea pig basilar artery. Eur J Pharmacol 2001;414:249-258.
147. Edvinsson L, Cantera L, Jansen-Olesen I, Uddman R. Expression of calcitonin gene-related peptide1 receptor mRNA in human trigeminal ganglia and cerebral arteries. Neurosci Lett 1997;229:209-211.
148. Jansen-Olesen I, Mortensen A, Edvinsson L. Calcitonin gene-related peptide is released from capsaicin-sensitive nerve fibres and induces vasodilatation of human cerebral arteries concomitant with activation of adenylyl cyclase. Cephalalgia 1996;16:310-316.
149. Sams A, Yenidunya A, Engberg J, Jansen-Olesen I. Equipotent in vitro actions of alpha- and beta-CGRP on guinea pig basilar artery are likely to be mediated via CRLR derived CGRP receptors. Regul Pept 1999;85:67-75.
150. Sams A, Knyihar-Csillik E, Engberg J, Szok D, Tajti J, Bodi I, Edvinsson L, Vecsei L, Jansen-Olesen I. CGRP and adrenomedullin receptor populations in human cerebral arteries: in vitro pharmacological and molecular investigations in different artery sizes. Eur J Pharmacol 2000;408:183-193.

CHAPTER 2

Effects of BIBN4096BS on regional cardiac output distribution and on CGRP-induced carotid haemodynamic responses in the pig

Based on: Kapoor K, Arulmani U, Heiligers JPC, Willems EW, Doods H, Villalón CM, Saxena PR. Effects of BIBN4096BS on regional cardiac output distribution and on CGRP-induced carotid haemodynamic responses in the pig. Eur J Pharmacol 2003;475:69-77.

2. Effects of BIBN4096BS on regional cardiac output distribution and on CGRP-induced carotid haemodynamic responses in the pig

2.1. Introduction

Calcitonin gene related peptide (CGRP), a 37 amino acid neuropeptide generated by alternative splicing of the calcitonin gene (1), is widely distributed in the body, including in trigeminal sensory nerve fibres innervating central and peripheral blood vessels, where it is co-localised with other vasoactive neuropeptides, such as substance P and neurokinin A (2, 3). CGRP is a potent vasodilator agent in a wide variety of tissues (4-7) and, although exogenous α-CGRP has potent systemic and regional haemodynamic effects (8), the physiological role of endogenous CGRP is not clear (9). This is mainly due to the unavailability of potent and selective CGRP receptor antagonists; the most widely used CGRP receptor antagonist thus far, $CGRP_{8-37}$, is not very potent and displays partial agonist properties (10, 11). Clearly, the advent of 'silent', selective and potent non-peptide CGRP receptor antagonists would be valuable in this regard.

Interestingly, CGRP has been implicated in the pathogenesis of migraine (12-15), and it can mediate neurogenic dilatation of cranial blood vessels as well as sensory nerve transmission between the first and second order afferent input from these vessels during migraine headache (3, 16-18). Significantly, plasma levels of CGRP, but not of other neurotransmitter (e.g. neuropeptide Y, vasoactive intestinal peptide or substance P), are elevated during migraine and, after sumatriptan, these levels are normalised paralleling the resolution of headache (12, 19). Therefore, inhibition of α-CGRP release or blockade of α-CGRP-induced vasodilatation may be a novel approach in the management of acute migraine headache.

Doods and colleagues (20) have recently described a small molecule CGRP receptor antagonist, BIBN4096BS (1-piperidinecarboxamide, N-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-piperazinyl] carbonyl]pentyl] amino]- 1-[(3,5-dibromo-4-hydroxyphenyl) methyl]- 2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2H)-quinazolinyl)-, [R-(R*,S*)]-), which possesses over 200 fold higher affinity for human (SK-N-MC cells; K_i: 14 pM) than for rat (spleen; K_i: 3.4 nM) CGRP receptors. BIBN4096BS as well as the endogenous ligand CGRP and its analogues concentration-dependently displace [^{3}H]BIBN4096BS from SK-N-MC cell membranes with the rank order of affinity: BIBN4096BS > human α-CGRP = human β-CGRP > [$Cys(Et)^{2,7}$] human α-CGRP > adrenomedullin (high affinity site) = human α-$CGRP_{8-37}$ = human β-$CGRP_{8-37}$ >> calcitonin = amylin (21). The compound inhibits vasodilatation evoked by trigeminal ganglion stimulation in marmosets (20) and by CGRP in several human isolated blood vessels (22-24). The purpose of the present study in anaesthetised pigs was to investigate the effects of BIBN4096BS on: (i) the complete distribution of cardiac output to assess the potential role of endogenous CGRP in regulating basal vascular tone and thereby the cardiovascular safety of BIBN4096BS, and (ii) the haemodynamic responses produced by intracarotid arterial (i.c.) infusion of α-CGRP in a model predictive of antimigraine activity (25, 26).

2.2. Methods

2.2.1. General

After an overnight fast, 25 domestic pigs (Yorkshire x Landrace, females, 10-14 kg) were sedated with intramuscular injections of azaperone (120 mg) and midazolam hydrochloride (10 mg) and then anaesthetised with sodium pentobarbital (600 mg, i.v.). After tracheal intubation, the animals were connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; pO_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of sodium pentobarbital (12-20 $mg.kg^{-1}.h^{-1}$). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by electrocardiogram signals. A catheter was placed in the inferior vena cava via the right femoral vein for the administration of vehicle and BIBN4096BS. Another catheter was placed in the aortic arch *via* the left femoral artery for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) and arterial blood withdrawal for the measurement of blood gases (ABL-510; Radiometer, Copenhagen, Denmark). During the experiment, body temperature was kept around 37°C and the animal was continuously infused with physiological saline to compensate for fluid losses.

Heart rate and systolic, diastolic and mean arterial blood pressure as well as the pulsatile and mean carotid artery blood flows (see later) were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands).

2.2.2. Cardiac output and its distribution

Cardiac output was measured by the thermodilution method using a 6F Swan-Ganz catheter (Braun Melsungen AG, Melsungen, Germany) introduced into the pulmonary artery via the left femoral vein.

The distribution of cardiac output was determined with 15.5±0.1 (s.d.) μm diameter microspheres labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, USA). For each measurement, a suspension of about 1,000,000 microspheres, labelled with one of the isotopes, was injected into the left ventricle via a catheter guided by way of the left carotid artery. Starting 15 s before microsphere injection and lasting 70 s, a reference arterial blood sample was withdrawn (Withdrawal pump, Harvard Apparatus Company, Southnatick, Mass, USA; rate: 6 $ml.min^{-1}$) via a catheter placed into the right femoral artery. An infusion of the corresponding volume of Haemaccel compensated blood loss during this procedure.

At the end of the experiment, the animal was killed using an overdose of pentobarbital. Subsequently, a number of tissues (lungs, kidneys, heart, stomach, small intestine, spleen, liver, adrenals, brain, skin and skeletal muscles) were dissected out, weighed and put into vials. The radioactivity in these vials was counted for 5 min in a γ-scintillation counter (Packard, Minaxi autogamma 5000), using suitable windows for the discrimination of the different isotopes (^{141}Ce: 120-167 KeV, ^{113}Sn: 355-435 KeV, ^{103}Ru: 450-548 KeV, ^{95}Nb: 706-829 KeV and ^{46}Sc: 830-965 KeV). All data were processed by a set of specially designed computer programs (27), using a personal computer. Tissue blood flows were calculated by multiplying the ratio of tissue and reference blood sample radioactivities by the blood withdrawal rate (6 $ml.min^{-1}$) and normalised to 100 g tissue weight. Systemic and tissue vascular conductances were calculated by dividing cardiac output ($ml.min^{-1}$) and tissue blood flows ($ml.min^{-1}$/100 g tissue), respectively, by mean arterial blood pressure (mmHg). Radioactivity in the lungs mainly represents peripheral arteriovenous anastomotic blood flow

(the non-nutrient part of the cardiac output), although a small part (1-1.5% of cardiac output) is derived from the bronchial arteries (28).

2.2.3. Carotid haemodynamic responses to CGRP

Both common carotid arteries and the external jugular veins were dissected free and the accompanying vagosympathetic trunks were cut between two ligatures in order to prevent a possible influence of CGRP via baroreceptor reflexes. Pulsatile and mean blood flows were measured in the right common carotid artery with a flow probe (internal diameter: 2.5 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands). The amplitude of carotid blood flow signals provided an index of carotid flow pulse. Carotid vascular conductance was calculated by dividing carotid blood flow ($ml.min^{-1}$) by mean arterial blood pressure (mmHg).

The right external jugular vein was catheterised for obtaining jugular venous blood samples to determine blood gases. Two hub-less needles, connected to polyethylene tubes, were inserted into the right common carotid artery and used for intracarotid (i.c.) infusions of phenylephrine (α_1-adrenoceptor agonist) and α-CGRP, respectively. It should be noted that under pentobarbital anaesthesia carotid arteriovenous anastomoses are dilated (29) and, therefore, to elicit vasodilator responses to CGRP, a continuous infusion of phenylephrine was used throughout the experiment. We have previously reported that phenylephrine decreases total carotid blood flow and conductance exclusively due to constriction of carotid arteriovenous anastomoses (30), resulting in an increase in the difference between arterial and jugular venous oxygen saturations (A-V SO_2 difference) (31).

2.2.4. Experimental protocols

In the case of cardiac output distribution experiments (n=12), baseline values of heart rate, mean arterial blood pressure, cardiac output and its distribution to the various tissues (see above) were determined after a stabilisation period of at least 90 min. The animals were then divided into two groups (n=6 each) receiving three i.v. infusions (rate: 0.5 $ml.min^{-1}$) of either BIBN4096BS (100, 300 and 1000 $\mu g.kg^{-1}$) or its vehicle (5 ml of acidified distilled water); each dose was given over 10 min with an intervening period of 10 min before the next dose. At the end of each infusion, the above mentioned haemodynamic variables were collated again. Lastly, the final measurements were made 40 min after the third dose of vehicle or BIBN4096BS (recovery).

In the case of the carotid artery experiments (n=13), phenylephrine (10 $\mu g.kg^{-1}.min^{-1}$ for 10 min, followed by 3-6 $\mu g.kg^{-1}.min^{-1}$ throughout the rest of the experiment) was infused into the right common carotid artery to maintain carotid blood flow at a constant low level. After a stabilisation period of at least 90 min, values of heart rate, arterial blood pressure, total carotid blood flow and A-V SO_2 difference were collated. The animal was then given three sequential i.c. infusions (rate: 0.083-1 $ml.min^{-1}$, depending on the weight of the animal) of CGRP (10, 30 and 100 $pmol.kg^{-1}.min^{-1}$) for 3 min and the above variables (except the A-V SO_2 difference, which was determined only after the highest dose) were collated again. After the highest dose of α-CGRP, a recovery period of 20 min was allowed to elapse when all haemodynamic parameters returned to baseline levels. At this point, the animals were divided into two groups receiving three i.v. infusions (rate: 0.5 $ml.min^{-1}$) of either BIBN4096BS (100, 300 and 1000 $\mu g.kg^{-1}$; n=7) or its vehicle (5 ml of acidified distilled water; n=6); each dose was given over a period of 10 min with an intervening period of about 10 min before the next dose. Ten min after each treatment, the values of mean arterial blood pressure, heart rate, total carotid blood flow and A-V SO_2 difference were collated. CGRP was infused as above after each treatment and data were collated again.

It may be mentioned that the vehicle of α-CGRP (distilled water) was devoid of any systemic and carotid haemodynamic responses (data not shown).

2.2.5. Data presentation and statistical analysis

All data have been expressed as mean±s.e.mean, unless stated otherwise. The significance of changes from baseline values within one group (vehicle or BIBN4096BS) was evaluated with Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations (27, 32). The differences in baseline haemodynamic values and percent change (from baseline values) in haemodynamic variables by corresponding doses of the vehicle and BIBN4096BS (between group comparisons) were evaluated by Student's unpaired *t*-test. Student's unpaired *t*-test was also applied to compare the changes in the effects of CGRP observed after different corresponding doses of the vehicle and BIBN4096BS. Statistical significance was accepted at $P<0.05$ (two-tailed).

2.2.6. Ethical approval

The Ethics Committee of the Erasmus MC, Rotterdam, dealing with the use of animals in scientific experiments, approved investigation protocols, which adhere to EEC guidelines.

2.2.7. Compounds

The following compounds were used: azaperone (Stresnil®; Janssen Pharmaceuticals, Beerse, Belgium), BIBN4096BS and human α-CGRP (Boehringer Ingelheim Pharma KG, Biberach, Germany), heparin sodium (to prevent blood clotting in catheters; Leo Pharmaceutical Products, Weesp, The Netherlands), midazolam hydrochloride (Dormicum®; Hoffmann La Roche b.v., Mijdrecht, The Netherlands), phenylephrine hydrochloride (Sigma-Aldrich Chemie b.v., Zwijndrecht, The Netherlands) and sodium pentobarbital (Sanofi Sante b.v., Maasluis, The Netherlands).

Phenylephrine and α-CGRP were dissolved in distilled water, while BIBN4096BS was initially dissolved in 0.5 ml of 1N HCl and subsequently diluted with 4 ml of distilled water, and then adjusted to pH 6.5 with 1N NaOH.

2.3. Results

2.3.1. Effect of BIBN4096BS on cardiac output and its distribution

2.3.1.1. Baseline values

Baseline values of heart rate, mean arterial blood pressure, cardiac output (expressed as cardiac index) and systemic vascular conductance in anaesthetised pigs (n=12) were: 108±3 beats.min^{-1}, 102±2 mmHg, 133±4 ml.min^{-1}.kg^{-1} and 1491±54 ml.min^{-1}.mmHg^{-1}, respectively. Baseline values of regional vascular conductances (ml.min^{-1}.mmHg^{-1}/100 g tissue) were: brain, 31±3; heart, 104±10; liver, 34±8; stomach, 24±2; lungs (mainly systemic arteriovenous anastomoses), 229±37; adrenals, 138±10; kidneys, 263±14; spleen, 126±15; skeletal muscles, 3.3±0.3; and skin, 11±2.

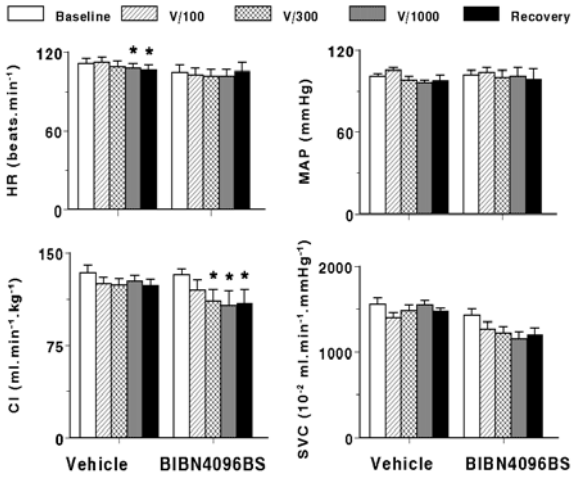


Figure 2.1. Heart rate (HR), mean arterial blood pressure (MAP), cardiac index (CI) and systemic vascular conductance (SVC) measured at baseline, after i.v. treatments with either vehicle (V, three times 5 ml; n=6) or BIBN4096BS (BIBN; 100, 300 and 1000 μg.kg^{-1}; n=7) and after 40 min recovery. All values are presented as mean±s.e.mean. *, P<0.05 vs. baseline. The changes after BIBN4096BS are not significantly different from those in the corresponding vehicle group.

2.3.1.2. Systemic and regional haemodynamic changes

Systemic haemodynamic values collated at baseline, after vehicle or BIBN4096BS (100, 300 and 1000 μg.kg^{-1}, i.v.) and after a 40-min recovery period are shown in Figure 2.1. There were no statistically significant differences (P>0.05) in baseline values in the vehicle and BIBN4096BS groups. Except for small decreases in heart rate by the vehicle (maximum change: 4±1%) and cardiac index by BIBN4096BS (maximum change: 19±8%), no other changes were observed. The changes in cardiac index by BIBN4096BS did not differ significantly (P>0.05) from those in the vehicle-treated animals (maximum change: 7±3%).

Figure 2.2 presents regional vascular conductances in a number of tissues in animals treated with either vehicle or BIBN4096BS (100, 300 and 1000 μg.kg^{-1}, i.v.). Baseline values in the two groups were not significantly different (P>0.05) in any of the tissues, including the liver, lungs and skin. Apart from decreases in liver conductance, no other changes in regional vascular conductances were noticed in the vehicle-treated group. BIBN4096BS produced small decreases in vascular conductance to liver, and with the highest dose (1000 μg.kg^{-1}) in lungs, adrenals, kidneys and spleen. Only the latter changes were significant when compared with the corresponding changes in the vehicle-treated animals.

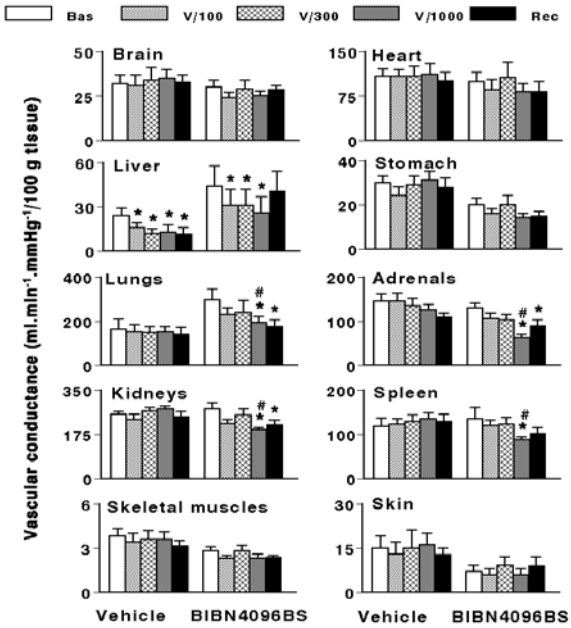


Figure 2.2. Regional vascular conductances at baseline (Bas), after i.v. treatments with either vehicle (V, three times 5 ml; n=6) or BIBN4096BS (100, 300, and 1000 μg.kg^{-1}, i.v.; n=6) and after 40 min recovery (Rec). All values are presented as mean±s.e.mean. *, P<0.05 vs. baseline. #, P<0.05 vs. the corresponding change in animals treated with vehicle.

2.3.2. Effect of BIBN4096BS on the haemodynamic responses to i.c. infusions of α-CGRP

2.3.2.1. Baseline values

Baseline values in anaesthetised pigs (n=13) were: heart rate, 129±5 beats.min^{-1}; mean arterial blood pressure, 122±4 mmHg; carotid flow pulse, 1.7±0.1 arbitrary units (a.u.); total carotid blood flow, 67±7 ml.min^{-1}; total carotid vascular conductance, 56±5 10^{-2} ml.min^{-1}.mmHg^{-1} and A-V SO_2 difference, 26±3%. Baseline values in the two groups of animals (vehicle and BIBN4096BS) did not differ significantly.

2.3.2.2. Systemic and carotid haemodynamic responses

Figure 2.3 shows the original tracings illustrating the systemic (blood pressure and heart rate) and carotid (flow pulse and total carotid blood flow) haemodynamic responses in anaesthetised pigs obtained with α-CGRP (10, 30 and 100 pmol.kg^{-1}.min^{-1}, i.c.) before and after i.v. treatments with three doses of vehicle (5 ml each time; *upper panel*) or BIBN4096BS (100, 300 and 1000 μg.kg^{-1}; *lower panel*). The infusions of α-CGRP did not affect heart rate, but decreased arterial blood pressure and increased carotid flow pulse and blood flow. These changes were accompanied by a redness of head skin and ears on the side of infusion (not shown in the figure). The effects of α-CGRP were clearly attenuated in the animals receiving BIBN4096BS, but not in the ones treated with vehicle.

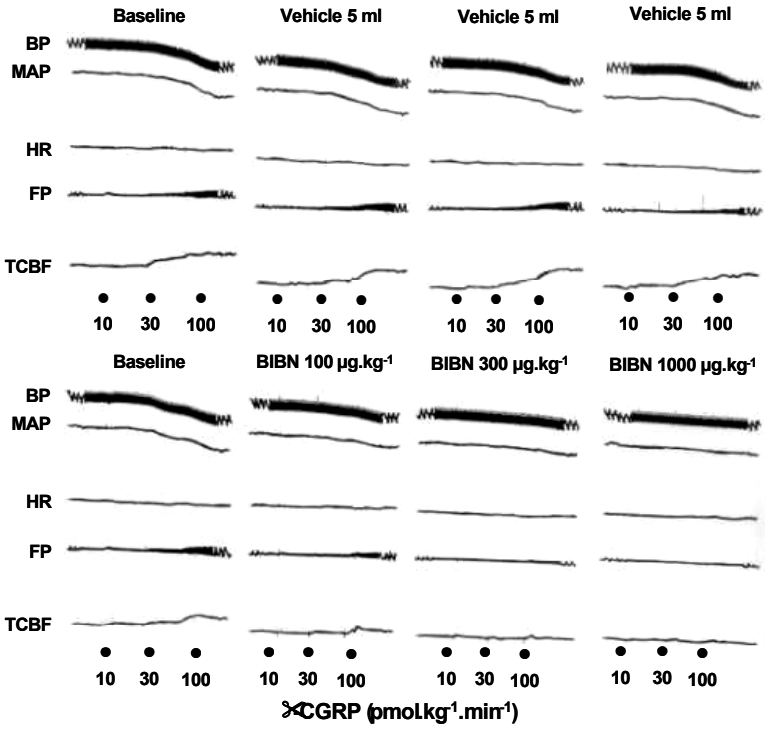


Figure 2.3. Original tracings from experiments in anaes-thetised pigs illustrating systemic and carotid haemo-dynamic responses to infusions of α-CGRP (•; 10, 30 or 100 pmol.kg^{-1}.min^{-1}, i.c.) given before and after i.v. treatments with either vehicle (three times 5 ml; *upper panel*) or BIBN4096BS (BIBN, 100, 300 and 1000 μg.kg^{-1}; *lower panel*). BP; systolic and diastolic arterial blood pressures; MAP, mean arterial blood pressure; HR, heart rate; FP, carotid blood flow pulse; TCBF, total carotid blood flow.

The effects of α-CGRP (10, 30 and 100 pmol.kg^{-1}.min^{-1}, i.c.) in the animals treated with vehicle or BIBN4096BS (100, 300 and 1000 μg.kg^{-1}, i.v.) were quantified as percent changes from baseline values (Figure 2.4). In both groups, infusions of α-CGRP before treatments with vehicle or BIBN4096 (control infusions) produced dose-dependent decreases in mean arterial blood pressure and increases in total carotid blood flow (data not shown) and conductance; heart rate was not affected (data not shown). These responses to α-CGRP remained unaffected after vehicle, but, in contrast, were dose-dependently antagonised by BIBN4096BS (Figure 2.4).

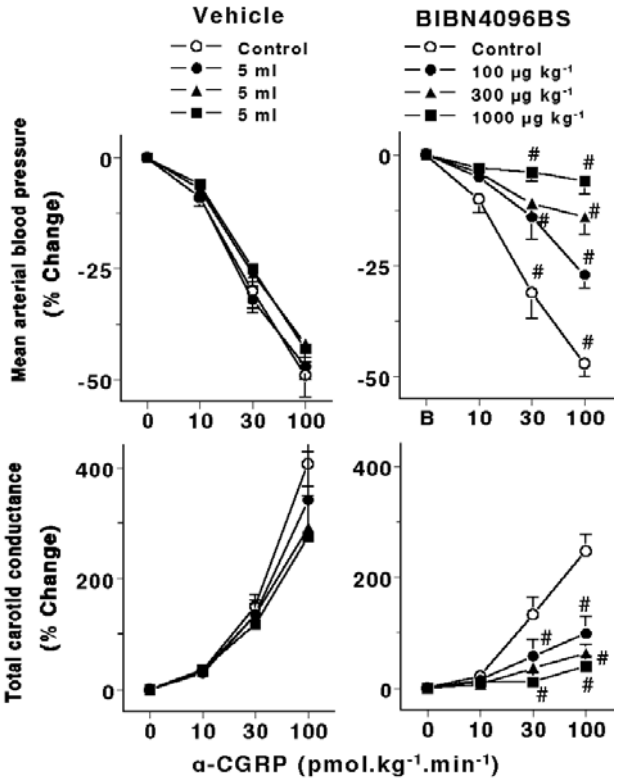


Figure 2.4. Changes in mean arterial blood pressure and total carotid vascular conductance from baseline values by i.c. infusion of α-CGRP in anaesthetised pigs given before (Control) and after i.v. treatments with vehicle (three times 5 ml; n=6) or BIBN4096BS (100, 300 and 1000 $\mu g.kg^{-1}$, n=7). All values are expressed as mean±s.e.mean. The two highest doses of α-CGRP significantly decreased mean arterial blood pressure and increased total carotid conductance (significance not shown for the sake of clarity). These effects of α-CGRP were dose-dependently antagonised by BIBN4096BS. #, P<0.05 vs. response after the corresponding volume of vehicle.

As shown in Figure 2.5, infusions of α-CGRP (100 $pmol.kg^{-1}.min^{-1}$, i.c.) clearly increased carotid blood flow (depicted as the maximum changes) and carotid blood flow pulsations (compare baseline and control values). While there was little change in animals treated with vehicle, BIBN4096BS (100, 300 and 1000 $\mu g.kg^{-1}$, i.v.) dose-dependently antagonised the responses to α-CGRP.

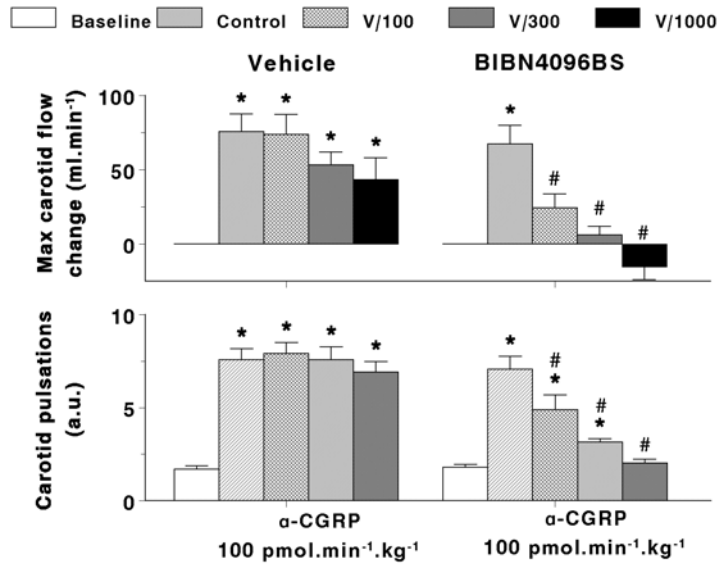


Figure 2.5. Maximum carotid blood flow changes and carotid blood flow pulsations measured at baseline and following infusions of α-CGRP (100 pmol. $kg^{-1}.min^{-1}$, i.c.) given in anaesthetised pigs before (Control) and after i.v. treatments with vehicle (V, 5 ml three times; n=6) or BIBN4096BS (100, 300 and 1000 $\mu g.kg^{-1}$, n=7). All values are expressed as mean± s.e.mean. a.u., Arbitrary units. *, P < 0.05 vs. baseline values; #, P<0.05 vs. response after the corresponding volume of vehicle.

2.3.2.3. Changes in the A-V SO_2 difference

α-CGRP (100 $pmol.kg^{-1}.min^{-}1$, i.c.) produced a significant reduction in the A-V SO_2 difference in both groups of animals (Figure 2.6; compare baseline and control values). The response to CGRP remained largely unaffected after treatments with vehicle, but

BIBN4096BS (100, 300 and 1000 μg.kg^{-1}, i.v.) dose-dependently blocked the reduction in the A-V SO_2 difference by α-CGRP. In fact, the CGRP-induced decrease in the A-V SO_2 difference was enhanced after the highest dose of BIBN4096BS (Figure 2.6).

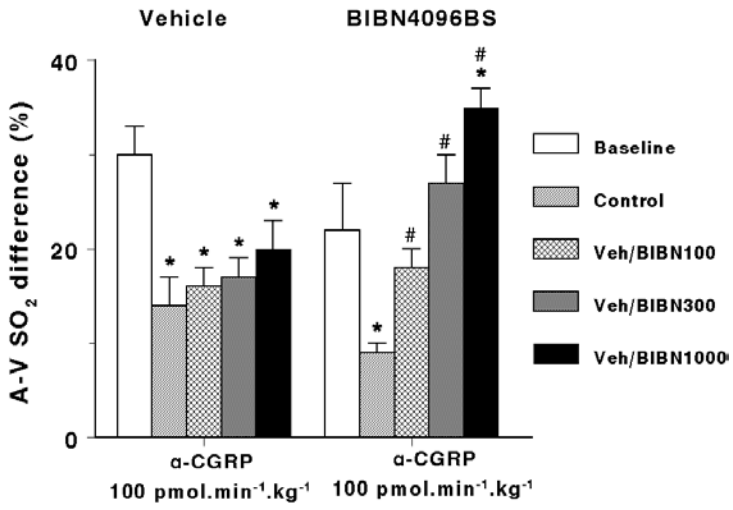


Figure 2.6. Differences between arterial and jugular venous oxygen saturations (A-V SO_2 difference) measured at baseline and after infusions of α-CGRP (100 pmol.kg^{-1}. min^{-1}, i.c.) given in anaesthe-tised pigs before (Control) and after i.v. treatments with vehicle (Veh, 5 ml three times; n=6) or BIBN4096BS (100, 300 and 1000 μg.kg^{-1}, n=7). All values are expressed as mean±s.e. mean. *, $P < 0.05$ vs. baseline values; #, $P<0.05$ vs. response after the corresponding volume of vehicle.

2.4. Discussion

2.4.1. General

Undoubtedly, a remarkable progress has been achieved in acute antimigraine therapy (33). Notwithstanding, the exact pathophysiological mechanisms underlying migraine remain unclear. There is, however, evidence supporting the involvement of the trigeminovascular system in migraine pathophysiology (17, 19, 34, 35). Thus, activation of the trigeminovascular system leads to neuropeptide release, including that of CGRP, and neurogenic dural vasodilatation (17). Of particular relevance is the finding that plasma concentration of CGRP is elevated during the headache phase of migraine, and this is normalised after treatment with sumatriptan (12, 19, 34). Hence, it is reasonable to assume that a potent CGRP receptor antagonist, such as BIBN4096BS (20), might be useful in migraine therapy. BIBN4096BS behaves as a 'silent' competitive antagonist at CGRP receptors mediating relaxation of human temporal, cranial and coronary arteries (22-24). The present study in anaesthetised pigs was designed: (i) to analyse, using BIBN4096BS, the potential role of endogenous CGRP in regulating vascular tone *in vivo*; and (ii) to investigate the effects of BIBN4096BS on the systemic and carotid haemodynamic responses produced by α-CGRP.

2.4.2. Systemic and regional haemodynamic effects of BIBN4096BS

It is well known that CGRP-immunoreactive nerve fibres are widely distributed in the cardiovascular system, with a higher preponderance in arteries than in veins (36). CGRP decreases blood pressure and has positive inotropic and chronotropic effects on the heart (37), which are mainly mediated via $CGRP_1$ receptors (36, 38, 39). Though CGRP has diverse biological actions within the cardiovascular system, our experiments showing few systemic

haemodynamic changes with BIBN4096BS do not support a major role for CGRP in the regulation of cardiovascular function in the anaesthetised pig.

As far as regional haemodynamics is concerned, a moderate decrease (compared to vehicle) in vascular conductances in the lungs, adrenals, kidneys and spleen was observed with the highest dose (1000 $\mu g.kg^{-1}$) of BIBN4096BS (Figure 2.2). Similarly, renal vasoconstriction was noticed in conscious rats with a high (300 $nmol.kg^{-1}.min^{-1}$), but not with a low (30 $nmol.kg^{-1}.min^{-1}$) dose of $CGRP_{8-37}$ (8). Since both BIBN4096BS and $CGRP_{8-37}$ caused renal changes only in doses that were considerably higher than those needed for CGRP antagonism, it does not appear that endogenous CGRP regulates renal vascular tone. Also, Shen et al. (9) recently reported that 30 $\mu g.kg^{-1}.min^{-1}$ (~10 $nmol.kg^{-1}.min^{-1}$) of $CGRP_{8-37}$, which antagonised CGRP-induced haemodynamic responses, caused little regional haemodynamic effects in conscious dogs as well as anaesthetised rats, thereby not supporting an important physiological role for endogenous CGRP in regulating vascular tone. Although we cannot rule out the involvement of CGRP in certain other circumstances, for example, cardiac preconditioning or coronary artery disease (40-42), the present results imply cardiovascular safety of BIBN4096BS. Nevertheless, one will have to explore the role of CGRP in cardiovascular pathophysiology before establishing whether or not CGRP receptor antagonists are completely safe in patients afflicted with cardiovascular disorders.

2.4.3. CGRP-induced haemodynamic responses and antagonism by BIBN4096BS

Activation of CGRP receptors elicits dilatation in different vascular beds in several species (8, 9, 43). Consistent with these studies, our experiments show that i.c. infusions of α-CGRP produced a marked vasodilatation in the porcine carotid circulation, with accompanying fall in arterial blood pressure. The fact that the animals were systematically vagosympathectomised may explain why the hypotension was not accompanied by a baroreflex-mediated tachycardia, as reported earlier (43). Interestingly, the ipsilateral skin redness, together with the marked decrease in A-V SO_2 difference by CGRP, indicates that porcine carotid arteriovenous anastomoses dilated in response to α-CGRP (31). However, we previously reported that i.c. infusions of α-CGRP failed to increase porcine arteriovenous anastomotic blood flow, despite a marked increase in the total carotid and capillary blood flows (43). Admittedly, arteriovenous anastomotic blood flow was not directly measured in these experiments, but we have recently observed that i.c. infusions capsaicin, which released CGRP, did increase carotid arteriovenous anastomotic blood flow with a concomitant decrease in the A-V SO_2 difference (44). Thus, it appears that the discrepancy between the two investigations may be due to different anaesthetic regimens employed (pentobarbital and fentanyl/thiopental, respectively) and, particularly, the use of phenylephrine in the present experiments. Phenylephrine potently constricts arteriovenous anastomoses (30).

In the present experimental study in anaesthetised pigs, BIBN4096BS proved to be an effective antagonist at the CGRP receptors mediating the systemic (hypotension) as well as the carotid (increased blood flow, pulsations and skin redness) haemodynamic responses to α-CGRP. The fact that BIBN4096BS also abolished α-CGRP-induced decreases in the A-V SO_2 difference suggests its action on carotid arteriovenous anastomoses; for further considerations, see Saxena (31). Interestingly, BIBN4096BS also antagonised the capsaicin-induced increases in carotid arteriovenous anastomotic blood flow as well as decreases in the A-V SO_2 difference, but not the plasma CGRP concentrations (44).

One cannot be certain about the nature of CGRP receptors that mediate porcine carotid vascular responses, but cardiac inotropic and vasodilator responses are mediated predominantly by $CGRP_1$ receptors (39), where BIBN4096BS has a very high affinity (7, 20).

2.4.4. Potential therapeutic efficacy of BIBN4096BS in the treatment migraine

Considering that plasma CGRP levels are elevated during the headache phase of migraine (34) and that BIBN4096BS dose-dependently blocked α-CGRP-induced carotid haemodynamic responses, it is likely that BIBN4096BS may be effective in migraine. The compound is presently under clinical investigation for the acute treatment of migraine and the results are awaited with great interest.

In conclusion, our study clearly demonstrates that BIBN4096BS is an effective antagonist at vascular CGRP receptors in anaesthetised pigs, but has little haemodynamic effects of its own, a finding that negates a major physiological role for CGRP in cardiovascular regulation. The potent blockade of the carotid haemodynamic effects of CGRP does suggest that BIBN4096BS may be effective in migraine treatment.

References

1. Amara SG, Jonas V, Rosenfeld MG, Ong ES, Evans RM. Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. *Nature* 1982;298:240-244.
2. Gulbenkian S, Barroso CP, Cunha e Sa M, Edvinsson L. The peptidergic innervation of human coronary and cerebral vessels. *Ital J Anat Embryol* 1995;100:317-327.
3. Gulbenkian S, Uddman R, Edvinsson L. Neuronal messengers in the human cerebral circulation. *Peptides* 2001;22:995-1007.
4. Brain SD, Williams TJ, Tippins JR, Morris HR, MacIntyre I. Calcitonin gene-related peptide is a potent vasodilator. *Nature* 1985;313:54-56.
5. Van Rossum D, Hanisch UK, Quirion R. Neuroanatomical localization, pharmacological characterization and functions of CGRP-related peptides and their receptors. *Neurosci Biobehav Rev* 1997;21:649-678.
6. Juaneda C, Dumont Y, Quirion R. The molecular pharmacology of CGRP and related peptide receptor subtypes. *Trends Pharmacol Sci* 2000;21:432-438.
7. Poyner D, Marshall I. CGRP receptors: beyond the $CGRP_1$-$CGRP_2$ subdivision? *Trends Pharmacol Sci* 2001;22:223.
8. Gardiner SM, Compton AM, Kemp PA, Bennett T, Bose C, Foulkes R, Hughes B. Antagonistic effect of human α-$CGRP_{8-37}$ on the in vivo regional haemodynamic actions of human α-CGRP. *Biochem Biophys Res Commun* 1990;171:938-943.
9. Shen YT, Pittman TJ, Buie PS, Bolduc DL, Kane SA, Koblan KS, Gould RJ, Lynch JJ, Jr. Functional role of α-calcitonin gene-related peptide in the regulation of the cardiovascular system. *J Pharmacol Exp Ther* 2001;298:551-558.
10. Wisskirchen FM, Burt RP, Marshall I. Pharmacological characterization of CGRP receptors mediating relaxation of the rat pulmonary artery and inhibition of twitch responses of the rat vas deferens. *Br J Pharmacol* 1998;123:1673-1683.
11. Waugh DJ, Bockman CS, Smith DD, Abel PW. Limitations in using peptide drugs to characterize calcitonin gene-related peptide receptors. *J Pharmacol Exp Ther* 1999;289:1419-1426.
12. Goadsby PJ, Edvinsson L, Ekman R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. *Ann Neurol* 1990;28:183-187.
13. Ashina M, Bendtsen L, Jensen R, Schifter S, Olesen J. Evidence for increased plasma levels of calcitonin gene-related peptide in migraine outside of attacks. *Pain* 2000;86:133-138.

14. Edvinsson L. Calcitonin gene-related peptide (CGRP) and the pathophysiology of headache: therapeutic implications. *CNS Drugs* 2001;15:745-753.
15. Durham P, Russo A. New insights into the molecular actions of serotonergic antimigraine drugs. *Pharmacol Ther* 2002;94:77-92.
16. Goadsby PJ, Lipton RB, Ferrari MD. Migraine - current understanding and treatment. *N Engl J Med* 2002;346:257-270.
17. Williamson DJ, Hargreaves RJ. Neurogenic inflammation in the context of migraine. *Microsc Res Tech* 2001;53:167-178.
18. Smith D, Hill RG, Edvinsson L, Longmore J. An immunocytochemical investigation of human trigeminal nucleus caudalis: CGRP, substance P and 5-HT_{1D}-receptor immunoreactivities are expressed by trigeminal sensory fibres. *Cephalalgia* 2002;22:424-431.
19. Goadsby PJ. Advances in the pharmacotherapy of migraine. How knowledge of pathophysiology is guiding drug development. *Drug Res Dev* 1999;2:361-374.
20. Doods H, Hallermayer G, Wu D, Entzeroth M, Rudolf K, Engel W, Eberlein W. Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist. *Br J Pharmacol* 2000;129:420-423.
21. Schindler M, Doods HN. Binding properties of the novel, non-peptide CGRP antagonist radioligand, [^{3}H]BIBN4096BS. *Eur J Pharmacol* 2002;442:187-193.
22. Verheggen R, Bumann K, Kaumann AJ. BIBN4096BS is a potent competitive antagonist of the relaxant effects of α-CGRP on human temporal artery: comparison with $CGRP_{8-37}$. *Br J Pharmacol* 2002;136:120-126.
23. Edvinsson L, Alm R, Shaw D, Rutledge RZ, Koblan KS, Longmore J, Kane SA. Effect of the CGRP receptor antagonist BIBN4096BS in human cerebral, coronary and omental arteries and in SK-N-MC cells. *Eur J Pharmacol* 2002;434:49-53.
24. Moreno MJ, Abounader R, Hebert E, Doods H, Hamel E. Efficacy of the non-peptide CGRP receptor antagonist BIBN4096BS in blocking CGRP-induced dilations in human and bovine cerebral arteries: potential implications in acute migraine treatment. *Neuropharmacology* 2002;42:568-576.
25. Saxena PR. Cranial arteriovenous shunting, an *in vivo* animal model for migraine. In: Olesen J, Moskowitz MA, eds. *Experimental headache models.* Philadelphia, USA: Lippincott-Raven Publishers, 1995:189-198. vol 27).
26. De Vries P, Villalón CM, Saxena PR. Pharmacological aspects of experimental headache models in relation to acute antimigraine therapy. *Eur J Pharmacol* 1999;375:61-74.
27. Saxena PR, Schamhardt HC, Forsyth RP, Hoeve J. Computer programs for the radioactive microsphere technique. Determination of regional blood flows and other haemodynamic variables in different experimental circumstances. *Comput Programs Biomed* 1980;12:63-84.
28. Baile EM, Nelems JM, Schulzer M, Pare PD. Measurement of regional bronchial arterial blood flow and bronchovascular resistance in dogs. *J Appl Physiol* 1982;53:1044-1049.
29. Den Boer MO, Van Woerkens LJ, Somers JA, Duncker DJ, Lachmann B, Saxena PR, Verdouw PD. On the preservation and regulation of vascular tone in arteriovenous anastomoses during anesthesia. *J Appl Physiol* 1993;75:782-789.
30. Willems EW, Trion M, De Vries P, Heiligers JPC, Villalón CM, Saxena PR. Pharmacological evidence that α_1- and α_2-adrenoceptors mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetized pigs. *Br J Pharmacol* 1999;127:1263-1271.

31. Saxena PR. Arteriovenous anastomoses and veins in migraine research. In: Blau JN, ed. *Migraine, clinical, therapeutic, conceptual and research aspects.* London, UK: Chapman and Hall medicin, 1987:581-596.
32. Steel RGD, Torrie JH. *Principles and procedures of statistics. A biomedical approach (2nd edition)*. Tokyo: McGraw-Hill Kogakusha Ltd, 1980.
33. De Vries P, Heiligers JP, Villalón CM, Saxena PR. Blockade of porcine carotid vascular responses to sumatriptan by GR 127935, a selective 5-HT_{1D} receptor antagonist. *Br J Pharmacol* 1996;118:85-92.
34. Goadsby PJ. Current concepts of the pathophysiology of migraine. *Neurol Clin* 1997;15:27-42.
35. Hargreaves RJ, Williamson DJ, Shepheard SL. Neurogenic inflammation: relation to novel antimigraine drugs. In: Edvinsson L, ed. *Migraine and headache pathophysiology.* London: Martin Dunitz, 1999:93-101.
36. Bell D, McDermott BJ. Calcitonin gene-related peptide in the cardiovascular system: characterization of receptor populations and their (patho)physiological significance. *Pharmacol Rev* 1996;48:253-288.
37. Wimalawansa SJ. Calcitonin gene-related peptide and its receptors: molecular genetics, physiology, pathophysiology, and therapeutic potentials. *Endocr Rev* 1996;17:533-585.
38. Saetrum Opgaard O, Hasbak P, De Vries R, Saxena PR, Edvinsson L. Positive inotropy mediated via CGRP receptors in isolated human myocardial trabeculae. *Eur J Pharmacol* 2000;397:373-382.
39. Saetrum Opgaard O, de Vries R, Tom B, Edvinsson L, Saxena PR. Positive inotropy of calcitonin gene-related peptide and amylin on porcine isolated myocardium. *Eur J Pharmacol* 1999;385:147-154.
40. Lu R, Li YJ, Deng HW. Evidence for calcitonin gene-related peptide-mediated ischemic preconditioning in the rat heart. *Regul Pept* 1999;82:53-57.
41. Peng J, Xiao J, Ye F, Deng HW, Li YJ. Inhibition of cardiac tumor necrosis factor-alpha production by calcitonin gene-related peptide-mediated ischemic preconditioning in isolated rat hearts. *Eur J Pharmacol* 2000;407:303-308.
42. Wu D-M, Van Zwieten PA, Doods HN. Effects of calcitonin gene-related peptide and BIBN4096BS on myocardial ischaemia in anaesthetized rats. *Acta Pharmacol Sin* 2001;22:588-594.
43. Van Gelderen EM, Du XY, Schoemaker RG, Saxena PR. Carotid blood flow distribution, haemodynamics and inotropic responses following calcitonin gene-related peptide in the pig. *Eur J Pharmacol* 1995;284:51-60.
44. Kapoor K, Arulmani U, Heiligers JPC, Garrelds IM, Willems EW, Doods H, Villalón CM, Saxena PR. Effects of the CGRP receptor antagonist BIBN4096BS on capsaicin-induced carotid haemodynamic changes in anaesthetized pigs. *Br J Pharmacol* 2003:In press.

CHAPTER 3

Effects of the CGRP receptor antagonist BIBN4096BS on capsaicin-induced carotid haemodynamic changes in anaesthetised pigs

Based on: Kapoor K, Arulmani U, Heiligers JPC, Garrelds IM, Willems EW, Doods H, Villalón CM, Saxena PR. Effects of the CGRP receptor antagonist BIBN4096BS on capsaicin-induced carotid haemodynamic changes in anaesthetised pigs. Br J Pharmacol 2003;140:329-338.

3. Effects of the CGRP receptor antagonist BIBN4096BS on capsaicin-induced carotid haemodynamic changes in anaesthetised pigs

3.1. Introduction

Although a complete understanding of the pathogenesis of migraine remains elusive thus far, there seems little doubt that dilatation of cranial blood vessels, including carotid arteriovenous anastomoses, is involved in the headache phase (1). Moreover, evidence is accumulating that a release of vasoactive neuropeptides from the trigeminal sensory nerves may be an important factor in the genesis of migraine (2). In this respect, a high circulating plasma concentration of calcitonin gene related peptide (CGRP) has been demonstrated during migraine headache (3) and these concentrations can be normalised by triptans in parallel with alleviation of headache (3, 4). Indeed, CGRP is widely distributed in the body, including the central and peripheral parts of the trigeminovascular system (5-8), where it is co-localised with substance P, neurokinin A and/or 5-HT_{1D} receptors (9-11). CGRP can mediate neurogenic dilatation of cranial blood vessels as well as sensory nerve transmission between the first and second order afferent input from these vessels during migraine headache (2, 10-12). Thus, it follows that inhibition of CGRP-mediated cranial vasodilatation and sensory nerve transmission with a potent and selective CGRP receptor antagonist may prove a novel strategy in treating migraine.

The recent discovery of a di-peptide CGRP receptor antagonist BIBN4096BS (1-piperidinecarboxamide, N-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-piperazinyl]carbonyl] pentyl] amino]-1-[(3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]- 4-(1,4-dihydro-2-oxo-3(2H)-quinazolinyl)-, [R-(R*,S*)]-) (13, 14) represents a significant advance in exploring the pathophysiological role of CGRP in migraine. BIBN4096BS displays a very high affinity for human CGRP receptors (13, 15-18). This compound is undergoing clinical trials for aborting migraine headache and the clinical results are awaited with great interest.

Using an animal model that seems to be predictive of antimigraine activity (1, 19-23), the present study in anaesthetised pigs was designed (i) to investigate the effects of capsaicin (pungent substance in red chilli pepper), which releases neuropeptides, including CGRP (24-27), on systemic and carotid haemodynamics, and (ii) to establish if BIBN4096BS is able to attenuate the responses induced by capsaicin. A preliminary account of this investigation was presented at the XIVth World Congress of Pharmacology (28).

3.2. Materials and methods

3.2.1. General

After an overnight fast, a total of 22 pigs (Yorkshire x Landrace, females, 10-14 kg; n=11 each for vehicle and BIBN4096BS) were sedated with azaperone (120 mg, i.m.) and midazolam hydrochloride (10 mg, i.m.) and then anaesthetised with sodium pentobarbital (600 mg, i.v.). After tracheal intubation, the animals were connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2:

35-48 mmHg; pO_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of sodium pentobarbital (12-20 $mg.kg^{-1}\ h^{-1}$). This anaesthetic regimen, together with bilateral vagosympathectomy (see below), increases heart rate and markedly dilates carotid arterioles and arteriovenous anastomoses due to a loss of parasympathetic and sympathetic tone, respectively. Consequently, carotid blood flow, particularly its arteriovenous anastomotic fraction, is considerably higher in these pigs than in conscious or thiopental-anaesthetised pigs (29).

Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by electrocardiogram signals. Both common carotid arteries were dissected free and the accompanying vagosympathetic trunks were cut between two ligatures to prevent any possible influence via baroreceptor reflexes on the carotid vascular responses produced by capsaicin. Pulsatile and mean carotid blood flows were measured in the right common carotid artery with a flow probe (internal diameter: 2.5 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands). The amplitude of carotid blood flow signals provided an *index* of carotid flow pulse. Subsequently, three hub-less needles, connected to a polyethylene tube, were inserted into the right common carotid artery for the administration of capsaicin, radioactive microspheres and the α_1-adrenoceptor agonist phenylephrine. The use of phenylephrine is necessitated by the fact that the carotid arterioles and arteriovenous anastomoses are already in a dilated state under the present anaesthetic regime (29) and, therefore, to study the effects of vasodilator agents (in the present case capsaicin) one has to constrict them first. As described earlier (30), phenylephrine decreases total carotid conductance exclusively by constricting carotid arteriovenous anastomoses, which results in an increase in the difference between arterial and jugular venous oxygen saturations (A-V SO_2 difference) (31).

Lastly, catheters were placed in the right external jugular vein for the withdrawal of venous blood samples to measure blood gases (ABL-510; Radiometer, Copenhagen, Denmark) and plasma concentrations of CGRP (see below), inferior vena cava (via the left femoral vein) for the administration of the vehicle or BIBN4096BS and aortic arch (via the left femoral artery) for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) as well as withdrawal of arterial blood samples to measure blood gases.

Heart rate and systolic, diastolic and mean arterial blood pressures as well as mean and pulsatile carotid artery blood flows were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). Vascular conductances were calculated by dividing respective blood flows ($ml.min^{-1}$) by mean arterial blood pressure (mmHg), multiplied by one hundred and expressed as 10^{-2} $ml.min^{-1}.mmHg^{-1}$. During the experiment, body temperature was maintained at 37±1°C by a heating pad and the animal was infused with physiological saline to compensate for fluid losses.

3.2.2. Distribution of carotid blood flow

The distribution of common carotid blood flow into tissue (capillary) and arteriovenous anastomotic fractions was determined in 13 pigs (later receiving vehicle, n=7 or BIBN4096BS, n=6) with radioactive microspheres (diameter: 15.5±0.1 μm; S.D.), labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, USA). For each measurement, a suspension of about 200,000 microspheres, labelled with one of the isotopes, was mixed and injected into the carotid artery. At the end of the experiment, the animal was killed using an overdose of pentobarbital and the heart, kidneys, lungs and different cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 5 min in a γ-scintillation counter (Packard, Minaxi autogamma 5000), using suitable windows for discriminating the different isotopes (^{141}Ce: 120-167 KeV, ^{113}Sn: 355-435 KeV,

^{103}Ru: 450-548 KeV, ^{95}Nb: 706-829 KeV and ^{46}Sc: 830-965 KeV). All data were processed by a set of specially designed computer programs (32).

The distribution of total carotid blood flow to different tissues (Q_{tis}) was calculated by the formula: $Q_{tis} = (I_{tis}/I_{total}) \times Q_{carotid}$, where I_{tis} is tissue radioactivity, I_{total} is the sum of radioactivity counted in tissues and $Q_{carotid}$ is the total common carotid blood flow at the time of microsphere injection. Since little or no radioactivity was detected in the heart or kidneys, it can be assumed that all microspheres trapped in lungs reach the lungs from the venous side after escaping via carotid arteriovenous anastomoses. Therefore, the amount of radioactivity in the lungs can be used as an *index* of the arteriovenous anastomotic fraction of carotid blood flow (32, 33).

3.2.3. Determination of plasma concentration of CGRP

Jugular venous blood samples were obtained from 12 pigs, receiving vehicle or BIBN4096BS (n=6 each). Four of these animals (2 each for vehicle and BIBN4096BS) had been used for carotid haemodynamic experiments, while the other 8 were separate experiments using the same protocol except that the radioactive microspheres were not used. Blood was transferred immediately into a polypropylene tube containing ethylene dinitro-tetraacetic acid (1 mg ml^{-1} of blood) and aprotinin (500 KIU ml^{-1} of blood). Aprotinin was used to inhibit endogenous plasma proteases, since we observed that CGRP is not detectable in biological samples without aprotinin (unpublished). After centrifugation at 1600 g for 15 min, plasma samples were coded and stored at -80°C until CGRP measurements were performed. The person measuring CGRP concentrations remained blind to the treatments, until all data had been collated.

CGRP was extracted from plasma using a C_{18} SEP-COLUMN, dried by lypholisation, and measured by radioimmunoassay (34), as per protocol of the Peninsula Laboratories, Inc (Belmont, CA, U.S.A.). The recovery of CGRP from the extraction procedure was ascertained by assaying control samples paired with a duplicate sample spiked with known quantities of CGRP. The column recovery values were 85, 79, 81, 89 and 92% (Mean=85.2; Standard deviation=5.4; Coefficient of variation=6.3%). The CGRP concentrations measured in the actual samples were, however, not corrected for the loss in the extraction procedure.

3.2.4. Experimental protocol

Following surgery and after haemodynamic condition of the animals (n=22) had been stable for 15-20 min (heart rate: 107±4 beats.min^{-1}, mean arterial blood pressure: 95±2 mmHg, mean carotid blood flow: 120±12 ml.min^{-1} and A-V SO_2 difference: 7.6±1.1%), phenylephrine was infused into the right common carotid artery at a rate of 10 µg.kg^{-1}.min^{-1} for 10 min, followed by 3-6 µg.kg^{-1}.min^{-1} throughout the rest of the experiment. The latter dose of phenylephrine was chosen so that the external jugular venous oxygen saturation was between 60-70% and mean carotid blood flow was about 40% of the original value. After a period during which haemodynamic variables remained constant for at least 60 min (heart rate: 130±4 beats.min^{-1}, mean arterial blood pressure: 105±2 mmHg, mean carotid blood flow: 48±5 ml.min^{-1} and A-V SO_2 difference: 31±2.3%; n=22), the animals received consecutive infusions (0.15, 0.45, 1.5 and 4.5 ml, i.c. during 3 min each) of capsaicin vehicle (see Compounds and kits section). It is important to mention that the vehicle of capsaicin was devoid of any systemic and carotid haemodynamic responses (data not shown).

Five to 10 min after the last infusion of capsaicin vehicle, blood samples were obtained for the measurements of blood gases and CGRP concentration and values of heart rate, arterial blood pressure and total carotid blood flow and conductance were collated (baseline values; 11 pigs each for vehicle and BIBN4096BS). In 12 of the 22 pigs (6 each for vehicle

and BIBN4096BS) the first batch of radioactive microspheres was injected for determining the baseline distribution of carotid blood flow. The animals then received consecutive infusions of capsaicin (0.3, 1, 3 and 10 $\mu g.kg^{-1}.min^{-1}$, i.c. for 3 min each) and heart rate, arterial blood pressure and total carotid blood flow were determined at the end of each infusion. In addition, after the last infusion of capsaicin (10 $\mu g.kg^{-1}.min^{-1}$), blood gases, plasma CGRP concentration and carotid blood flow distribution were measured as described above (control values). Subsequently, a recovery period of 20 min was allowed until all haemodynamic parameters had returned to baseline levels. At this point, the animals were divided into two groups, which were treated with i.v. infusions (rate: 0.5 $ml.min^{-1}$ for 10 min) of either vehicle (three times 5 ml of acidified distilled water) or BIBN4096BS (100, 300 and 1000 $\mu g.kg^{-1}$). Ten min after each infusion, capsaicin was given and haemodynamic and biochemical variables were measured again, as described above.

3.2.5. Data presentation and statistical analysis

All data are presented as mean±s.e.mean, unless stated otherwise. The statistical analysis was performed using the SPSS package for windows (version 10.0; SPSS Inc., Chicago, IL, USA). The significance of changes within one group (vehicle or BIBN4096BS) was analysed with repeated-measures ANOVA, followed by Greenhouse-Geisser correction for serial autocorrelation (35) and Bonferroni correction for multiple comparisons (36). The significance of the between-group changes (vehicle versus BIBN4096BS treatments) was first analysed with repeated-measures ANOVA, including baseline measurements as a covariate (37) and the Greenhouse-Geisser correction. If the two groups differed significantly, pairwise comparisons of corresponding values in the vehicle- and BIBN4096BS-treated groups were performed using univariate analysis (38), followed by Bonferroni correction. Statistical significance was accepted at $P<0.05$ (two-tailed).

3.2.6. Ethical approval

The Ethics Committee of the Erasmus MC, Rotterdam, dealing with the use of animals in scientific experiments, approved the protocols for this investigation.

3.2.7. Compounds and kits

The following compounds were used: aprotinin (5850 KIU mg^{-1}; Roth, Karlsruhe, Germany), azaperone (Stresnil®; Janssen Pharmaceuticals, Beerse, Belgium), BIBN4096BS (gift from Boehringer Ingelheim Pharma KG, Biberach, Germany), capsaicin, tween 80, ethanol and phenylephrine hydrochloride (all from Sigma-Aldrich Chemie b.v., Zwijndrecht, The Netherlands), ethylene dinitro-tetraacetic acid (Merck, Darmstadt, Germany), heparin sodium (to prevent blood clotting in catheters; Leo Pharmaceutical Products, Weesp, The Netherlands), midazolam hydrochloride (Dormicum®; Hoffmann La Roche b.v., Mijdrecht, The Netherlands), phenylephrine hydrochloride (Sigma-Aldrich Chemie b.v., Zwijndrecht, The Netherlands) and sodium pentobarbital (Sanofi Sante b.v., Maasluis, The Netherlands). The radioimmunoassay kit for CGRP was purchased from Peninsula Laboratories, Inc. (Belmont, CA, U.S.A.).

Capsaicin was initially dissolved in tween 80, ethanol and physiological saline in the ratio of 0.5:1:8.5 ml, respectively. Phenylephrine was dissolved in distilled water, while BIBN4096BS was initially dissolved in 0.5 ml of 1N HCl, then diluted with 4 ml of distilled water and adjusted to pH 6.5 by 1N NaOH.

3.3. Results

3.3.1. Baseline values

Baseline values in the 22 pigs used were: heart rate, 126±3 beats.min^{-1}; mean arterial blood pressure, 105±3 mmHg; total carotid blood flow, 40±5 ml.min^{-1}; total carotid vascular conductance, 39±5 10^{-2} ml.min^{-1}.mmHg^{-1} and A-V SO_2 difference, 38±2%. No significant differences in baseline values were found between the two groups of animals (n=11 each) that later received vehicle or BIBN4096BS.

3.3.2. Effect of different doses of capsaicin on heart rate, blood pressure and carotid blood flow

Figure 3.1 depicts heart rate, mean arterial blood pressure and total carotid blood flow and conductance changes produced by the infusions of capsaicin (0.3, 1, 3 and 10 μg.kg^{-1}.min^{-1}, i.c.) before (control response) and after treatments with BIBN4096BS (100, 300 and 1000 μg.kg^{-1}.min^{-1}, i.v.) or the corresponding volumes of vehicle. In both groups of animals, capsaicin elicited dose-dependent increases in mean arterial blood pressure as well as total carotid blood flow and conductance, without significantly affecting heart rate. These effects

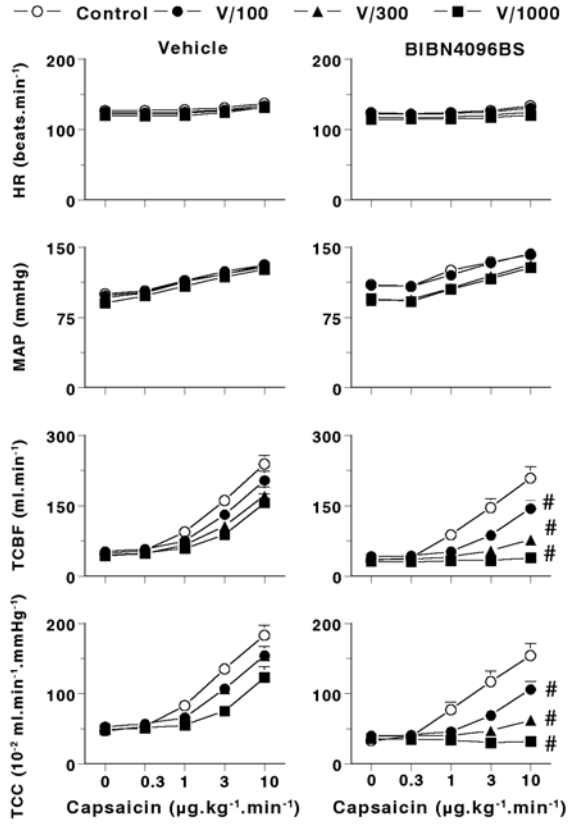


Figure 3.1. Heart rate (HR), mean arterial blood pressure (MAP) and total carotid blood flow (TCBF) and vascular conducta-nce (TCC) values at baseline (B) and following infusions of capsaicin (0.3, 1, 3, 10 μg.kg^{-1}.min^{-1}, i.c.) in anaesthetised pigs before (Control) and after i.v. administra-tions of vehicle (V, 5 ml three times; n=11) or BIBN4096BS (100, 300 and 1000 μg.kg^{-1}, n=11). All values are expressed as mean±s.e.mean. While the heart rate was not affected, capsaicin increased the mean arterial blood pressure as well as total carotid blood flow and conductance (significance not shown for the sake of clarity). BIBN4096BS dose-dependently antagonised capsaicin-induced carotid haemodynamic changes, but not the increase in arterial blood pressure. #, P<0.05 vs. response after the corresponding volume of vehicle.

of capsaicin remained essentially unchanged after the administration of vehicle (0.5 ml), except that a slight attenuation was noticed in the increases in carotid blood flow and conductance after the third dose of vehicle. In contrast, BIBN4096BS produced a dose-dependent attenuation of capsaicin-induced increases in total carotid blood flow and conductance, but not in blood pressure.

3.3.3. Carotid haemodynamic changes following capsaicin infusion

The carotid haemodynamic effects observed after the highest infusion (10 μg.kg^{-1}.min^{-1}) of capsaicin were examined in more detail in animals receiving vehicle or BIBN4096BS.

3.3.3.1. Effect on carotid blood flow and pulsations

As shown in Figure 3.2, i.c. infusions of capsaicin (10 μg.kg^{-1}.min^{-1}) clearly increased carotid blood flow and conductance (both depicted as maximum absolute changes) as well as pulsations. In animals treated with the vehicle, there was some decrease in the responses to capsaicin, but these responses were significantly more attenuated in animals treated with BIBN4096BS, particularly the two highest doses.

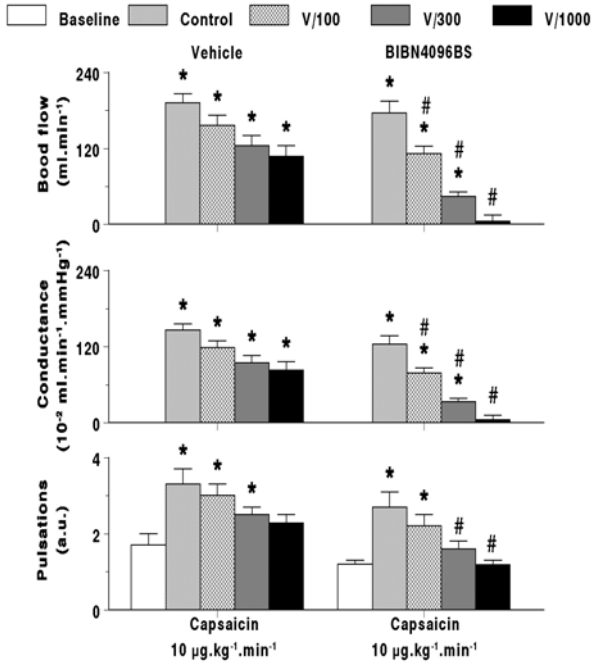


Figure 3.2. Maximum changes in carotid blood flow, vascular conductance and pulsations measured at baseline and following infusions of capsaicin (10 μg.kg^{-1}.min^{-1}, i.c.) given in anaesthetised pigs before (Control) and after i.v. administrations of vehicle (V, 5 ml three times; n=11) or BIBN4096BS (100, 300 and 1000 μg.kg^{-1}, n=11). All values are expressed as mean±s.e.mean. a.u., Arbitrary units. *, $P < 0.05$ vs. baseline values; #, $P<0.05$ vs. response after the corresponding volume of vehicle.

3.3.3.2. Fractionation of carotid blood flow and vascular conductance

In both vehicle and BIBN4096BS groups, capsaicin (10 μg.kg^{-1}.min^{-1}, i.c.) significantly increased total carotid blood flow and conductance as well as those distributed to arteriovenous anastomoses and capillaries. The increases from baseline values in blood flows and vascular conductances were, respectively: total carotid, 494±59% and 362±40%; arteriovenous anastomotic fraction, 726±282% and 505±188% and capillary fraction, 526±48% and 389±32% (n=13 in each case).

The effects of BIBN4096 as well as of its vehicle on the carotid haemodynamic responses to capsaicin are illustrated in Figure 3.3. Compared to the corresponding volumes of vehicle, the increases in total, arteriovenous anastomotic as well as capillary blood flows and vascular conductances were clearly antagonised after the two highest infusions (300 and 1000 μg.kg^{-1}.min^{-1}) of BIBN4096BS.

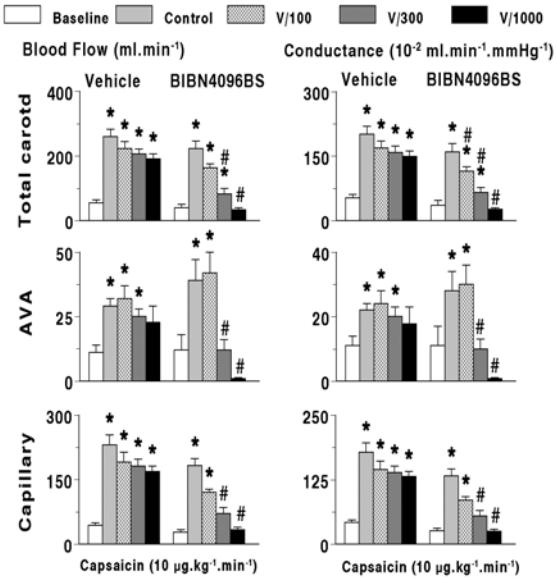


Figure 3.3. Total carotid, arteriovenous anastomotic (AVA) and capillary blood flows (*left panel*) and vascular conductances (*right panel*) measured at baseline and following infusions of capsaicin (10 µg.kg^{-1}.min^{-1}, i.c.) given in anaesthetised pigs before (Control) and after i.v. administrations of vehicle (V, 5 ml three times; n=7) or BIBN4096BS (100, 300 and 1000 µg.kg^{-1}, n=6). All values are expressed as mean±s.e.mean. *, $P < 0.05$ vs. baseline values; #, $P<0.05$ vs. response after the corresponding volume of vehicle.

Figure 3.4 shows that capsaicin (10 µg.kg^{-1}.min^{-1}, i.c.) increased vascular conductances to the different cranial tissues, including the skin, ear, skeletal muscles, fat, bone, eye, tongue and dura mater, but not in the brain or salivary glands. As has been described with 5-hydroxytryptamine (33), the increase in skin blood flow was most likely responsible for the redness of skin on the side of capsaicin infusion (not shown in the figure). These effects of capsaicin were significantly and dose-dependently antagonised by BIBN4096BS (100, 300 and 1000 µg.kg^{-1}.min^{-1}, i.v.), but not by the corresponding volumes of vehicle.

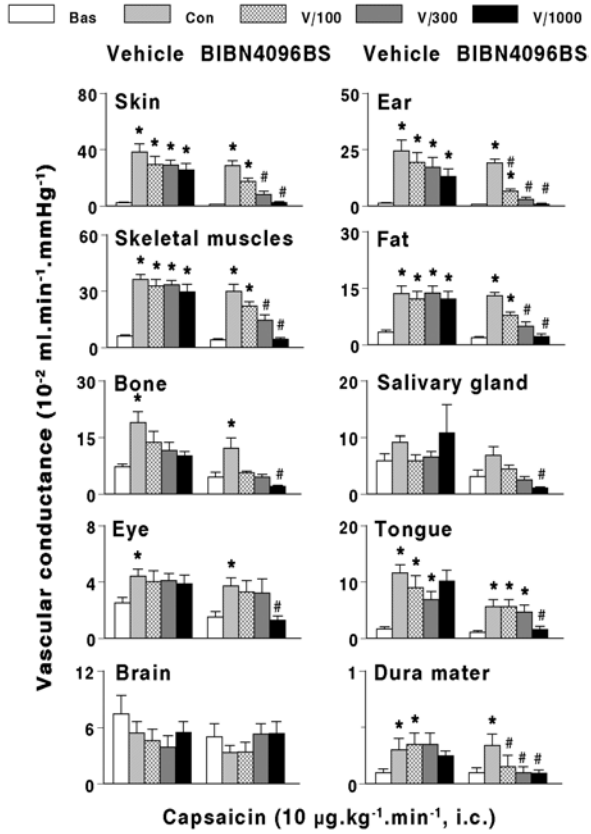


Figure 3.4. Distribution of carotid vascular conductances to head tissues measured at baseline (Bas) and following infusions of capsaicin (10 µg kg^{-1}.min^{-1}, i.c.) given in anaesthetised pigs before (Con) and after i.v. administrations of vehicle (V, 5 ml three times; n=7) or BIBN4096BS (100, 300 and 1000 µg.kg^{-1}, n=6). All values are expressed as mean±s.e.mean. *, $P < 0.05$ vs. baseline values; #, $P<0.05$ vs. response after the corresponding volume of vehicle.

3.3.4. A-V SO_2 difference

Consistent with the increase in arteriovenous anastomotic blood flow, capsaicin (10 μg.kg^{-1}.min^{-1}, i.c.) significantly decreased A-V SO_2 difference from baseline values of 38±2% to 4.5±0.4% (n=22). This response remained unaffected in animals treated with the vehicle, but was dose-dependently antagonised by BIBN4096BS (Figure 3.5).

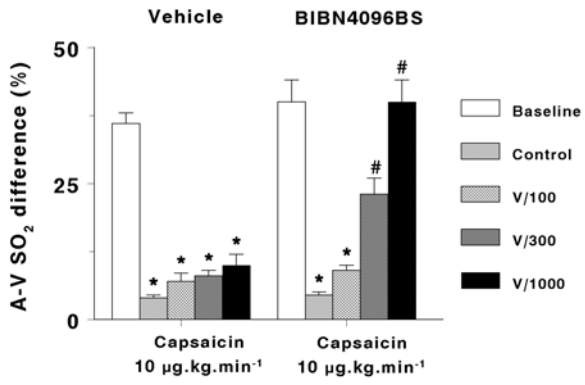


Figure 3.5. Difference between arterial and jugular venous oxygen saturations (A-V SO_2 difference) measured at baseline and following infusions of capsaicin (10 μg. kg^{-1}.min^{-1}, i.c.) given in anaesthetised pigs before (Control) and after i.v. administrations of the vehicle (V, 5 ml three times; n=11) or BIBN4096BS (100, 300 and 1000 μg.kg^{-1}, n=11). All values are expressed as mean±s.e.mean. *, P < 0.05 vs. baseline values; #, P<0.05 vs. response after the corresponding volume of the vehicle.

3.3.5. Jugular venous plasma concentrations of CGRP

In the 12 pigs used for this purpose, the baseline value of CGRP concentration in jugular venous plasma was 27±2 pg ml^{-1} and following capsaicin infusion (10 μg.kg^{-1}.min^{-1}, i.c.) it increased by 119±17% to 58±5 pg ml^{-1}.

Figure 3.6 shows the effects of capsaicin (10 μg.kg^{-1}.min^{-1}, i.c.) on jugular venous plasma concentration of CGRP in pigs receiving either three i.v. infusions of vehicle (5 ml each) or BIBN4096BS (100, 300 and 1000 μg.kg^{-1}). Capsaicin increased plasma CGRP concentration in both animal groups by a similar magnitude and this increase was not attenuated in either vehicle- or BIBN4096BS-treated group of animals. Interestingly, following the two highest doses of BIBN4096BS (300 and 1000 μg.kg^{-1}, i.v.) there was even a potentiation of capsaicin-induced increases in plasma CGRP concentrations (control response: 138±29%; response after BIBN4096BS: 211±30% and 211±38%, respectively; n=6).

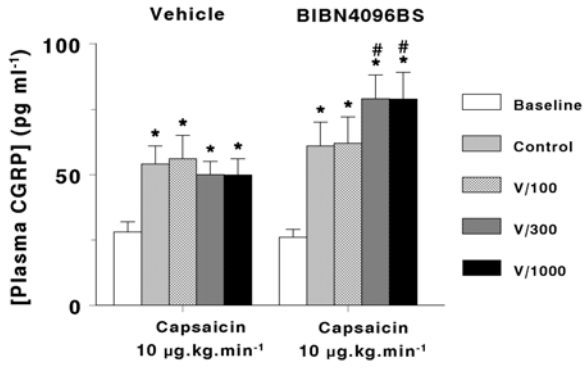


Figure 3.6. Jugular venous plasma CGRP concentrations measured at baseline and after infusions of capsaicin (10 μg kg^{-1}.min^{-1}, i.c.) given in anaesthetised pigs before (Control) and after i.v. administrations of the vehicle (V, 5 ml three times; n=6) or BIBN4096BS (100, 300 and 1000 μg.kg^{-1}, i.v., n=6). All values are expressed as mean±s.e.mean. *, P < 0.05 vs. baseline values; #, P<0.05 vs. response after the corresponding volume of the vehicle.

3.4. Discussion

3.4.1. General

Although there is much debate about the pathogenesis of migraine, there seems to be a general agreement regarding its neurovascular nature (1, 2, 39, 40). Thus, there is a release of vasoactive peptides producing intense cranial vasodilatation, increased arterial pulsations and a sterile inflammatory reaction with pain (1, 41). Amongst these neuropeptides, CGRP is considered as a biological marker in migraine pathogenesis (2, 42, 43). Moreover, stimulation of trigeminal sensory neurones with electrical procedures or chemical substances, like capsaicin, releases endogenously-stored CGRP (26, 44) that, in turn, dilates cranial vessels (12), including carotid arteriovenous anastomoses (45). In addition, CGRP may also facilitate sensory nerve transmission between the first and second order afferent input from these vessels during migraine headache (2, 10, 11). On this basis, it is reasonable to assume that CGRP receptor antagonists can be a novel approach to antimigraine therapy. In this respect, recent *in vitro* studies have shown that, BIBN4096BS, a potent and 'silent' CGRP receptor antagonist (13), inhibits CGRP-induced dilatation of isolated cranial blood vessels (16, 18). BIBN4096BS can also effectively antagonise CGRP-induced carotid vasodilatation in anaesthetised pigs (46). Therefore, it seems important to investigate the effects of BIBN4096BS on the carotid haemodynamic responses produced by endogenous CGRP released by capsaicin in a porcine model predictive of antimigraine activity (1, 22, 23). Our results show that: (i) i.c. administration of capsaicin increased blood pressure, but dilated carotid arteriovenous anastomoses and arterioles, together with an increase in carotid pulsations and a narrowing of A-V SO_2 difference as well as an elevation of jugular venous plasma CGRP concentration; and (ii) BIBN4096BS dose-dependently antagonised the changes in carotid haemodynamics and A-VSO_2 difference caused by capsaicin, but it enhanced the capsaicin-induced increase in jugular venous plasma CGRP concentration.

3.4.2. Systemic haemodynamic responses to capsaicin

The widespread distribution of CGRP immunoreactivity in cardiovascular tissues suggests that CGRP may play a role in the regulation of systemic and regional haemodynamics (42, 47). In fact, several *in vivo* studies have evidenced a hypotensive response to CGRP due to its potent vasodilator action (47, 48). In contrast, our study shows a significant increase in mean blood pressure following i.c. capsaicin, and this increase was not abolished by BIBN4096BS. Despite the absence of clear tachycardic responses to i.c. capsaicin, the simplest interpretation of these findings may be that the vasopressor response to capsaicin is not mediated via CGRP receptors, but is rather due to an interaction with vasoconstrictor mechanisms. Indeed, not only do high subcutaneous doses (50 $mg.kg^{-1}$) of capsaicin increase plasma CGRP concentrations, but also plasma catecholamines, neurokinin A and neuropeptide Y concentrations (25).

3.4.3. Carotid haemodynamics

Stimulation of the trigeminal ganglion increases cerebral blood flow and releases endogenous vasoactive neuropeptides, including CGRP (49). Vasoactive neuropeptides are also released from sensory afferent nerves by capsaicin, but its relaxant effect on isolated cerebral blood vessels is mediated by CGRP, rather than by substance P or neurokinin A (27, 50, 51). These findings are in full agreement with our results in anaesthetised pigs showing dose-dependent vasodilator responses to capsaicin in the carotid circulation, including arteriovenous anastomoses and arterioles. Admittedly, as reported earlier (24), vasodilator responses to capsaicin tended to wear off in vehicle-treated animals, suggestive of tachyphylaxis. This

tachyphylaxis was rather limited, possibly due to a neuronal reuptake of released CGRP into capsaicin-sensitive perivascular nerves (52). However, compared to the vehicle-treated animals, the carotid haemodynamic effects of capsaicin were clearly much more attenuated by the potent and selective CGRP receptor antagonist BIBN4096BS (13-15, 53). BIBN4096BS has also been demonstrated to effectively block the relaxation of blood vessels by CGRP, both *in vitro* (13, 16-18, 53) and *in vivo* (13), including the porcine carotid vascular bed (46). Therefore, it is clear that carotid vasodilatation by capsaicin in the present investigation is mediated by the release of CGRP.

3.4.4. A-V SO_2 difference

During the headache phase of migraine, the A-V SO_2 difference is abnormally low, presumably due to an opening of arteriovenous shunts (54). Thus, a reduction of carotid arteriovenous anastomotic blood flow, with a consequent normalisation of the A-V SO_2 difference, makes our porcine vascular model highly predictive of antimigraine activity (1, 22, 31). In the present study, i.c. infusions of capsaicin significantly decreased A-V SO_2 difference together with dilatation of carotid arteriovenous anastomoses. Since both these effects of capsaicin were effectively blocked by BIBN4096BS, it confirms that capsaicin-induced responses are mediated via the release of CGRP. Indeed, CGRP also decreases A-V SO_2 difference and this effect is antagonised by BIBN4096BS (46).

3.4.5. Plasma concentrations of CGRP

The release of CGRP by capsaicin is mediated by selective activation of the Aδ- and C-fibre sensory neurones *via* vanilloid receptors (26, 55, 56). Our results showing an increase in plasma concentrations of CGRP after capsaicin (see Figure 3.6) are consistent with the above observations. Interestingly, not only did BIBN4096BS fail to block capsaicin-induced CGRP release, but also there was a modest enhancement of CGRP release. There is evidence for uptake of CGRP into perivascular, capsaicin-sensitive neurones in the guinea-pig isolated basilar artery (52). Therefore, it may well be that blockade of prejunctional 'inhibitory' CGRP autoreceptors by BIBN4096BS led to increased release of CGRP by capsaicin, similar to the modulation of sympathetic neurotransmission by presynaptic α-adrenoceptors (57).

It may be noted that plasma CGRP concentrations measured by us at baseline (27±2 pmol ml^{-1}, n=12) as well as after capsaicin treatment (58±5 pmol ml^{-1}, n=12) are in agreement with those previously reported in pigs (Table 3.1) (25, 58, 59).

Table 3.1. Plasma CGRP concentration range (pmol.ml^{-1}) in pigs

Baseline	Capsaicin	Sampled from	Reference
10	36	Femoral artery	Alving et al. (25)
11-16	Not measured	Femoral artery and interventricular vein	Kallner et al. (58)
4-12	Not measured	Carotid artery	Arden et al. (59)
14-38	27-88	External jugular vein	Present investigation

3.4.6. Possible clinical implications

Lastly, we would like to consider the possible clinical implications of our results with BIBN4096BS within the context of antimigraine therapy. Indeed, the trigeminovascular system, a functional network of cranial blood vessels and their trigeminal innervation, seems to be activated during migraine (2), thereby provoking CGRP release and cranial blood vessel dilatation. Thus, a blockade of the release and/or the effects of CGRP are likely to provide novel avenues for developing antimigraine drugs without associated vasoconstriction. BIBN4096BS may be such a compound and the present findings demonstrating that it effectively antagonises the carotid vasodilator responses elicited by capsaicin are indeed encouraging. Obviously, the results of currently undergoing clinical trials with BIBN4096BS are awaited with great interest; these would be crucial in determining not only the role of CGRP in the pathophysiology of migraine, but also of such compounds as therapeutic agents.

References

1. De Vries P, Villalón CM, Saxena PR. Pharmacological aspects of experimental headache models in relation to acute antimigraine therapy. *Eur J Pharmacol* 1999;375:61-74.
2. Goadsby PJ, Lipton RB, Ferrari MD. Migraine - current understanding and treatment. *N Engl J Med* 2002;346:257-270.
3. Goadsby PJ, Edvinsson L, Ekman R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. *Ann Neurol* 1990;28:183-187.
4. Ashina M, Bendtsen L, Jensen R, Schifter S, Olesen J. Evidence for increased plasma levels of calcitonin gene-related peptide in migraine outside of attacks. *Pain* 2000;86:133-138.
5. Brain SD, Williams TJ, Tippins JR, Morris HR, MacIntyre I. Calcitonin gene-related peptide is a potent vasodilator. *Nature* 1985;313:54-56.
6. Juaneda C, Dumont Y, Quirion R. The molecular pharmacology of CGRP and related peptide receptor subtypes. *Trends Pharmacol Sci* 2000;21:432-438.
7. Van Rossum D, Hanisch UK, Quirion R. Neuroanatomical localization, pharmacological characterization and functions of CGRP-related peptides and their receptors. *Neurosci Biobehav Rev* 1997;21:649-678.
8. Poyner D, Marshall I. CGRP receptors: beyond the $CGRP_1$-$CGRP_2$ subdivision? *Trends Pharmacol Sci* 2001;22:223.
9. Gulbenkian S, Barroso CP, Cunha e Sa M, Edvinsson L. The peptidergic innervation of human coronary and cerebral vessels. *Ital J Anat Embryol* 1995;100:317-327.
10. Gulbenkian S, Uddman R, Edvinsson L. Neuronal messengers in the human cerebral circulation. *Peptides* 2001;22:995-1007.
11. Smith D, Hill RG, Edvinsson L, Longmore J. An immunocytochemical investigation of human trigeminal nucleus caudalis: CGRP, substance P and $5\text{-}HT_{1D}$-receptor immunoreactivities are expressed by trigeminal sensory fibres. *Cephalalgia* 2002;22:424-431.
12. Williamson DJ, Hargreaves RJ. Neurogenic inflammation in the context of migraine. *Microsc Res Tech* 2001;53:167-178.
13. Doods H, Hallermayer G, Wu D, Entzeroth M, Rudolf K, Engel W, Eberlein W. Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist. *Br J Pharmacol* 2000;129:420-423.
14. Doods H. Development of CGRP antagonists for the treatment of migraine. *Curr Opin Investig Drugs* 2001;2:1261-1268.

15. Wu D, Eberlein W, Rudolf K, Engel W, Hallermayer G, Doods H. Characterisation of calcitonin gene-related peptide receptors in rat atrium and vas deferens: evidence for a [Cys(Et)(2,7)]hCGRP-preferring receptor. *Eur J Pharmacol* 2000;400:313-319.
16. Edvinsson L, Alm R, Shaw D, Rutledge RZ, Koblan KS, Longmore J, Kane SA. Effect of the CGRP receptor antagonist BIBN4096BS in human cerebral, coronary and omental arteries and in SK-N-MC cells. *Eur J Pharmacol* 2002;434:49-53.
17. Moreno MJ, Abounader R, Hebert E, Doods H, Hamel E. Efficacy of the non-peptide CGRP receptor antagonist BIBN4096BS in blocking CGRP-induced dilations in human and bovine cerebral arteries: potential implications in acute migraine treatment. *Neuropharmacology* 2002;42:568-576.
18. Verheggen R, Bumann K, Kaumann AJ. BIBN4096BS is a potent competitive antagonist of the relaxant effects of alpha-CGRP on human temporal artery: comparison with CGRP(8-37). *Br J Pharmacol* 2002;136:120-126.
19. Spierings EL, Saxena PR. Effect of isometheptene on the distribution and shunting of 15 μM microspheres throughout the cephalic circulation of the cat. *Headache* 1980;20:103-106.
20. Villalón CM, Terrón JA. Characterization of the mechanisms involved in the effects of catecholamines on the canine external carotid blood flow. *Can J Physiol Pharmacol* 1994;72:165.
21. Saxena PR, De Vries P, Heiligers JP, Bax WA, Maassen VanDenBrink A, Yocca FD. BMS-181885, a 5-$HT_{1B/1D}$ receptor ligand, in experimental models predictive of antimigraine activity and coronary side-effect potential. *Eur J Pharmacol* 1998;351:329-339.
22. Saxena PR. Cranial arteriovenous shunting, an *in vivo* animal model for migraine. In: Olesen J, Moskowitz MA, eds. *Experimental headache models.* Philadelphia, USA: Lippincott-Raven Publishers, 1995:189-198. vol 27).
23. Tfelt-Hansen P, De Vries P, Saxena PR. Triptans in migraine: a comparative review of pharmacology, pharmacokinetics and efficacy. *Drugs* 2000;60:1259-1287.
24. Szallasi A, Blumberg PM. Vanilloid (capsaicin) receptors and mechanisms. *Pharmacol Rev* 1999;51:159-212.
25. Alving K, Matran R, Lundberg JM. Capsaicin-induced local effector responses, autonomic reflexes and sensory neuropeptide depletion in the pig. *Naunyn-Schmiedeberg's Arch Pharmacol* 1991;343:37-45.
26. Eltorp CT, Jansen-Olesen I, Hansen AJ. Release of calcitonin gene-related peptide (CGRP) from guinea pig dura mater in vitro is inhibited by sumatriptan but unaffected by nitric oxide. *Cephalalgia* 2000;20:838-844.
27. Jansen-Olesen I, Mortensen A, Edvinsson L. Calcitonin gene-related peptide is released from capsaicin-sensitive nerve fibres and induces vasodilatation of human cerebral arteries concomitant with activation of adenylyl cyclase. *Cephalalgia* 1996;16:310-316.
28. Kapoor K, Heiligers JPC, Willems EW, Saxena PR. Effects of BIBN4096BS - a novel CGRP antagonist on the capsaicin-induced haemodynamic changes in anaesthetized pigs. *Pharmacologist* 2002;44 (Suppl. 1):A151.
29. Den Boer MO, Van Woerkens LJ, Somers JA, Duncker DJ, Lachmann B, Saxena PR, Verdouw PD. On the preservation and regulation of vascular tone in arteriovenous anastomoses during anesthesia. *J Appl Physiol* 1993;75:782-789.
30. Willems EW, Trion M, De Vries P, Heiligers JPC, Villalón CM, Saxena PR. Pharmacological evidence that α_1- and α_2-adrenoceptors mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetized pigs. *Br J Pharmacol* 1999;127:1263-1271.

31. Saxena PR. Arteriovenous anastomoses and veins in migraine research. In: Blau JN, ed. *Migraine, clinical, therapeutic, conceptual and research aspects.* London, UK: Chapman and Hall medicin, 1987:581-596.
32. Saxena PR, Schamhardt HC, Forsyth RP, Hoeve J. Computer programs for the radioactive microsphere technique. Determination of regional blood flows and other haemodynamic variables in different experimental circumstances. *Comput Programs Biomed* 1980;12:63-84.
33. Saxena PR, Verdouw PD. Redistribution by 5-hydroxytryptamine of carotid arterial blood at the expense of arteriovenous anastomotic blood flow. *J Physiol (Lond)* 1982;332:501-520.
34. Dwenger A. Radioimmunoassay: an overview. *J Clin Chem Clin Biochem* 1984;22:883-894.
35. Ludbrook J. Repeated measurements and multiple comparisons in cardiovascular research. *Cardiovasc Res* 1994;28:303-311.
36. Overall JE, Doyle SR. False-positive error rates in routine application of repeated measurements ANOVA. *J Biopharm Stat* 1996;6:69-81.
37. Overall JE, Doyle SR. Implications of chance baseline differences in repeated measurement designs. *J Biopharm Stat* 1994;4:199-216.
38. Overall JE, Atlas RS. Power of univariate and multivariate analyses of repeated measurements in controlled clinical trials. *J Clin Psychol* 1999;55:465-485.
39. Goadsby PJ, Edvinsson L. The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats. *Ann Neurol* 1993;33:48-56.
40. Villalón CM, Centurión D, Valdivia LF, De Vries P, Saxena PR. An introduction to migraine: from ancient treatment to functional pharmacology and antimigraine therapy. *Proc West Pharmacol Soc* 2002;45:199-210.
41. Moskowitz MA, Buzzi MG, Sakas DE, Linnik MD. Pain mechanisms underlying vascular headaches. Progress Report 1989. *Rev Neurol (Paris)* 1989;145:181-193.
42. Hagner S, Stahl U, Knoblauch B, McGregor GP, Lang RE. Calcitonin receptor-like receptor: identification and distribution in human peripheral tissues. *Cell Tissue Res* 2002;310:41-50.
43. Van Rossum D, Hanisch UK, Quirion R. Neuroanatomical localization, pharmacological characterization and functions of CGRP, related peptides and their receptors. *Neurosci Biobehav Rev* 1997;21:649-678.
44. Buzzi MG, Bonamini M, Moskowitz MA. Neurogenic model of migraine. *Cephalalgia* 1995;15:277-280.
45. Van Gelderen EM, Du XY, Schoemaker RG, Saxena PR. Carotid blood flow distribution, haemodynamics and inotropic responses following calcitonin gene-related peptide in the pig. *Eur J Pharmacol* 1995;284:51-60.
46. Kapoor K, Arulmani U, Heiligers JPC, Willems EW, Doods H, Villalón CM, Saxena PR. Effects of BIBN4096BS on cardiac output distribution and on CGRP-induced carotid haemodynamic changes in the pig. *Eur J Pharmacol* 2003:In press.
47. Bell D, McDermott BJ. Calcitonin gene-related peptide in the cardiovascular system: characterization of receptor populations and their (patho)physiological significance. *Pharmacol Rev* 1996;48:253-288.
48. Shen YT, Pittman TJ, Buie PS, Bolduc DL, Kane SA, Koblan KS, Gould RJ, Lynch JJ, Jr. Functional role of alpha-calcitonin gene-related peptide in the regulation of the cardiovascular system. *J Pharmacol Exp Ther* 2001;298:551-558.

49. Goadsby PJ, Edvinsson L, Ekman R. Release of vasoactive peptides in the extracerebral circulation of humans and the cat during activation of the trigeminovascular system. *Ann Neurol* 1988;23:193-196.
50. Jansen I, Alafaci C, Uddman R, Edvinsson L. Evidence that calcitonin gene-related peptide contributes to the capsaicin-induced relaxation of guinea pig cerebral arteries. *Regul Pept* 1990;31:167-178.
51. O'Shaughnessy CT, Waldron GJ, Connor HE. Lack of effect of sumatriptan and UK-14,304 on capsaicin-induced relaxation of guinea-pig isolated basilar artery. *Br J Pharmacol* 1993;108:191-195.
52. Sams-Nielsen A, Orskov C, Jansen-Olesen I. Pharmacological evidence for CGRP uptake into perivascular capsaicin sensitive nerve terminals. *Br J Pharmacol* 2001;132:1145-1153.
53. Wu D, Doods HN, Arndt K, Schindler M. Development and potential of non-peptide antagonists for calcitonin-gene-related peptide (CGRP) receptors: evidence for CGRP receptor heterogeneity. *Biochem Soc Trans* 2002;30:468-473.
54. Heyck H. Pathogenesis of migraine. *Res Clin Stud Headache* 1969;2:1-28.
55. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997;389:816-824.
56. Ebersberger A, Averbeck B, Messlinger K, Reeh PW. Release of substance P, calcitonin gene-related peptide and prostaglandin E2 from rat dura mater encephali following electrical and chemical stimulation in vitro. *Neuroscience* 1999;89:901-907.
57. Langer SZ. Presynaptic regulation of the release of catecholamines. *Pharmacol Rev* 1980;32:337-362.
58. Kallner G, Gonon A, Franco-Cereceda A. Calcitonin gene-related peptide in myocardial ischaemia and reperfusion in the pig. *Cardiovasc Res* 1998;38:493-499.
59. Arden WA, Fiscus RR, Wang X, Yang L, Maley R, Nielsen M, Lanzo S, Gross DR. Elevations in circulating calcitonin gene-related peptide correlate with hemodynamic deterioration during endotoxic shock in pigs. *Circ Shock* 1994;42:147-153.

CHAPTER 4

α_1-Adrenoceptor subtypes mediating vasoconstriction in the carotid circulation of anaesthetised pigs: possible avenues for antimigraine drug development

Based on: Willems EW, Heiligers JPC, De Vries P, Kapoor K, Tom B, Villalón CM, Saxena PR. α_1-adrenoceptor subtypes mediating vasoconstriction in the carotid circulation of anaesthetized pigs: possible avenues for anti-migraine drug development. Cephalalgia 2001;21:110-119.

4. α_1-Adrenoceptor subtypes mediating vasoconstriction in the carotid circulation of anaesthetised pigs: possible avenues for antimigraine drug development

4.1. Introduction

Although the pathophysiology of migraine has not yet been completely unravelled, there is little doubt that dilatation of large cephalic arteries and, possibly, arteriovenous anastomoses is involved in the headache phase of migraine (4-7). Indeed, over the years we have shown that two important groups of drugs that are highly effective in the acute treatment of migraine, i.e. the triptans and ergot alkaloids, potently constrict carotid arteriovenous anastomoses (7-9). While the carotid vasoconstrictor effect of sumatriptan as well as some other triptans is mediated by the 5-HT_{1B} receptor (7, 9, 10), the ergot-induced vasoconstriction involves, in addition to 5-HT_{1B} receptors (11), also α-adrenoceptors (12).

There are several reasons to believe that α-adrenoceptors may regulate vascular tone of carotid arteriovenous anastomoses, providing a potential avenue for the development of new antimigraine drugs. It is well known that α-adrenoceptors play an important role in the regulation of vascular tone and blood pressure (13) and that stimulation of the receptors results in constriction of the isolated carotid artery (14-18). Furthermore, administration of α-adrenoceptor antagonists to conscious pigs as well as pigs under thiopentone anaesthesia results in an increase in arteriovenous anastomotic blood flow (19, 20). More recently, we have shown that both α_1- and α_2-adrenoceptors mediate the constriction of carotid arteriovenous anastomoses in anaesthetised pigs (21).

Table 4.1. Binding affinity constants (pK_i values) for cloned human α_1-adrenoceptor subtypes.

Compound	α_{1a}	α_{1b}	α_{1d}
Prazosin	9.4[a]	10.2[a]	9.8[a]
5-Methylurapidil	8.7[a]	7.6[a]	7.5[a]
L-765,314	6.4[b]	8.7[b]	7.5[b]
BMY 7378	6.6[c]	7.2[c]	9.4[c]

Data from: [a], Weinberg *et al*. (1); [b], Patane *et al*. (2); [c], Goetz *et al*. (3).

The objective of the present study was to elucidate the subtype(s) of α_1-adrenoceptors –α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes (22-25) – involved in the constriction of carotid arteriovenous anastomoses in anaesthetised pigs. For this purpose, we investigated the effects of 5-methylurapidil, L-765,314 and BMY 7378, which are preferential antagonists, respectively, at α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors (see Table 4.1 for affinity constants) (1-3), on the carotid vasoconstriction induced by the α_1-adrenoceptor agonist phenylephrine in a well-defined *in vivo* animal model predictive for antimigraine activity (8, 26). Unfortunately, selective agonists at the α_1-adrenoceptor subtypes are not available.

4.2. Materials and methods

4.2.1. General

After an overnight fast, 41 domestic pigs (Yorkshire x Landrace; female; 10-14 kg) were anaesthetised with azaperone (120 mg, i.m.), midazolam hydrochloride (5 mg, i.m.) and sodium pentobarbital (600 mg, i.v.). After tracheal intubation, the animals were connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; pO_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of sodium pentobarbital (20 $mg.kg^{-1}.h^{-1}$). It may be pointed out that this anaesthetic regimen, together with bilateral vagosympathectomy (see below), leads to an increase in heart rate and dilatation of arteriovenous anastomoses due to a loss of parasympathetic and sympathetic tone, respectively. Indeed, basal arteriovenous anastomotic blood flow is considerably higher in sodium pentobarbital-anaesthetised pigs (70-80% of carotid blood flow; present results) than in conscious (<5% of carotid blood flow; 19) or fentanyl/thiopental anaesthetised (~19% of carotid blood flow; 20) pigs. A high basal carotid arteriovenous anastomotic blood flow is particularly useful for investigating the effects of drugs that constrict these 'shunt' vessels.

A catheter was placed in the inferior vena cava *via* the left femoral vein for the administration of sodium pentobarbital, vehicle (distilled water) or the antagonists. Another catheter was placed in the aortic arch *via* the left femoral artery for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) and arterial blood withdrawal for the measurement of blood gases (ABL-510; Radiometer, Copenhagen, Denmark). Subsequently, bilateral vagosympathectomy was performed in order to prevent the possible influence *via* baroreceptor reflexes on phenylephrine-induced carotid vascular responses. Two hub-less needles, each connected to a polyethylene tube, used for the administration of radioactive microspheres and phenylephrine, respectively, were inserted into the right common carotid artery against the direction of blood flow for uniform mixing.

Total common carotid blood flow was measured with a flow probe (internal diameter: 2.5 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands), as describe elsewhere (8, 27). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by electrocardiogram signals. Arterial blood pressure, heart rate and total carotid blood flow were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). During the experiment, body temperature was kept about 37 °C and the animal was continuously infused with physiological saline to compensate fluid losses.

The Ethics Committee of the Erasmus University Rotterdam, dealing with the use of animals in scientific experiments, approved the protocols followed in this investigation.

4.2.2. Distribution of carotid blood flow

As described in detail previously (27-29), the distribution of carotid blood flow was determined with 15.5±0.1 μm (s.d.) diameter microspheres labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, USA). For each measurement, about 200,000 microspheres, labelled with one of the radioisotopes, were mixed and injected into the right common carotid artery. At the end of the experiment, the animal was killed by an overdose

of sodium pentobarbital and the heart, lungs, kidneys and all ipsilateral cranial tissues were dissected out, weighed and put in plastic vials. The radioactivity in these vials was counted for 10 min in a γ-scintillation counter (Packard, Minaxi autogamma 5000), using suitable windows to discriminate the different isotopes (^{141}Ce: 120-167 KeV, ^{113}Sn: 355-435 KeV, ^{103}Ru: 450-548 KeV, ^{95}Nb: 706-829 KeV and ^{46}Sc: 830-965 KeV). All data were processed by a set of specially designed computer programs (27). The fraction of carotid blood flow distributed to the different tissues ($CaBF_{tis}$) was calculated by the following equation: $CaBF_{tis} = (I_{tis}/I_{tot}) \times CaBF$, where I_{tis} and I_{tot} denote the tissue and total (i.e. the sum of all tissue) radioactivity, respectively, of each radioisotope and CaBF represents the common carotid artery blood flow at the time of microsphere injection. Since little or no radioactivity was detected in the heart and kidneys, all microspheres trapped in lungs reached this tissue from the venous side after escaping *via* carotid arteriovenous anastomoses. Therefore, the amount of radioactivity in the lungs was used as an *index* of the arteriovenous anastomotic fraction of the total carotid blood flow (8). Vascular conductance ($ml.min^{-1}.mmHg^{-1}$) was calculated by dividing blood flow ($ml.min^{-1}$) by mean arterial blood pressure (mmHg).

4.2.3. Experimental protocol

After a stabilisation period of at least 60 min, baseline values of heart rate, mean arterial blood pressure, total carotid blood flow and its distribution into arteriovenous anastomotic and capillary fractions, as well as arterial blood gases were measured. Thereafter, the animals were divided into seven groups, receiving i.v. infusions (0.5 $ml.min^{-1}$ for 10 min) of either vehicle (distilled water; n=8), 5-methylurapidil (300 or 1000 $\mu g.kg^{-1}$; n=6 each dose), L-765,314 (300 or 1000 $\mu g.kg^{-1}$; n=6 and 3, respectively) or BMY 7378 (300 or 1000 $\mu g.kg^{-1}$; n=6 each dose). After a waiting period of 15 min, all variables were reassessed. Subsequently, the animals received cumulative doses of phenylephrine (1, 3 and 10 $\mu g.kg^{-1}.min^{-1}$) infused into the right common carotid artery (0.1 $ml.min^{-1}$ for 10 min). All variables were collated again 10 min after the start of each agonist infusion.

At least 90 min after the last microsphere injection, the animals received i.v. bolus injections of phenylephrine (3 and 10 $\mu g.kg^{-1}$) and peak changes in mean arterial blood pressure were noted.

4.2.4. Data presentation and statistical analysis

All data have been expressed as mean±s.e.m. In order to correct for potential baseline differences caused by the antagonists or vehicle, the percent changes induced by phenylephrine from the values after administration of the different antagonists or vehicle were calculated in each group. The significance of the percent changes induced by the different doses of phenylephrine within one group was evaluated with Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations (30). Percent changes caused by phenylephrine in the different treatment groups were compared to the percent changes caused by the corresponding phenylephrine dose in the vehicle-treated group using Student's unpaired *t*-test. Statistical significance was accepted at $P<0.05$ (two-tailed).

4.2.5. Drugs

Apart from the anaesthetics azaperone (Stresnil®; Janssen Pharmaceuticals, Beerse, Belgium), midazolam hydrochloride (Dormicum®; Hoffmann La Roche b.v. Mijdrecht, The Netherlands) and sodium pentobarbital (Apharmo, Arnhem, The Netherlands), the compounds used in this study were: L-phenylephrine hydrochloride, 5-methylurapidil, BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro [4,5]decane-

7,9-dione dihydrochloride) dihydrochloride (all from Sigma-Aldrich Chemie b.v., Zwijndrecht, The Netherlands) and L-765,314 (4-amino-2-[4-[1-(benzyloxycarbonyl)-2(S)-[[(1,1-dimethylethyl)amino] carbonyl]-piperazinyl]-6,7-dimethoxyquinazoline hydrochloride; Merck & Co., Inc., West Point, PA 19486, USA). Finally, heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) was used to prevent clotting of blood in the catheters.

All drugs were dissolved in distilled water. A short period of heating was needed to dissolve 5-methylurapidil (acidified to pH=6.8-7.0 with 0.1 M HCl) and L-765,314. The doses of the drugs refer to their respective salts.

4.3. Results

4.3.1. Baseline values and effect of antagonists per se

Baseline values of haemodynamic variables in anaesthetised pigs (n=41) were: heart rate (100±2 beats.min^{-1}), mean arterial blood pressure (93±2 mmHg), total carotid blood flow (123±6 ml.min^{-1}) and conductance (132±6 10^{-2} ml.min^{-1}.mmHg^{-1}), arteriovenous anastomotic blood flow (95±6 ml.min^{-1}) and conductance (102±6 10^{-2} ml.min^{-1}.mmHg^{-1}) and capillary blood flow (28±2 ml.min^{-1}) and conductance (30±2 10^{-2} ml.min^{-1}.mmHg^{-1}). There were no major differences between the baseline values in the different groups of animals (data not shown).

No haemodynamic changes were observed with the vehicle or BMY 7378 (data not shown). 5-Methylurapidil (1000 μg.kg^{-1}) slightly decreased mean arterial blood pressure (-8±4%) and increased heart rate (9±1%) as well as capillary blood flow (35±4%) and conductance (49±9%). L-765,314 (1000 μg.kg^{-1}) decreased mean arterial blood pressure (-9±4%) and increased capillary conductance (27±11%).

4.3.2. Systemic haemodynamic responses to intracarotid infusions of phenylephrine

As shown in Table 4.2, after treatment with the vehicle intracarotid infusions of phenylephrine (1, 3 and 10 μg.kg^{-1}.min^{-1}) caused a dose-dependent increase in heart rate by up to 28±5%, without affecting mean arterial blood pressure. This phenylephrine-induced tachycardia was slightly less (maximal increase: 11±3%) after 300 μg.kg^{-1} of 5-methylurapidil (probably due to higher initial value), but was not different after the highest dose of 5-methylurapidil (1000 μg.kg^{-1}). In animals treated with either 1000 μg.kg^{-1} of L-765,314 or BMY7378, a small decrease in mean arterial blood pressure (9±1 or 8±4%, respectively) was observed with phenylephrine; however, when compared to the corresponding blood pressure change in vehicle-treated animals, statistical significance was not reached.

4.3.3. Carotid haemodynamic responses to intracarotid infusions of phenylephrine

Absolute values of total carotid, arteriovenous anastomotic and capillary vascular conductances in the different groups of animals before and after intracarotid infusions of phenylephrine are shown in Figure 4.1. In animals treated with vehicle, phenylephrine produced a dose-dependent decrease in total carotid conductance by up to 75±4%. Since phenylephrine did not change the vascular conductance in the capillary fraction, the decrease in total carotid conductance was exclusively caused by a decrease in the arteriovenous anastomotic fraction (maximal response: 92±3%).

Table 4.2. Changes in heart rate and mean arterial blood pressure induced by 10-min intracarotid infusions of phenylephrine in anaesthetised pigs treated i.v. with either vehicle, 5-methylurapidil, L-765,314 or BMY 7378.

Variables and Treatment groups	Dose ($\mu g.kg^{-1}$)	Values before phenylephrine*	Phenylephrine ($\mu g.kg^{-1}.min^{-1}$)		
			1	3	10
Heart rate (beats.min^{-1})					
Vehicle	5 ml	95±2	98±2	105±3[a]	121±5[a]
5-Methylurapidil	300	112±5	111±6[b]	112±5[b]	123±4[ab]
	1000	101±3	103±4	106±4	117±3[a]
L-765,314	300	98±5	101±5	107±5[a]	129±7[a]
	1000	93±2	93±3	97±4	112±6[a]
BMY 7378	300	99±4	101±3	104±3	119±5[a]
	1000	109±6	112±6	117±6[a]	127±6[a]
Mean arterial blood pressure (mmHg)					
Vehicle	5 ml	97±2	97±3	96±3	99±5
5-Methylurapidil	300	80±5	82±5	79±6	83±6
	1000	95±5	89±5[b]	87±5	89±6
L-765,314	300	84±6	83±7	84±8	87±8
	1000	84±3	80±4[a]	78±2[a]	79±4[a]
BMY 7378	300	85±3	85±4	84±3	90±3
	1000	91±5	88±5	86±4	84±4[a]

*, Values after treatment with respective antagonist or vehicle.
a, $P<0.05$ vs. baseline.
b, $P<0.05$ vs. response (% response from respective baseline) to the corresponding phenylephrine dose in animals treated with vehicle.

As shown in Figures 4.1 and 4.2, the constrictor effect of phenylephrine on carotid arteriovenous anastomoses was not affected by 300 $\mu g.kg^{-1}$ of either 5-methylurapidil or BMY 7378, but was significantly attenuated by 300 $\mu g.kg^{-1}$ of L-765,314 and1000 $\mu g.kg^{-1}$ of 5-methylurapidil and BMY 7378. Furthermore, after the highest dose of L-765,314, the responses to phenylephrine were clearly abolished and the values did not significantly differ from those before phenylephrine infusion. Since mean arterial blood pressure was little affected by the intracarotid infusions of phenylephrine, the responses of vascular conductances were qualitatively and quantitatively similar to those of blood flow (data not shown).

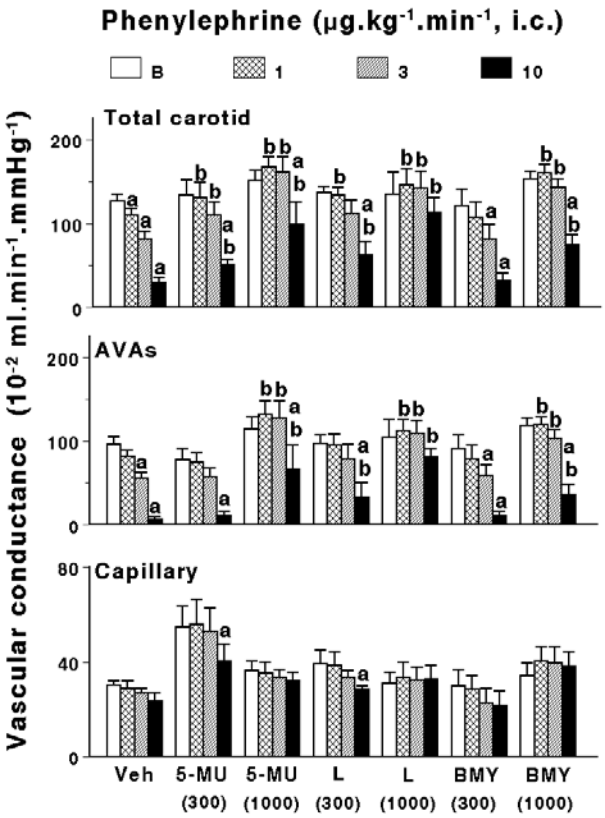


Figure 4.1. Effects of 10-min intracarotid infusions of phenylephrine on total carotid, arteriovenous anastomotic (AVA) and capillary vascular conductances in anaesthetised pigs treated i.v. with either vehicle (Veh; n=8), 5-methylurapidil (5-MU; 300 or 1000 µg.kg^{-1}; n=6 each dose), L-765,314 (L; 300 or 1000 µg.kg^{-1}; n=6 and 3, respectively) or BMY 7378 (BMY; 300 or 1000 µg.kg^{-1}; n=6 each dose). a, P<0.05 vs. baseline (B; values after treatment); b, P<0.05 vs. response (% response from respective baseline) of the corresponding phenylephrine dose in vehicle-treated animals.

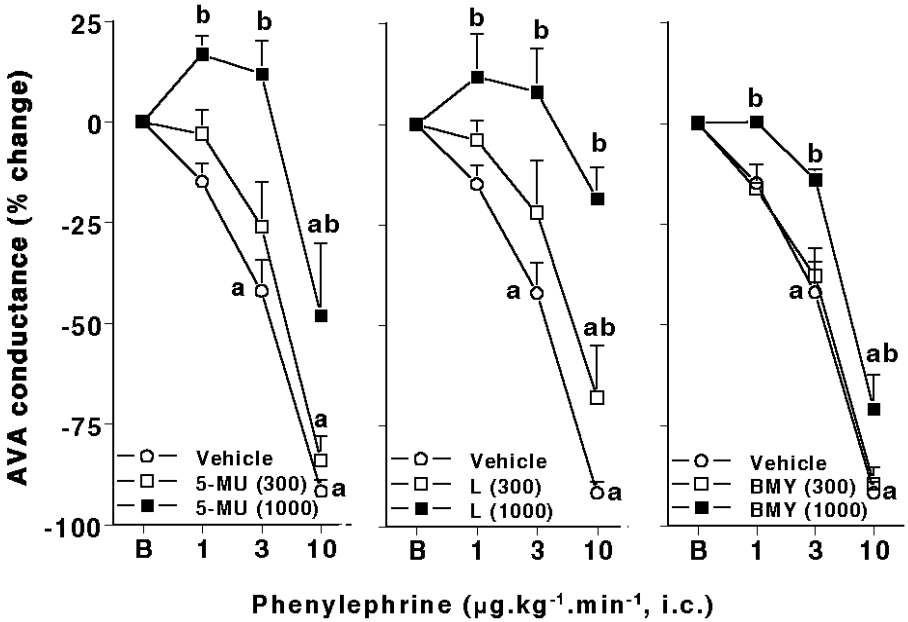


Figure 4.2. Percent changes in arteriovenous anastomotic (AVA) conductance induced by 10-min intracarotid infusions of phenylephrine in anaesthetised pigs treated i.v. with either vehicle (Veh; n=8), 5-methylurapidil (5-MU; 300 or 1000 µg.kg^{-1}; n=6 each dose; *left graph*), L-765,314 (L; 300 or 1000 µg.kg^{-1}; n=6 and 3, respectively; *middle graph*) or BMY 7378 (BMY; 300 or 1000 µg.kg^{-1}; n=6 each dose; *right graph*). a, P<0.05 vs. baseline (B; values after treatments); b, P<0.05 vs. response (% response from respective baseline) of the corresponding phenylephrine dose in vehicle-treated animals. Note that the control curves are identical in each graph.

4.3.4. Changes in mean arterial blood pressure by i.v. bolus administration of phenylephrine

In vehicle-treated animals, bolus injections of phenylephrine (3 and 10 μg.kg^{-1}, i.v) produced a dose-dependent increase in mean arterial blood pressure, yielding peak responses of 28±3 and 51±3%, respectively (Figure 4.3). These phenylephrine-induced vasopressor responses

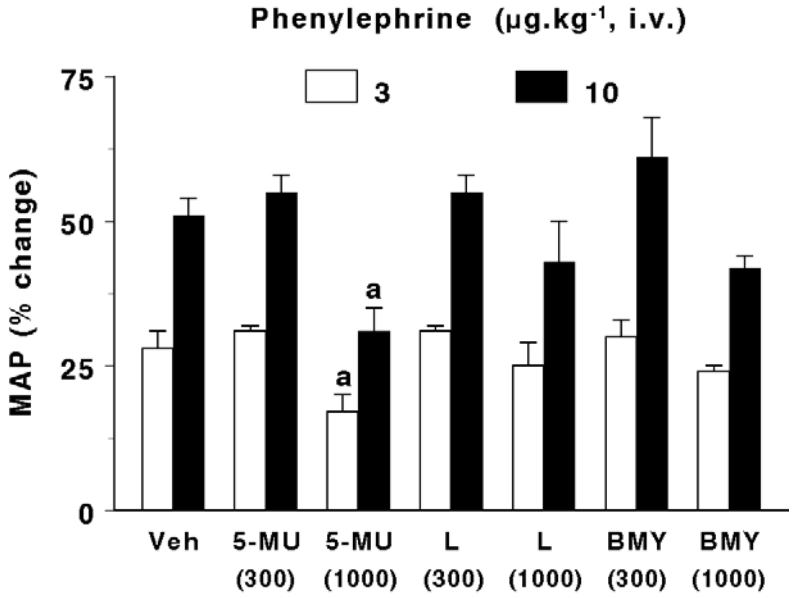


Figure 4.3. Peak changes in mean arterial blood pressure (MAP) induced by bolus injection of phenylephrine (3 or 10 μg.kg^{-1}, i.v.) in animals treated i.v. with either vehicle (Veh; n=8), 5-methylurapidil (5-MU; 300 or 1000 μg.kg^{-1}; n=6 each dose), L-765,314 (L; 300 or 1000 μg.kg^{-1}; n=6 and 3, respectively) or BMY 7378 (BMY; 300 or 1000 μg.kg^{-1}; n=6 each dose). a, P<0.05 vs. percent response of the corresponding phenylephrine dose in vehicle-treated animals.

were significantly attenuated by 1000 μg.kg^{-1} of 5-methylurapidil (peak responses: 17±3 and 31±4%, respectively), but were not affected by L-765,314 (300 or 1000 μg.kg^{-1}), BMY 7378 (300 or 1000 μg.kg^{-1}) or 300 μg.kg^{-1} of 5-methylurapidil.

4.4. Discussion

4.4.1. General

We have recently shown that both α_1- and α_2-adrenoceptors mediate constriction of arteriovenous anastomoses within the carotid vascular bed in anaesthetised pigs (21). This conclusion was based on the findings that (*i*) intracarotid administration of phenylephrine (α_1-adrenoceptor agonist) and BHT933 (α_2-adrenoceptor agonist) decreased the total carotid blood flow exclusively confined to the arteriovenous anastomotic fraction, without affecting mean arterial blood pressure; and (*ii*) these effects of phenylephrine and BHT933 were selectively antagonised by the α_1- and α_2-adrenoceptor antagonists, prazosin and rauwolscine, respectively (21). This is also in agreement with recent findings demonstrated in the external carotid vascular bed of anaesthetised dogs (31).

Based on radioligand binding, molecular biology and isolated tissue experiments, it is known that α_1-adrenoceptors are a heterogeneous group of receptors, currently subdivided into α_{1A}, α_{1B} and α_{1D} subtypes (24, 32). As reviewed by Vargas and Gorman (13), the α_{1A}-adrenoceptor subtype, which is widely distributed throughout the body and is the major subtype regulating systemic vascular resistance and blood pressure; α_{1D}-adrenoceptors seem to play only a minor role in blood pressure regulation (33). Whereas there is limited information concerning the vascular effects mediated by α_{1B}-adrenoceptors, it has been shown that constriction of the isolated carotid artery of the dog and rabbit mainly resembles the cloned α_{1B}-adrenoceptor (13). However, no information is available on the subtype mediating vasoconstrictor effects within the carotid arterial bed *in vivo*. Therefore, the objective of the present study was to identify the α_1-adrenoceptor subtype(s) that mediate constriction in the carotid vasculature in anaesthetised pigs, with particular emphasis on the arteriovenous anastomotic fraction, which may be of relevance to migraine therapy (4, 8, 10).

In recent years, several selective antagonists at α_1-adrenoceptor subtypes have been developed (Table 4.1). In the present study, we made use of 5-methylurapidil and BMY 7378, which have frequently been used to characterise α_{1A}- and α_{1D}-adrenoceptors, respectively (Table 4.1; 3, 25, 32, 34, 35-37). To block α_{1B}-adrenoceptors, we employed L-765,314, which shows a moderate to high selectivity for cloned α_{1b}-adrenoceptors over cloned α_{1a}- or α_{1d}-adrenoceptors (2). Many studies have used the clonidine derivative, chloroethylclonidine, for this purpose (32, 38). However, it is now evident that chloroethylclonidine alkylates several other receptors as well (32, 39, 40).

4.4.2. Systemic and carotid haemodynamic effects of different antagonists

Whereas the vehicle was devoid of any systemic and carotid haemodynamic effects, administration of the α_{1A}-adrenoceptor antagonist 5-methylurapidil produced a small hypotension and tachycardia (Table 4.2). Similar hypotensive effect was observed earlier with 5-methylurapidil (41) and may be related to either blockade of vascular smooth muscle α_{1A}-adrenoceptors, which play an important role in the maintenance of vascular tone (13) or to its agonist properties at central 5-HT_{1A} receptors (42). Admittedly, we do not have a clear-cut explanation for the slight hypotension and increase in capillary conductance produced by L-765,314, since it has been shown by Piascik *et al*. (43) that α_{1B}-adrenoceptors play only a minor role in the contraction of peripheral blood vessels *in vitro*.

4.4.3. Changes in heart rate and mean arterial blood pressure by phenylephrine

Intracarotid infusions of phenylephrine produced only minor systemic haemodynamic responses in vehicle-treated animals. The tachycardia (Table 4.2), which was also observed with i.v. phenylephrine (data not shown), most likely involves an interaction with β-adrenoceptors (21). Furthermore, i.v. administration of phenylephrine (3 and 10 $\mu g.kg^{-1}$) induced a dose-dependent increase in blood pressure (Figure 4.3), which was antagonised by 100 $\mu g.kg^{-1}$ of prazosin, but not by 300 $\mu g.kg^{-1}$ of rauwolscine (Willems *et al*., unpublished observations). In view of the antagonism of this response by 5-methylurapidil, but not by L-765,314 (α_{1B}-adrenoceptor antagonist) or BMY 7378 (α_{1D}-adrenoceptor antagonist) (Figure 4.3), the pressor response to phenylephrine is likely to be mediated by α_{1A}-adrenoceptors. In keeping with the approximately 10-fold higher affinity at the cloned α_{1a}-adrenoceptor displayed by prazosin compared to 5-methylurapidil (Table 4.1), a 10-fold higher dose of 5-methylurapidil (1000 $\mu g.kg^{-1}$) was needed to produce antagonism of the phenylephrine-induced vasopressor response. Thus, as previously discussed (13), these

results support the role of α_{1A}-adrenoceptors in the increase in peripheral vascular resistance and concomitant hypertensive effect upon activation.

4.4.4. Carotid haemodynamic responses to intracarotid infusions of phenylephrine

As previously reported (21), phenylephrine caused a pronounced and dose-dependent decrease in total carotid conductance, which was exclusively caused by constriction of carotid arteriovenous anastomoses; nutrient vascular conductance was not modified (Figure 4.1). These carotid vasoconstrictor responses were not affected by treatment with 300 $\mu g.kg^{-1}$ of the α_{1D}-adrenoceptor antagonist BMY 7378. This dose of BMY 7378 should be sufficient to block α_{1D}-adrenoceptors in view of comparable affinities of prazosin and BMY 7378 at the cloned human α_{1d}-adrenoceptor (Table 4.1) and the fact that 100 $\mu g.kg^{-1}$ of prazosin abolished this response (21). On this basis, the involvement of α_{1D}-adrenoceptors in the cranial vasoconstriction induced by phenylephrine in anaesthetised pigs seems highly unlikely. Nevertheless, as shown in Figures 4.1 and 4.2, the higher dose of BMY 7378 (1000 $\mu g.kg^{-1}$) produced a slight, but significant, attenuation in the phenylephrine-induced total carotid and arteriovenous anastomotic vasoconstriction. Since BMY7378 displays a moderate affinity at α_{1A}- and α_{1B}-adrenoceptors (pK_i: 6.6 and 7.2, respectively; Table 4.1), a non-selective blockade of these receptors (which can mediate carotid vasoconstriction; see below) is a likely explanation. Indeed, in 2 experiments a combination of 5-methylurapidil (1000 $\mu g.kg^{-1}$) and BMY 7378 (1000 $\mu g.kg^{-1}$) did not cause any more attenuation of the phenylephrine-induced arteriovenous constriction than 5-methylurapidil (1000 $\mu g.kg^{-1}$) alone (see below).

The α_{1B}-adrenoceptor antagonist L-765,314 abolished the constriction of carotid arteriovenous anastomoses by phenylephrine, a finding that supports the role of α_{1B}-adrenoceptors in this effect. Interestingly, the α_{1A}-adrenoceptor antagonist, 5-methylurapidil was also able to antagonise the cranial vasoconstrictor effects of phenylephrine, but in contrast to L-765,314, the highest dose of phenylephrine still elicited a 50% decrease in arteriovenous anastomotic conductance (Figures 4.2 and 4.3). Thus, at similar doses, L-765,314 acted as a more potent antagonist when compared to 5-methylurapidil. The above findings lead us to conclude that both α_{1A}- and α_{1B}-adrenoceptors mediate the phenylephrine-induced constriction of carotid arteriovenous anastomoses, whereas the α_{1D}-adrenoceptor plays a minor role, if any.

Admittedly, a critique of the above conclusion may be that, unlike *in vitro* studies, the exact concentration of α_1-adrenoceptor antagonists at the receptor site can be influenced by pharmacokinetic differences in such an *in vivo* investigation. However, current techniques do not allow us to study carotid arteriovenous anastomoses *in vitro* and, to some extent, we have tried to ensure effective antagonist concentration at the receptor site by using as high dose of antagonists as possible. Moreover, we would like to emphasis the fact that selective agonists at the different α_1-adrenoceptor subtypes are currently unavailable. Since porcine α_1-adrenoceptor subtypes have not yet been cloned, the selectivity of the subtype-selective antagonists (see Table 4.1) is based on their affinity for human cloned receptors.

4.4.5. Possible clinical implications

Lastly, we would like to consider possible clinical implications of the present results showing that α_{1A}- and α_{1B}-adrenoceptors mediate the constriction of carotid arteriovenous anastomoses in anaesthetised pigs. To date all acutely acting antimigraine agents, such as the triptans and ergot alkaloids, potently constrict carotid arteriovenous anastomoses (44, 45). Moreover, dilatation of these 'shunt' vessels may be involved in the pathophysiology of the

headache phase of migraine (4, 8, 10). Since a vasoconstrictor effect in this experimental model seems to be highly predictive for antimigraine efficacy, a α_{1A}- (such as A61603, see ref. 46) or α_{1B}-adrenoceptor agonist (which is yet to be developed) should be able to abort migraine headaches. Of the two α_1-adrenoceptor subtypes, the α_{1B}-adrenoceptor is an interesting target for future antimigraine drugs, especially when considering that this receptor, unlike the α_{1A}-adrenoceptor, does not seem to be much involved in the constriction of the peripheral blood vessels (13, 43). Indeed, our results suggest that the hypertensive effect produced by intravenous administration of phenylephrine is predominantly mediated via the α_{1A}-, but not α_{1B}-adrenoceptor (Figure 4.3). An α_{1B}-adrenoceptor agonist may have a major advantage over the currently available acute antimigraine drugs, which all constrict human isolated coronary artery (47), where, importantly, the α_{1B}-adrenoceptor is not present (48).

In conclusion, the present study shows that both α_{1A}- and α_{1B}-adrenoceptors mediate constriction of porcine carotid arteriovenous anastomoses produced by phenylephrine. Since the α_{1B}-adrenoceptor subtype is not much involved in constriction of the systemic vasculature, a cranioselective vasoconstriction may be achieved using selective α_{1B}-adrenoceptor agonists, which may prove effective in migraine.

References

1. Weinberg DH, Trivedi P, Tan CP, Mitra S, Perkins-Barrow A, Borkowski D, Strader CD, Bayne M. Cloning, expression and characterization of human alpha adrenergic receptors alpha 1a, alpha 1b and alpha 1c. Biochem Biophys Res Commun 1994;201:1296-1304.
2. Patane MA, Scott AL, Broten TP, Chang RSL, Ransom RW, DiSalvo J, Forray C, Bock MG. 4-Amino-2-[4-[1-(benzyloxycarbonyl)-2(S)-[[(1,1- dimethylethyl) amino] carbonyl]-piperazinyl]-6, 7-dimethoxyquinazoline (L- 765,314): a potent and selective alpha1b adrenergic receptor antagonist. J Med Chem 1998;41:1205-1208.
3. Goetz AS, King HK, Ward SD, True TA, Rimele TJ, Saussy DL, Jr. BMY 7378 is a selective antagonist of the D subtype of alpha 1-adrenoceptors. Eur J Pharmacol 1995;272:R5-R6.
4. Heyck H. Pathogenesis of migraine. Res Clin Stud Headache 1969;2:1-28.
5. Humphrey PPA, Goadsby PJ. The mode of action of sumatriptan is vascular? A debate. Cephalalgia 1994;14:401-410.
6. Goadsby PJ. Advances in the pharmacotherapy of migraine. How knowledge of pathophysiology is guiding drug development. Drugs R D 1999;2:361-374.
7. Saxena PR, Tfelt-Hansen P. Triptans, 5-$HT_{1B/1D}$ receptor agonists in the acute treatment of migraine. In: Olesen J, Tfelt-Hansen P, Welch KMA, eds. The headaches. New York: Lippincott, Williams & Wilkins, 2000:411-438.
8. Saxena PR. Cranial arteriovenous shunting, an *in vivo* animal model for migraine. In: Olesen J, Moskowitz MA, eds. Experimental headache models. Vol. 27. Philadelphia, USA: Lippincott-Raven Publishers, 1995:189-198.
9. De Vries P, Villalon CM, Saxena PR. Pharmacological aspects of experimental headache models in relation to acute antimigraine therapy. Eur J Pharmacol 1999;375:61-74.
10. De Vries P, Willems EW, Heiligers JPC, Villalón CM, Saxena PR. Constriction of porcine carotid arteriovenous anastomoses as indicator of antimigraine activity: the role

of 5-$HT_{1B/1D}$, as well as unidentified receptors. In: Edvinsson L, ed. Migraine & Headache Pathophysiology. London: Martin Dunitz Ltd., 1999:119-132.
11. De Vries P, Villalón CM, Heiligers JPC, Saxena PR. Characterization of 5-HT receptors mediating constriction of porcine carotid arteriovenous anastomoses; involvement of 5-$HT_{1B/1D}$ and novel receptors. Br J Pharmacol 1998;123:1561-1570.
12. Villalón CM, De Vries P, Rabelo G, Centurión D, Sánchez-López A, Saxena PR. Canine external carotid vasoconstriction to methysergide, ergotamine and dihydroergotamine: a role of 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors. Br J Pharmacol 1999;126:385-394.
13. Vargas HM, Gorman AJ. Vascular alpha-1 adrenergic receptor subtypes in the regulation of arterial pressure. Life Sci 1995;57:2291-2308.
14. Kawai Y, Kobayashi S, Ohhashi T. Existence of two types of postjunctional α-adrenoceptors in the isolated canine internal carotid artery. Can J Physiol Pharmacol 1988;66:655-659.
15. Kohno Y, Saito H, Takita M, Kigoshi S, Muramatsu I. Heterogeneity of alpha 1-adrenoceptor subtypes involved in adrenergic contractions of dog blood vessels. Br J Pharmacol 1994;112:1167-1173.
16. Muramatsu I, Kigoshi S, Ohmura T. Subtypes of alpha 1-adrenoceptors involved in noradrenaline-induced contractions of rat thoracic aorta and dog carotid artery. Jpn J Pharmacol 1991;57:535-544.
17. Muramatsu I. Relation between adrenergic neurogenic contraction and alpha 1-adrenoceptor subtypes in dog mesenteric and carotid arteries and rabbit carotid arteries. Br J Pharmacol 1991;102:210-214.
18. Ohgushi M, Yasue H, Kugiyama K, Murohara T, Sakaino N. Contraction and endothelium dependent relaxation via alpha adrenoceptors are variable in various pig arteries. Cardiovasc Res 1993;27:779-784.
19. Van Woerkens LJ, Duncker DJ, Huigen RJ, van der Giessen WJ, Verdouw PD. Redistribution of cardiac output caused by opening of arteriovenous anastomoses by a combination of azaperone and metomidate. Br J Anaesth 1990;65:393-399.
20. Den Boer MO, Van Woerkens LJ, Somers JA, Duncker DJ, Lachmann B, Saxena PR, Verdouw PD. On the preservation and regulation of vascular tone in arteriovenous anastomoses during anesthesia. J Appl Physiol 1993;75:782-789.
21. Willems EW, Trion M, De Vries P, Heiligers JPC, Villalon CM, Saxena PR. Pharmacological evidence that α_1- and α_2-adrenoceptors mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetized pigs. Br J Pharmacol 1999;127:1263-1271.
22. Bylund DB, Eikenberg DC, Hieble JP, Langer SZ, Lefkowitz RJ, Minneman KP, Molinoff PB, Ruffolo RR, Jr., Trendelenburg U. International Union of Pharmacology nomenclature of adrenoceptors. Pharmacol Rev 1994;46:121-136.
23. Hieble JP, Bondinell WE, Ruffolo RRJ. α- and β-Adrenoceptors: from the gene to the clinic. 1. Molecular biology and adrenoceptor subclassification. J Med Chem 1995;38:3415-3444.
24. Hieble JP, Bylund DB, Clarke DE, Eikenburg DC, Langer SZ, Lefkowitz RJ, Minneman KP, Ruffolo RR, Jr. International Union of Pharmacology. X. Recommendation for nomenclature of alpha 1-adrenoceptors: consensus update. Pharmacol Rev 1995;47:267-270.
25. Zhong H, Minneman KP. Alpha1-adrenoceptor subtypes. Eur J Pharmacol 1999;375:261-276.

26. Saxena PR. Is there still a case for the shunt hypothesis in migraine? In: Sandler M, Collins GM, eds. Migraine: a spectrum of ideas. Oxford: Oxford University Press, 1990:191-199.
27. Saxena PR, Schamhardt HC, Forsyth RP, Hoeve J. Computer programs for the radioactive microsphere technique. Determination of regional blood flows and other haemodynamic variables in different experimental circumstances. Comput Programs Biomed 1980;12:63-84.
28. Johnston BM, Saxena PR. The effect of ergotamine on tissue blood flow and the arteriovenous shunting of radioactive microspheres in the head. Br J Pharmacol 1978;63:541-549.
29. Saxena PR, Verdouw PD. Redistribution by 5-hydroxytryptamine of carotid arterial blood at the expense of arteriovenous anastomotic blood flow. J Physiol (Lond) 1982;332:501-520.
30. Steel RGD, Torrie JH. Principles and procedures of statistics. A biomedical approach. Tokyo, Japan: McGraw-Hill Kogakusha Ltd., 1980
31. Willems E, Valdivia L, San-Juan E, Saxena P, Villalón C. Pharmacological identification of the major subtypes of adrenoceptors involved in the external carotid vascular effects of adrenaline and noradrenaline in anaesthetised dogs. Life Sci 2000:In press.
32. Hieble JP, Ruffolo RR, Jr. Subclassification and nomenclature of α_1- and α_2-adrenoceptors. Prog Drug Res 1996;47:81-130.
33. Villalobos-Molina R, Lopez-Guerrero J, Ibarra M. Functional evidence of α_{1D}-adrenoceptors in the vasculature of young and adult spontaneously hypertensive rats. Br J Pharmacol 1999;126:1534-1536.
34. Kenny BA, Chalmers DH, Philpott PC, Naylor AM. Characterization of an alpha 1D-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. Br J Pharmacol 1995;115:981-986.
35. Schwinn DA, Johnston GI, Page SO, Mosley MJ, Wilson KH, Worman NP, Campbell S, Fidock MD, Furness LM, Parry-Smith DJ. Cloning and pharmacological characterization of human alpha-1 adrenergic receptors: sequence corrections and direct comparison with other species homologues. J Pharmacol Exp Ther 1995;272:134-142.
36. Forray C, Bard JA, Wetzel JM, Chiu G, Shapiro E, Tang R, Lepor H, Hartig PR, Weinshank RL, Branchek TA. The alpha 1-adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned human alpha 1c subtype. Mol Pharmacol 1994;45:703-708.
37. Testa R, Destefani C, Guarneri L, Poggesi E, Simonazzi I, Tadei C, Leonardi A. The α_{1d}-adrenoceptor subtype is involved in the noradrenaline-induced contractions of rat aorta. Life Sci 1995;57:PL159-PL163.
38. Docherty JR. Subtypes of functional alpha1- and alpha2-adrenoceptors. Eur J Pharmacol 1998;361:1-15.
39. Piascik MT, Soltis EE, Piascik MM, Macmillan LB. α-Adrenoceptors and vascular regulation: molecular, pharmacologic and clinical correlates. Pharmacol Ther 1996;72:215-241.
40. Docherty JR, O'Rourke M. The alpha-adrenoceptor-mediated actions of chloroethylclonidine. Gen Pharmacol 1997;28:197-201.
41. Valenta B, Singer EA. Hypotensive effects of 8-hydroxy-2-(di-n-propylamino)tetralin and 5- methylurapidil following stereotaxic microinjection into the ventral medulla of the rat. Br J Pharmacol 1990;99:713-716.

42. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol Rev 1994;46:157-203.
43. Piascik MT, Hrometz SL, Edelmann SE, Guarino RD, Hadley RW, Brown RD. Immunocytochemical localization of the alpha-1B adrenergic receptor and the contribution of this and the other subtypes to vascular smooth muscle contraction: analysis with selective ligands and antisense oligonucleotides. J Pharmacol Exp Ther 1997;283:854-868.
44. De Vries P, Villalón CM, Saxena PR. Pharmacology of triptans. Emerg Drugs 1999;4:107-125.
45. Saxena PR, Ferrari MD, De Vries P, Villalón CM. Pharmacological overview of new 5-HT_{1D} receptor agonists in development for the acute treatment of migraine. In: Olesen J, Tfelt-Hansen P, eds. Headache treatment: trial methodology and new drugs. New York: Lippincott-Raven publishers, 1997:229-241.
46. Knepper SM, Buckner SA, Brune ME, DeBernardis JF, Meyer MD, Hancock AA. A-61603, a potent alpha 1-adrenergic receptor agonist, selective for the alpha 1A receptor subtype. J Pharmacol Exp Ther 1995;274:97-103.
47. MaassenVanDenBrink A, Reekers M, Bax WA, Ferrari MD, Saxena PR. Coronary side-effect potential of current and prospective antimigraine drugs. Circulation 1998;98:25-30.
48. Rudner XL, Berkowitz DE, Booth JV, Funk BL, Cozart KL, D'Amico EB, El-Moalem H, Page SO, Richardson CD, Winters B, Marucci L, Schwinn DA. Subtype specific regulation of human vascular α_1-adrenergic receptors by vessel bed and age. Circulation 1999;100:2336-2343.

CHAPTER 5

A61603-induced vasoconstriction in porcine carotid vasculature: involvement of a non-adrenergic mechanism

Based on: Willems EW, Heiligers JP, De Vries P, Tom B, Kapoor K, Villalon CM, Saxena PR. A61603-induced vasoconstriction in porcine carotid vasculature: involvement of a non-adrenergic mechanism. Eur J Pharmacol 2001;417:195-201.

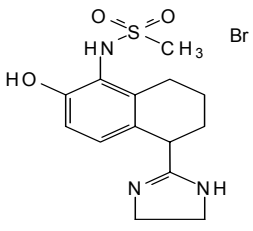
5. A61603-induced vasoconstriction in porcine carotid vasculature: involvement of a non-adrenergic mechanism

5.1. Introduction

There seems to be little doubt that the headache phase of migraine is associated with dilatation of cranial blood vessels. Indeed, sumatriptan as well as all 'second-generation' triptans potently constrict human isolated cranial arteries as well as carotid arteriovenous anastomoses in anaesthetised animals, mainly *via* the 5-HT_{1B} receptor (1, 2). In an attempt to explore new avenues for the development of antimigraine agents, we recently reported that phenylephrine and BHT933 (6-ethyl- 5,6,7,8-tetrahydro-4H-oxazolo [4,5-d] azepin-2-amine dihydrochloride) constrict carotid arteriovenous anastomoses in anaesthetised pigs *via* α_1- and α_2-adrenoceptors, respectively (3). Subsequent studies suggest that the phenylephrine-induced response is mediated by the α_{1A}- and α_{1B}- adrenoceptor subtypes, but not by the α_{1D} subtype (4).

To confirm the involvement of α_{1A}-adrenoceptors, in the present study we studied the effects of a potent and selective α_{1A}-adrenoceptor agonist, A61603 (N-[5-(4,5-dihydro-1H-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl] methane sulphonamide) (see Table 5.1, 5, 6), on regional carotid blood flow in anaesthetised pigs. The response to A61603 was characterised by using selective α-adrenoceptor antagonists, 5-methylurapidil (α_{1A}), prazosin (α_1) and a combination of prazosin (α_1) and rauwolscine (α_2). Similarly, the effects of GR127935 (N-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2'-methyl-4' (5-methyl-1, 2,4-oxadiazol-3-yl) [1,1,-biphenyl]-4-carboxamide hydrochloride monohydrate; 5-$HT_{1B/1D}$), ketanserin (5-HT_2, α_1) and methiothepin (5-$HT_{1/2}$), in doses sufficient to block their respective receptors (3, 7, 8), were also investigated. Surprisingly, the results suggest that A61603 constricts porcine arteriovenous anastomoses by a non-adrenergic mechanism.

Table 5.1. Chemical structure and binding affinity (pK_i), potency (pEC_{50}) and intrinsic activity (i.a.), of A61603 at several cloned human receptor subtypes (22).

1.1.1.1. Chemical structure		α_{1a}	α_{1b}	α_{1d}	α_{2a}	α_{2b}	α_{2c}	5-HT_{1B}	5-HT_{1D}
	**pK_i**	7.1	4.8	4.9	7.3	6.5	6.2	5.2	5.6
	pEC_{50}	8.9	4.4	4.6	7.5	7.1	7.7	ND	ND
	i.a.	1.2	0.1	0.1	0.8	0.8	0.9	ND	ND

At other receptor subtypes (histamine $H_{1/2}$, dopamine $D_{1/2/3}$, 5-$HT_{1/2/7}$ or β), $pK_i < 5.5$. pEC_{50} was determined in phosphoinositol breakdown assay. ND, not determined.

5.2. Materials and Methods

5.2.1. General

After an overnight fast, 33 domestic pigs (Yorkshire x Landrace; female; 10-14 kg) were anaesthetised with azaperone (120 mg, i.m.), midazolam hydrochloride (5 mg, i.m.) and sodium pentobarbital (600 mg, i.v.). After tracheal intubation, the animals were connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; pO_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of sodium pentobarbital (20 $mg.kg^{-1}.h^{-1}$). It may be pointed out that this anaesthetic regimen, together with bilateral vagosympathectomy (see below), leads to an increase in heart rate and dilatation of carotid arteriovenous anastomoses due to a loss of parasympathetic and sympathetic tone, respectively. Indeed, basal carotid arteriovenous anastomotic blood flow is considerably higher in sodium pentobarbital-anaesthetised pigs (70-80% of carotid blood flow; present results) than in conscious (<5% of carotid blood flow; 9) or fentanyl/thiopental anaesthetised pigs (~19% of carotid blood flow; 10). A high basal carotid arteriovenous anastomotic blood flow is particularly useful for investigating the effects of drugs that constrict these 'shunt' vessels.

A catheter was placed in the inferior vena cava *via* the left femoral vein for infusion of vehicle (distilled water), the antagonists and sodium pentobarbital. Another catheter was placed in the aortic arch *via* the left femoral artery for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) and arterial blood withdrawal for the measurement of blood gases (ABL-510; Radiometer, Copenhagen, Denmark). Subsequently, the right common carotid artery was dissected free and bilateral vagosympathectomy was performed in order to prevent a possible influence *via* baroreceptor reflexes on A61603-induced carotid vascular responses. Two hub-less needles, each connected to a polyethylene tube, used for the administration of radioactive microspheres and A61603, respectively, were inserted into the right common carotid artery against the direction of blood flow for uniform mixing.

Total common carotid blood flow was measured with a flow probe (internal diameter: 2.5 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by electrocardiogram signals. Arterial blood pressure, heart rate and carotid blood flow were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). During the experiment, body temperature was kept at about 37 °C and the animal was continuously infused with physiological saline to compensate fluid losses.

The Ethics Committee of the Erasmus University Medical Centre Rotterdam, dealing with the use of animals in scientific experiments, approved the protocols followed in this investigation.

5.2.2. Distribution of total common carotid blood flow

The distribution of total common carotid blood flow was determined with 15.5±0.1 μm (S.D.) diameter microspheres labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, USA). For each measurement, about 200,000 microspheres, labelled with one of the radioisotopes, were mixed and injected into the right common carotid artery. At the end of the experiment, the animal was killed by an overdose of sodium pentobarbital and the heart,

lungs, kidneys and all ipsilateral cranial tissues were dissected out, weighed and put in *via*ls. The radioactivity in these *via*ls was counted for 10 min in a γ-scintillation counter (Packard, Minaxi autogamma 5000), using suitable windows to discriminate the different isotopes (^{141}Ce: 120-167 KeV, ^{113}Sn: 355-435 KeV, ^{103}Ru: 450-548 KeV, ^{95}Nb: 706-829 KeV and ^{46}Sc: 830-965 KeV). All data were processed by a set of specially designed programs (11). The fraction of right common carotid blood flow distributed to the different tissues was calculated by multiplying the ratio of tissue and total radioactivity of each radioisotope by the common carotid blood flow at the time of the injection of microspheres, labeled with the respective isotope. Since little or no radioactivity was detected in the heart and kidneys, all microspheres trapped in lungs reached this tissue from the venous side after escaping *via* carotid arteriovenous anastomoses. Therefore, the amount of radioactivity in the lungs was used as an *index* of the arteriovenous anastomotic fraction of the total common carotid blood flow (12). Vascular conductance (10^{-2} ml.min^{-1}.mmHg^{-1}) was calculated by dividing blood flow (ml.min^{-1}) by mean arterial blood pressure (mmHg) multiplied by hundred.

5.2.3. Experimental protocol

After a stabilisation period of at least 60 min, values of heart rate, mean arterial blood pressure, total common carotid blood flow, as well as arterial blood gases were measured. Thereafter, the animals (n=33) were divided into seven groups, receiving i.v. infusions (0.5 ml.min^{-1} for 10 min) of either vehicle (distilled water; 5 ml, n=6), 5-methylurapidil (1000 μg.kg^{-1}, n=6), prazosin (100 μg.kg^{-1}, n=3), a combination of prazosin and rauwolscine (100 and 300 μg.kg^{-1}, respectively, n=6), GR127935 (500 μg.kg^{-1}, n=3), ketanserin (500 μg.kg^{-1}, n=3) or methiothepin (3000 μg.kg^{-1}, n=6). After 15 min, baseline values of heart rate, mean arterial blood pressure, arterial blood gases, total common carotid blood flow and its distribution into arteriovenous anastomotic and capillary fractions (injection of the first batch of microspheres) were measured. Subsequently, all animals received of A61603 (cumulative total doses: 0.3, 1, 3 and 10 μg.kg^{-1} at the rate of 0.1 ml.min^{-1} over 10 min infused into the right common carotid artery). Ten min after the start of each A61603 infusion, the animals received a different batch of microspheres and all variables were collated again.

After the carotid and systemic haemodynamic variables had returned to baseline values, we analysed the systemic haemodynamic effects of i.v. bolus injections of A61603 (1, 3, 10 and 30 μg.kg^{-1}) in the different groups of animals.

5.2.4. Data presentation and statistical analysis

All data are presented as the mean ± S.E.M. Percent changes from baseline values (i.e. after vehicle or the antagonists) caused by the different doses of A61603 within each group of animals were calculated. Duncan new multiple-range test, together with two-way Analysis of Variance (ANOVA; SigmaStat 1.0, Jandel Corporation, Chicago, IL, USA), was used to establish whether these changes were statistically significant ($P<0.05$, two-tailed) when compared to the baseline in each group as well as with the corresponding dose of A61603 in the vehicle-treated group.

5.2.5. Drugs

Apart from the anaesthetics azaperone (Stresnil®; Janssen Pharmaceuticals, Beerse, Belgium), midazolam hydrochloride (Dormicum®; Hoffmann La Roche b.v., Mijdrecht, The Netherlands) and sodium pentobarbital (Apharmo, Arnhem, The Netherlands), the compounds used in this study were: A61603 hydrobromide (Tocris Cookson Ltd., Bristol, UK), rauwolscine hydrochloride (Sigma-Aldrich Chemie b.v., Zwijndrecht, The Netherlands), 5-methylurapidil (Byk Gulden, Konstanz, Germany), prazosin hydrochloride

(Bufa Chemie b.v., Castricum, The Netherlands), GR127935, sumatriptan succinate (both from GlaxoWellcome, Herts, UK; courtesy: Dr. H.E. Connor), ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium) and methiothepin maleate (Hoffman La Roche b.v., Mijndrecht, The Netherlands). Finally, heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) was used to prevent clotting of blood in the catheters.
All drugs were dissolved in distilled water (vehicle). A short period of heating was needed to dissolve prazosin, rauwolscine, GR127935, 5-methylurapidil (acidified to pH=6.8-7.0 with 0.1 M HCl) and methiothepin (1 % of ascorbic acid was added). The doses of the drugs refer to their respective salts.

5.3. Results

5.3.1. Systemic and carotid haemodynamic variables after different antagonists

Baseline values of these variables in the 33 pigs used in the present investigation were: mean arterial blood pressure, 94±3 mmHg; heart rate, 101±3 beats.min^{-1}; total common carotid blood flow, 133±7 ml.min^{-1} and total common carotid conductance, 144±8 10^{-2} ml.min^{-1}.mmHg^{-1}. No significant differences were observed between the values of haemodynamic variables collated before and after the administration of the vehicle (distilled water) or different antagonists used in this study (Table 5.2).

5.3.2. Systemic haemodynamic responses to A61603

A61603 (0.3, 1, 3 and 10 μg.kg^{-1}, i.c.) produced a dose-dependent increase in mean arterial blood pressure (Figure 5.1; upper panel), without affecting heart rate (data not shown). This

Table 5.2. Absolute values in mean arterial blood pressure, heart rate and total carotid blood flow before and after i.v. administration of vehicle and various antagonists used in anaesthetised pigs.

Treatment	**Dose (μg.kg^{-1})**	**Mean arterial blood pressure (mmHg)**		**Heart rate (beats.min^{-1})**		**Total carotid blood flow (ml.min^{-1})**	
		Before	*After*	*Before*	*After*	*Before*	*After*
Vehicle	5 ml	96±4	94±5	106±3	105±3	145±13	139±12
5-Methylurapidil	1000	102±4	95±7	103±5	116±6	149±12	152±15
Prazosin	100	92±5	77±7	112±6	108±5	147±11	128±13
Prazosin and Rauwolscine	100 and 300	105±4	93±5	104±6	100±6	125±12	104±14
GR127935	500	102±3	91±7	96±3	92±3	183±31	178±26
Ketanserin	500	105±5	98±5	94±4	89±5	134±10	129±7
Methiothepin	3000	103±4	108±4	105±6	105±6	134±10	120±10

No significant ($P<0.05$) differences were noticed in corresponding values either when compared to the vehicle-treated group or between those ‘Before’ and ‘After’ treatments.

vasopressor response was antagonised (even reverted to hypotension) after treatment with methiothepin (3000 μg.kg^{-1}), but remained by 5-methylurapidil (1000 μg.kg^{-1}), prazosin (100 μg.kg^{-1}), a combination of prazosin and rauwolscine (100 and 300 μg.kg^{-1}, respectively), GR127935 (500 μg.kg^{-1}) or ketanserin (500 μg.kg^{-1}).

The pressor responses following i.v. bolus injection of A61603 (1, 3, 10 and 30 μg.kg^{-1}) are shown in Figure 5.1 (lower panel); heart rate remained unaffected (data not shown). Treatment with 5-methylurapidil slightly attenuated these responses, which were markedly reduced by methiothepin; the other antagonists were ineffective.

5.3.3. Carotid haemodynamic responses to A61603

Absolute values of total carotid, arteriovenous anastomotic and capillary conductances before and after i.c. infusions of A61603 (0.3-10 μg.kg^{-1}) in the seven groups of animals are shown

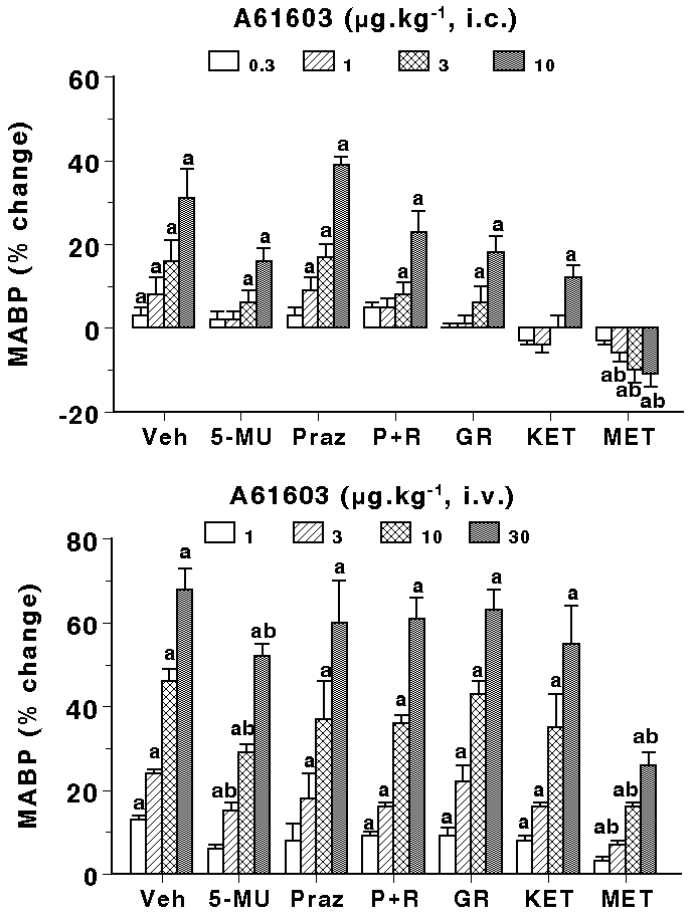


Figure 5.1. Changes in mean arterial blood pressure (MABP) following i.c. (upper panel) or i.v. (lower panel) administration of A61603 in anaesthetised pigs treated i.v. with either vehicle (Veh; 5 ml distilled water), 5-methylurapidil (5-MU; 1000 μg.kg^{-1}), prazosin (Praz; 100 μg.kg^{-1}), a combination of prazosin and rauwolscine (P+R; 100 and 300 μg.kg^{-1}, respectively), GR127935 (GR; 500 μg.kg^{-1}), ketanserin (KET; 500 μg.kg^{-1}) or methiothepin (MET; 3000 μg.kg^{-1}). Data are presented as mean ± S.E.M. a, P<0.05 vs. baseline; b, P<0.05 vs. the response produced by the corresponding dose of A61603 in the vehicle-treated group.

in Figure 5.2. In animals treated with vehicle, A61603 produced a dose-dependent decrease in total carotid conductance. This effect was restricted to the carotid arteriovenous anastomotic fraction, since the capillary fraction remained unmodified. The A61603-induced changes were clearly attenuated in animals treated with methiothepin, but not in those treated with prazosin, GR127935 or ketanserin. 5-Methylurapidil only attenuated the decrease in the total carotid blood flow by the highest dose of A61603. The treatment with prazosin and rauwolscine combination affected the A61603-induced decreases in the total carotid (highest two doses) and its carotid arteriovenous anastomotic fraction (highest dose).

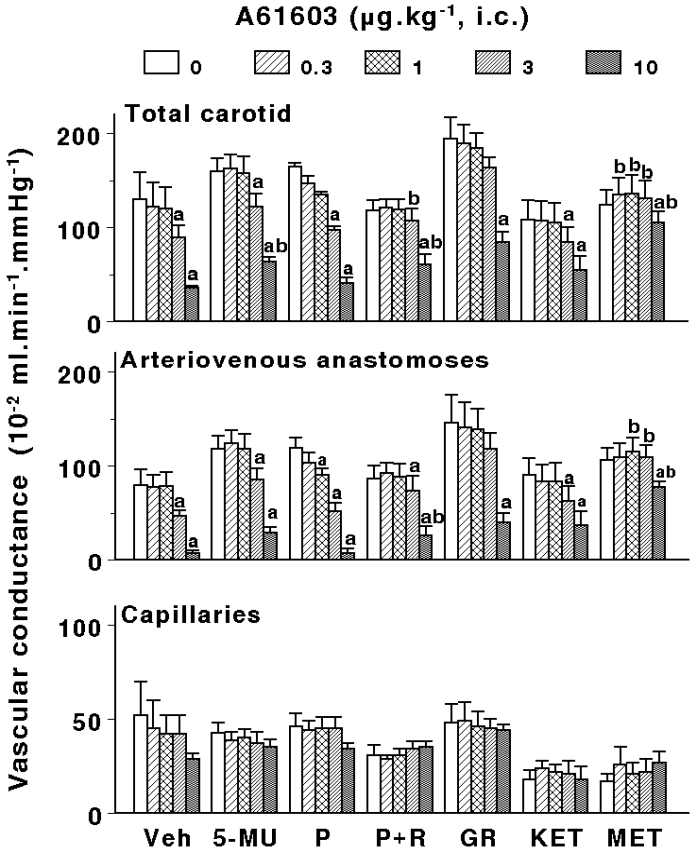


Figure 5.2. Total carotid, arteriovenous anastomotic and capillary vascular conductances before and after i.c. administration of A61603 in anaesthetised pigs treated i.v. with either vehicle (Veh; 5 ml distilled water), 5-methylurapidil (5-MU; 1000 µg.kg^{-1}), prazosin (Praz; 100 µg.kg^{-1}), a combination of prazosin and rauwolscine (P+R; 100 and 300 µg.kg^{-1}, respectively), GR127935 (GR; 500 µg.kg^{-1}), ketanserin (KET; 500 µg.kg^{-1}) or methiothepin (MET; 3000 µg.kg^{-1}). Data are presented as mean ± S.E.M. a, $P<0.05$ vs. baseline; b, $P<0.05$ vs. the response produced by the corresponding dose of A61603 in the vehicle-treated group.

Figure 5.3 compares decreases in carotid arteriovenous anastomotic blood flow by A61603 as percent changes from baseline values in control pigs (vehicle-treatment) and in pigs treated with the different antagonists. It can be observed that the responses to A61603 were clearly antagonised by methiothepin and only slightly by the combination of prazosin and rauwolscine; 5-methylurapidil, prazosin, GR127935 and ketanserin were ineffective.

5.4. Discussion

5.4.1. Consideration of known receptors that mediate carotid vasoconstriction

A number of studies have shown that sumatriptan produces vasoconstriction in the carotid vasculature of several species *via* GR127935-sensitive 5-HT$_{1B/1D}$ receptors; these species include the dog (13), pig (14) and rabbit (15, 16). It is now known that the receptor mediating vasoconstriction is of the 5-HT$_{1B}$ subtype (for references, see 17, 18). In line with this proposal, the therapeutic efficacy of sumatriptan in migraine can be explained by carotid vasoconstriction mediated by the 5-HT$_{1B}$ receptor. Furthermore, the canine external carotid vasoconstriction by ergotamine and dihydroergotamine involves 5-HT$_{1B/1D}$ receptors as well as α-adrenoceptors (19). Since the ergots display reasonable affinity at α-adrenoceptors (20), their carotid vasoconstrictor effects in the pig may also be explained by these receptors. In this respect, we recently showed that: (*i*) both α_1- and α_2-adrenoceptors mediate constriction of porcine carotid arteriovenous anastomoses (3); and (*ii*) these α_1-adrenoceptors belong to α_{1A} and α_{1B} subtypes, but not the α_{1D} subtype (4).

Except for A61603 (α_{1A}-adrenoceptor agonist), potent and selective agonists at α_1-adrenoceptor subtypes are unfortunately not available in order to verify this hypothesis.

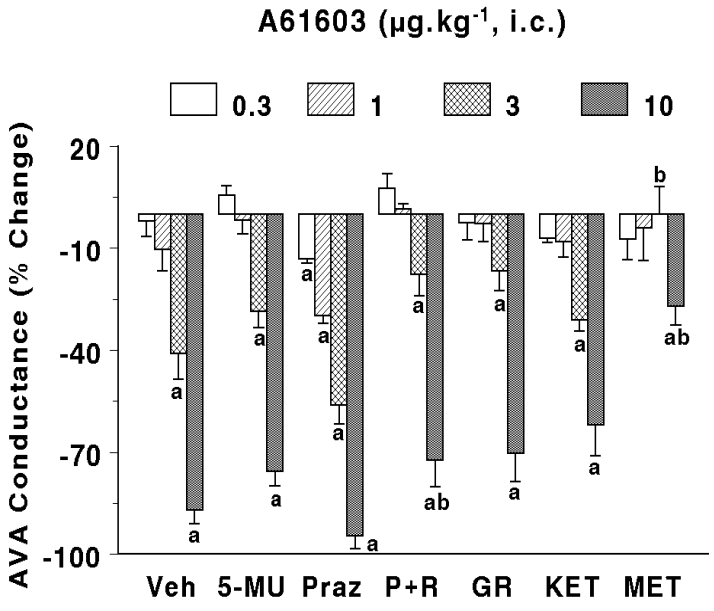


Figure 5.3. Changes in carotid arteriovenous anastomotic (AVA) conductance following i.c. administration of A61603 in anaesthetised pigs treated i.v. with either vehicle (Veh; 5 ml distilled water), 5-methylurapidil (5-MU; 1000 µg.kg^{-1}), prazosin (Praz; 100 µg.kg^{-1}), a combination of prazosin and rauwolscine (P+R; 100 and 300 µg.kg^{-1}, respectively), GR127935 (GR; 500 µg.kg^{-1}), ketanserin (KET; 500 µg.kg^{-1}) or methiothepin (MET; 3000 µg.kg^{-1}). Data are presented as mean ± S.E.M. a, P<0.05 vs. baseline; b, P<0.05 vs. the response produced by the corresponding dose of A61603 in the vehicle-treated group.

Therefore, in the present study we used A61603 to confirm the possible involvement of α_{1A}-adrenoceptors in the constriction of porcine carotid arteriovenous anastomoses.

5.4.2. Pharmacological profile of A61603

A61603 (Table 5.1) is a tetrahydro-1-napthyl imidazoline derivative that has been reported to show potent α_{1A}-adrenoceptor-agonist properties (5, 6). As described previously (5, 21), A61603 is 35-fold more potent at human cloned α_{1a}- than at α_{1b}- or α_{1d}-adrenoceptors in radioligand binding studies and 100 to 300-fold more potent than noradrenaline and phenylephrine in isolated canine prostate strips and rat vas deferens (α_{1A}-adrenoceptors). In contrast, A61603 is only 40-fold more potent than phenylephrine at α_{1B}-adrenoceptors (rat spleen) and 35-fold less potent at α_{1D}-adrenoceptors (rat aorta) (5, 21). Although the compound displays low affinity (pK_i<6) for other receptors, it has a reasonable affinity and agonist property at α_2-adrenoceptor subtypes (see Table 5.1; 22). In anaesthetised dogs, A61603 increases intra-urethral as well as diastolic arterial blood pressure (5). In agreement with the latter, A61603 produces pressor responses in conscious rats at 50- to 100-fold lower doses than those of phenylephrine, and tamsulosin (α_{1A}-adrenoceptor antagonist) causes a marked shift of the A61603-induced response curve (5).

5.4.3. Possible involvement of a novel mechanism?

A61603 produced a dose-dependent increase in blood pressure when administered by either i.c. (0.3-10 µg.kg^{-1}) or i.v. (1-30 µg.kg^{-1}) routes (Figure 5.1). In view of the high affinity of A61603 at the α_{1A}-adrenoceptor (Table 5.1) and the important role of α-adrenoceptors in the regulation of vascular tone (see review by 23), it was surprising that the hypertensive response to A61603 was little affected by 5-methylurapidil, prazosin or a combination of prazosin and rauwolscine. On the other hand, A61603-induced pressor response was markedly attenuated (i.v.) or even converted to hypotension (i.c.) by methiothepin.

Similar results were obtained with respect to the carotid haemodynamics. As shown in Figure 5.2, A61603 (0.3-10 µg.kg^{-1}, i.c.) produced a dose-dependent decrease in the porcine carotid blood flow, exclusively due to a constriction of carotid arteriovenous anastomoses.

This selective carotid vasoconstriction was apparently maximal because a higher dose of A61603 (30 $\mu g.kg^{-1}$) did not produce an additional decrease in total carotid conductance (maximal change: 80±4%; n=6).

The A61603-induced constriction of carotid arteriovenous anastomoses was, unexpectedly, resistant to blockade by the potent and selective α_{1A}-adrenoceptor antagonist 5-methylurapidil (24, 25). Since prazosin was also ineffective in attenuating this response, it seems plausible to conclude that α_1-adrenoceptors do not play an important role. As mentioned before, both α_1- and α_2-adrenoceptors can mediate vasoconstriction in the porcine carotid arterial bed (Willems et al., 1999). For this reason and for the fact that A61603 also has affinity for α_2-adrenoceptors (see Table 5.1, 22), we applied a combination of prazosin and rauwolscine to investigate the possible involvement of α_2-adrenoceptors. The combination of prazosin and rauwolscine produced only a slight attenuation in the A61603-induced constriction of carotid arteriovenous anastomoses, which implies, at most, a limited involvement of α_2-adrenoceptors. Similarly, the fact that GR127935 as well as ketanserin did not significantly modify this response excludes the possible involvement of 5-$HT_{1B/1D}$ and 5-HT_2 receptors, respectively. Although A61603 shows only a low affinity at these receptors (see Table 5.1; 22), the exclusion of 5-$HT_{1B/1D}$ receptors is of interest, considering the affinity of benzylimidazoline derivatives related in structure to A61603 at 5-$HT_{1B/1D}$ receptors (26). As reported elsewhere, the involvement of 5-HT_{1F} receptors in the carotid vasoconstriction of pigs and dogs (external) carotid vascular bed has been categorically excluded (14, 18). Moreover, an endothelium-dependent vasoconstriction *via* the release of pro-constrictor cyclo-oxygenase products (27, 28) also seems unlikely, based on the lack of effect of indomethacin (3000 $\mu g.kg^{-1}$, i.v.; data not shown) on A61603-induced decrease in total carotid conductance. Similarly, a combination of indomethacin, prazosin, rauwolscine, GR127935 and ketanserin, at the doses previously mentioned, also failed to attenuate the decreases in total carotid conductance produced by i.c. infusions of A61603 (data not shown).

Methiothepin displays high affinity at 5-$HT_{1/2}$ receptors (29) as well as $\alpha_{1/2}$ adrenoceptors (20). Therefore, we decided to test methiothepin against the A61603-induced constriction of arteriovenous anastomoses in this porcine model. It may be noted that a relatively high dose of methiothepin (3000 $\mu g.kg^{-1}$) was required to abolish sumatriptan-induced carotid vasoconstriction in anaesthetised dogs and pigs (8, 18, 30); while a lower dose (1000 $\mu g.kg^{-1}$) was ineffective. As shown in Figures. 5.2 and 5.3, treatment of the animals with methiothepin (3000 $\mu g.kg^{-1}$) markedly attenuated the A61603-induced vasoconstriction in the porcine carotid vascular bed. Since all currently known vasoconstrictor receptors/mechanisms ($\alpha_{1/2}$-adrenoceptors, 5-$HT_{1B/1D}$, 5-HT_2 and eicosanoid receptors) had already been excluded (see above), this latter finding implies the involvement of another, possibly novel mechanism in the constriction of carotid arteriovenous anastomoses by A61603. Because this *in vivo* animal model is predictive for antimigraine activity (12), this novel mechanism could be a potential new target for the development of antimigraine agents in the future. Admittedly, as an antimigraine drug, such an agonist must be devoid of systemic vasoconstrictor properties.

In conclusion, the present results show that A61603 does not behave as a potent and selective α_{1A}-adrenoceptor agonist in the pig and that the constriction of porcine carotid arteriovenous anastomoses by A61603 is primarily mediated by a novel non-adrenergic mechanism.

References

1. De Vries P, Heiligers JPC, Villalón CM, Saxena PR. Blockade of porcine carotid vascular response to sumatriptan by GR127935, a selective 5-HT_{1D} receptor antagonist. Br J Pharmacol 1996;118:85-92.
2. Saxena PR, Tfelt-Hansen P. Triptans, 5-$HT_{1B/1D}$ receptor agonists in the acute treatment of migraine. In: Olesen J, Tfelt-Hansen P, Welch KMA, eds. The Headaches. New York: Lippincott, Williams & Wilkins, 2000:411-438.
3. Willems EW, Trion M, De Vries P, Heiligers JPC, Villalón CM, Saxena PR. Pharmacological evidence that α_1- and α_2-adrenoceptors mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetized pigs. Br J Pharmacol 1999;127:1263-1271.
4. Willems EW, Heiligers JPC, De Vries P, Kapoor K, Tom B, Villalón CM, Saxena PR. α_1-adrenoceptor subtypes mediating vasoconstriction in the carotid circulation of anaesthetised pigs: possible avenues for antimigraine drug development. Cephalalgia 2000:in press.
5. Knepper SM, Buckner SA, Brune ME, DeBernardis JF, Meyer MD, Hancock AA. A-61603, a potent alpha$_1$-adrenergic receptor agonist, selective for the alpha$_{1A}$ receptor subtype. J Pharmacol Exp Ther 1995;274:97-103.
6. Docherty JR. Subtypes of functional alpha$_1$- and alpha$_2$-adrenoceptors. Eur J Pharmacol 1998;361:1-15.
7. Bom AH, Duncker DJ, Saxena PR, Verdouw PD. 5–Hydroxytryptamine-induced tachycardia in the pig: possible involvement of a new type of 5-hydroxytryptamine receptor. Br J Pharmacol 1988;93:663-671.
8. Villalón CM, Ramirez-San Juan E, Castillo C, Castillo E, López-Munoz FJ, Terrón JA. Pharmacological profile of the receptors that mediate external carotid vasoconstriction by 5-HT in vagosympathectomized dogs. Br J Pharmacol 1995;116:2778-2784.
9. Van Woerkens LJ, Duncker DJ, Huigen RJ, van der Giessen WJ, Verdouw PD. Redistribution of cardiac output caused by opening of arteriovenous anastomoses by a combination of azaperone and metomidate. Br J Anaesth 1990;65:393-399.
10. Den Boer MO, Van Woerkens LJ, Somers JA, Duncker DJ, Lachmann B, Saxena PR, Verdouw PD. On the preservation and regulation of vascular tone in arteriovenous anastomoses during anesthesia. J Appl Physiol 1993;75:782-789.
11. Saxena PR, Schamhardt HC, Forsyth RP, Hoeve J. Computer programs for the radioactive microsphere technique. Determination of regional blood flows and other haemodynamic variables in different experimental circumstances. Comput Programs Biomed 1980;12:63-84.
12. Saxena PR. Cranial arteriovenous shunting, an *in vivo* animal model for migraine. In: Olesen J, Moskowitz MA, eds. Experimental headache models. Vol. 27. Philadelphia, USA: Lippincott-Raven Publishers, 1995:189-198.
13. Villalón CM, Sánchez-López A, Centurión D. Operational characteristics of the 5-HT_1-like receptors mediating external carotid vasoconstriction in vagosympathectomized dogs. Close resemblance to the 5-HT_{1D} receptor subtype. Naunyn Schmiedebergs Arch Pharmacol 1996;354:550-556.
14. De Vries P, Villalón CM, Heiligers JPC, Saxena PR. Characterization of 5-HT receptors mediating constriction of porcine carotid arteriovenous anastomoses; involvement of 5-$HT_{1B/1D}$ and novel receptors. Br J Pharmacol 1998;123:1561-1570.

15. Choppin A, O'Connor SE. Influence of vascular tone on vasoconstrictor responses to the 5-HT_1-like receptor agonist sumatriptan in anaesthetised rabbits. Eur J Pharmacol 1996;304:87-92.
16. De Vries P, Apaydin S, Villalón CM, Heiligers JP, Saxena PR. Interactions of GR127935, a 5-$HT_{1B/D}$ receptor ligand, with functional 5-HT receptors. Naunyn-Schmiedeberg's Arch Pharmacol 1997;355:423-430.
17. De Vries P, Willems EW, Heiligers JPC, Villalón CM, Saxena PR. Investigations of the role of 5-HT_{1B} and 5-HT_{1D} receptors in the sumatriptan-induced constrction of porcine carotid arteriovenous anastomoses. Br J Pharmacol 1999;127:405-412.
18. Villalón CM, Centurión D, Sánchez-López A, De Vries P, Saxena PR. 5-HT receptors mediating external carotid vasoconstriction in vagosympathectomised dogs. Acta Pharmacol Sin 1999;20:1057-1067.
19. Villalón CM, De Vries P, Rabelo G, Centurión D, Sánchez-López A, Saxena PR. Canine external carotid vasoconstriction to methysergide, ergotamine and dihydroergotamine: a role of 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors. Br J Pharmacol 1999;126(3):385-394.
20. Leysen JE. Serotonergic binding sites. In: Vanhoutte PM, ed. Serotonin and the cardiovascular system. New York: Raven Press, 1985:43-62.
21. Meyer MD, Altenbach RJ, Hancock AA, Buckner SA, Knepper SM, Kerwin JF, Jr. Synthesis and in vitro characterization of N-[5-(4,5-dihydro-1H- imidazol-2-yl)-2-hydroxy-5,6,7,8- tetrahydronaphthalen-1- yl]methanesulfonamide and its enantiomers: a novel selective alpha 1A receptor agonist. J Med Chem 1996;39:4116-4119.
22. Craig DA, Forray CC, Gluchowski C, Branchek TA. Use of alpha1A-selective adrenoceptor agonists for the treatment of urinary incontinence. United States Patent (5,610,174). USA: Synaptic Pharmaceutical Corporation, Paramus, N.J., 1997
23. Vargas HM, Gorman AJ. Vascular alpha-1 adrenergic receptor subtypes in the regulation of arterial pressure. Life Sci 1995;57:2291-2308.
24. Hieble JP, Ruffolo RR, Jr. Subclassification and nomenclature of α_1- and α_2-adrenoceptors. Prog Drug Res 1996;47:81-130.
25. Zhong H, Minneman KP. $Alpha_1$-adrenoceptor subtypes. Eur J Pharmacol 1999;375:261-276.
26. Law H, Dukat M, Teitler M, Lee DK, Mazzocco L, Kamboj R, Rampersad V, Prisinzano T, Glennon RA. Benzylimidazolines as h5-$HT_{1B/1D}$ serotonin receptor ligands: a structure-affinity investigation. J Med Chem 1998;41:2243-2251.
27. Rosenblum WI, Nelson GH. Endothelium-dependent constriction demonstrated in vivo in mouse cerebral arterioles. Circ Res 1988;63:837-843.
28. Seager JM, Clark AH, Garland CJ. Endothelium-dependent contractile responses to 5-hydroxytryptamine in the rabbit basilar artery. Br J Pharmacol 1992;105:424-428.
29. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PPA. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol Rev 1994;46:157-203.
30. Den Boer MO, Villalón CM, Heiligers JP, Humphrey PP, Saxena PR. Role of 5-HT_1-like receptors in the reduction of porcine cranial arteriovenous anastomotic shunting by sumatriptan. Br J Pharmacol 1991;102:323-330.

CHAPTER 6

Assessment of antimigraine potential of a novel α-adrenoceptor agonist S19014: effects on porcine carotid and regional haemodynamics and human coronary artery

Based on: Kapoor K, Willems E W, MaassenVanDenBrink A, Heiligers JPC, Vayssettes-Courchay C, Verbeuren TJ, Cordi A, Villalón CM, Saxena PR. Assessment of antimigraine potential of a novel α-adrenoceptor agonist S19014: effects on porcine carotid and regional haemodynamics and human coronary artery. Cephalalgia 2003;In press.

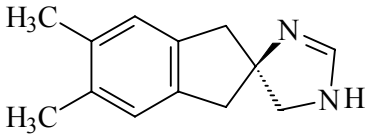
6. Assessment of antimigraine potential of a novel α-adrenoceptor agonist S19014: effects on porcine carotid and regional haemodynamics and human coronary artery

6.1. Introduction

Vasodilatation of cranial large arteries and arteriovenous anastomoses has been proposed to play an important role in the pathophysiology of migraine headache (1, 2). Indeed, to date all acutely acting antimigraine agents, i.e. the triptans and ergots, constrict isolated cranial vessels as well as arteriovenous anastomoses within the carotid vasculature (3, 4). While the vasoconstrictor effect of triptans seems to be mediated by the 5-HT_{1B} receptor, that of ergot alkaloids also involves other receptors (5, 6), including the α-adrenoceptors, which mediate the carotid vasoconstriction in anaesthetised dogs (7).

Stimulation of α-adrenoceptors produces contraction of the isolated carotid artery of several species, including the dog (8, 9), rabbit (10, 11) and pig (12). Also, *in vivo* studies have shown that sympathetic nerve stimulation as well as administration of the α-adrenoceptor agonists, phenylephrine and BHT 933, constrict carotid arteriovenous anastomoses (13) and there is evidence that α-adrenoceptors may regulate vascular tone of carotid arteriovenous anastomoses (14). Thus, it is possible that α-adrenoceptors may provide a target for the development of new antimigraine drugs (15).

Table 6.1. Chemical structure of S19014 and its binding affinities (pK_i) at human cloned α_1- and α_2-adrenoceptor subtypes.

Chemical structure	pK_i[a]	
	α_1-adrenoceptors	**α_2-adrenoceptors**
	α_{1a}: 7.66	α_{2a}: 8.98
	α_{1b}: 7.80	α_{2b}: 8.33
	α_{1d}: 7.65	α_{2c}: 8.75

[a], Unpublished data from the Institut de Recherches Internationales Servier, Courbevoie Cedex, France.

Cordi and his coworkers (16) described a series of compounds with α-adrenoceptor agonist activity and one such compound, S18148 ((5S)-spiro[(1,3-diazacyclopent-1-ene)-5:2'-(7'-methyl-1',2',3',4'-tetrahydronaphthalene)] fumarate) decreased carotid and cutaneous blood flows via activation of α_2-adrenoceptors (17). The follow-up compound, S19014 (spiro[(1,3-diazacyclopent-1-ene)-5:2'-(4',5'-dimethylindane)] fumarate), which exhibited a high affinity at both α_1- and α_2-adrenoceptor subtypes (Table 6.1), interestingly showed a wide variation in efficacy (maximum effect: E_{max}) and potency (concentration needed to elicit 50% of E_{max}: EC_{50}) in contracting rabbit, dog and human saphenous vein (EC_{50}: 18, 79 and 8500 nM, respectively; E_{max}: 92, 49 and 36% of K^+-induced contraction,

respectively), rabbit aorta (EC_{50}: 816 nM; E_{max}: 36% of K^+-induced contraction) and dog femoral artery (practically inactive) (18).

Over the years, the constriction of porcine carotid arteriovenous anastomoses has served as a predictive model for the antimigraine efficacy of triptans, which have an agonist action at the 5-HT_{1B} receptor (2, 3, 19). In addition, ergot alkaloids, which act via both 5-HT_{1B} receptor and α_2-adrenoceptors (5, 7) and isometheptene, which acts via α-adrenoceptors (20, 21), constrict carotid arteriovenous anastomoses. Therefore, the present study in anaesthetised pigs we set out to (i) investigate the effects of S19014 on the distribution of carotid blood flow into nutrient (capillary) and non-nutrient (arteriovenous anastomotic) fractions; and (ii) establish the cardiovascular safety of S19014 analysing its effects on cardiac output and its regional distribution to vital organs. The cardiovascular safety was also assessed in the human isolated coronary artery (22, 23).

6.2. Methods

6.2.1. Anaesthetised pigs

6.2.1.1. General

After an overnight fast, 39 domestic pigs (Yorkshire x Landrace; 10-14 kg) were sedated with intramuscular injections of azaperone (120 mg) and midazolam hydrochloride (10 mg) and then anaesthetised with sodium pentobarbital (600 mg, i.v.). After tracheal intubation, the animals were connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; pO_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of sodium pentobarbital (12-20 $mg.kg^{-1}.h^{-1}$). This anaesthetic regimen, together with bilateral vagosympathectomy (see below), increases heart rate and dilates carotid arteriovenous anastomoses. Thus, arteriovenous anastomotic blood flow is considerably high (70-80% of carotid blood flow) in these animals compared to pigs in a conscious state or under fentanyl/thiopental anaesthesia (~19% of carotid blood flow, 14), thereby producing one of the putative features of migraine (1, 2).

Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by electrocardiogram signals. A catheter was placed in the inferior vena cava via the right femoral vein for the administration of vehicle and S19014. Another catheter was placed in the aortic arch *via* the left femoral artery for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) and arterial blood withdrawal for the measurement of blood gases (ABL-510; Radiometer, Copenhagen, Denmark). During the experiment, body temperature was kept around 37°C and the animal was continuously infused with physiological saline to compensate for fluid losses.

6.2.1.2. Carotid blood flow experiments

The common carotid arteries, external jugular veins and vagosympathetic trunks were identified. The vagosympathetic trunks were cut between two ligatures to avoid reflex-mediated cardiovascular changes. Subsequently, the right common carotid artery was dissected free and a hub-less needle, connected to a polyethylene tube, was inserted against the direction of blood flow for the administration and uniform mixing of radioactive microspheres. Another catheter was placed in the right external jugular vein for the withdrawal of venous blood samples to determine blood gases and, subsequently, the difference between arterial and jugular venous oxygen saturations (A-V SO_2 difference).

Blood flow was measured in the right common carotid artery with a flow probe (internal diameter: 2.5 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands) and continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands).

The distribution of common carotid blood flow was determined with 15.5±0.1 μm (s.d.) diameter microspheres labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, USA). For each measurement, about 200,000 microspheres, labelled with one of the radioisotopes, were mixed and injected into the right common carotid artery. At the end of the experiment, the animal was killed by an overdose of sodium pentobarbital and the heart, lungs, kidneys and all ipsilateral cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 5 min in a γ-scintillation counter (Packard, Minaxi autogamma 5000), using suitable windows to discriminate the different isotopes (^{141}Ce: 120-167 KeV, ^{113}Sn: 355-435 KeV, ^{103}Ru: 450-548 KeV, ^{95}Nb: 706-829 KeV and ^{46}Sc: 830-965 KeV). All data were processed by a set of specially designed computer programs (24). The fraction of carotid blood flow distributed to the different tissues was calculated by multiplying the ratio of tissue and total radioactivity of each radioisotope by the total common carotid blood flow at the time of the injection of the microspheres labelled with the respective isotope. Since little or no radioactivity was detected in the heart and kidneys, all microspheres trapped in lungs reached this tissue from the venous side after escaping via carotid arteriovenous anastomoses. Therefore, the amount of radioactivity in the lungs was used as an *index* of the arteriovenous anastomotic fraction of the common carotid blood flow (24, 25). Vascular conductance was calculated by dividing blood flow (ml.min^{-1}) by mean arterial blood pressure (mmHg), multiplied by hundred.

6.2.1.3. Cardiac output experiments

A 6F Swan-Ganz thermodilution catheter (Braun Melsungen AG, Melsungen, Germany) was introduced into the pulmonary artery via the right femoral vein to measure cardiac output using a computerised cardiac output monitor (Erasmus MC, Rotterdam, The Netherlands). Another catheter, connected to a pressure transducer (Combitrans disposable pressure transducer; Braun, Melsungen, Germany), was guided via the left carotid artery into the left ventricle for the injection of radioactive microspheres. Lastly, a catheter was placed into the right femoral artery and connected to a Harvard pump for the withdrawal of reference blood samples during the injection of radioactive microspheres.

The distribution of cardiac output was determined using radioactive microspheres (see above). For each measurement about 1,000,000 microspheres, labelled with one of the isotopes, were injected into the left ventricle against the direction of blood flow over a 10 s period. Starting 10 s before microsphere injection and lasting 70 s, an arterial reference blood sample was withdrawn from the right femoral artery at a constant rate of 6 ml.min^{-1}. Blood loss during the experiment was compensated by infusing the corresponding volume of haemaccel. At the end of the experiments the animals were killed as described above and a number of tissues (lungs, kidneys, heart, stomach, small intestine, spleen, liver, adrenals, brain, skin and skeletal muscles) were dissected out, weighed and put into vials for counting radioactivity. As described by Saxena et al. (24), tissue blood flow was calculated by multiplying the ratio of tissue and reference blood sample radioactivity by the blood withdrawal rate (6 ml.min^{-1}) and normalised to 100 g tissue. Radioactivity in the lungs mainly represents the peripheral arteriovenous anastomotic blood flow (i.e. the non-nutrient part of the cardiac output), although a small amount (1-1.5% of cardiac output) is derived from the bronchial arteries (26). Vascular conductance was calculated by dividing blood flow (ml.min^{-1}.100 g^{-1}) or cardiac output (ml.min^{-1}) by mean arterial blood pressure (mmHg), multiplied by hundred. Stroke volume (cardiac output divided by heart rate) and nutrient

cardiac output (cardiac output minus lung blood flow, i.e. mainly total arteriovenous shunting) were also calculated.

6.2.1.4. Experimental protocols

After a stabilisation period of about 1 h, the animals were divided into two groups used for either carotid blood flow (n=27) or cardiac output (n=12) experiments.

This first group (carotid blood flow experiments) was divided into four subgroups. Whereas the first and second subgroups (n=6, each) remained untreated, the animals in the third (n=8) and fourth subgroups (n=7) received i.v. infusions (rate: 0.5 $ml.min^{-1}$ for 10 min) of prazosin (100 $\mu g.kg^{-1}$) or rauwolscine (300 $\mu g.kg^{-1}$) to block α_1- and α_2-adrenoceptors, respectively (13). Fifteen min later, baseline values of blood pressure, heart rate and total carotid blood flow were collated and the distribution of carotid blood flow into arteriovenous anastomotic and capillary fractions and A-V SO_2 difference were determined. Subsequently, the animals in the first subgroup received four consecutive i.v. infusions (1 $ml.min^{-1}$ for 3 min plus 2 min flush) of distilled water (vehicle), whereas those in the second (control), third (prazosin) and fourth (rauwolscine) subgroups received intravenous infusions of S19014 (cumulative doses: 1, 3, 10 and 30 $\mu g.kg^{-1}$) over a period of 3 min (1 $ml.min^{-1}$) plus 2 min flush. Systemic and carotid haemodynamic variables were reassessed 10 min after each administration of vehicle (first subgroup) or S19014 dose (other three subgroups).

The second group (cardiac output experiments) was divided into two subgroups (n=6, each). Whereas the first subgroup was treated with four consecutive infusions of 5 ml of distilled water (vehicle), the second subgroup received four cumulative doses of S19014 (1, 3, 10 and 30 $\mu g.kg^{-1}$) at the rate of 1 $ml.min^{-1}$ for 3 min plus 2 min of flushing. Systemic and regional haemodynamic variables were reassessed 10 min after every administration of vehicle or S19014 dose.

6.2.2. Human isolated coronary artery

6.2.2.1. Tissue preparation

The right epicardial coronary artery was obtained from eight heart-beating organ donors who died of noncardiac disorders less than 24 h before the tissue was taken to the laboratory (7 cerebrovascular accident, 1 hydrocephalus; 4 males, 4 females; age: 24-57 years). The hearts were provided by the Rotterdam Heart Valve Bank after donor mediation by Bio Implant Services Foundation / Eurotransplant Foundation (Leiden, The Netherlands) after removal of the aortic and pulmonary valves for homograft valve transplantation. The hearts were stored at 0 to 4°C in a sterile organ protecting solution (UW, EuroCollins, or HTK-Bretschneider) immediately following circulatory arrest. After arrival in the laboratory, the right coronary artery was removed and placed in a cold, oxygenated Krebs buffer solution of the following composition: 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$ and 8.3 mM glucose; pH 7.4.

Ring segments of approximately 3-4 mm length were prepared from the blood vessels, excluding macroscopically visible atherosclerotic lesions. The segments were suspended on stainless steel hooks in 15-ml organ baths containing Krebs buffer solution, aerated with 95% O_2 and 5% CO_2 and maintained at 37°C. After equilibration for at least 30 min and wash every 15 min, isometric tension was measured with a force transducer (Harvard, South Natick, MA, USA) and recorded on a flatbed recorder (Servogor 124, Goerz, Neudorf, Austria). The rings were stretched to a stable pre-tension of about 15 mN.

6.2.2.2. Experimental protocol

Segments were exposed to K^+ (30 mM) twice. After pre-contraction with prostaglandin $F_{2\alpha}$ (1 μM), the functional integrity of the endothelium was verified by observing relaxation to

substance P (1 nM). Following wash, the tissue was exposed to K^+ (100 mM) to determine the maximal contractile response to K^+. After a 30-min incubation period, concentration response curves to S19014 or sumatriptan (both from 1 nM to 100 μM) were constructed in a paired, parallel set-up (22). The contractions are expressed as a percentage of contraction to 100 mM K^+ to correct for differences in wall thickness or segment length between individual artery segments. In four out of eight experiments, concentration response curves to S19014 and sumatriptan were also constructed in the presence of the thromboxane A_2 analogue U46619 (9,11–dideoxy-11α,9α-epoxy-methano-prostaglandin $F_{2\alpha}$) in a concentration (30-100 nM) eliciting about 10% of the K^+-induced contraction. The contraction induced by U46619 was similar for the blood vessel segments that were used for concentration response curves to S19014 (10±2% of the contraction to K^+) or sumatriptan (10±5%).

6.2.3. Statistical analysis and data presentation

The significance of the difference between the haemodynamic variables within one group was evaluated with Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations (27). Percent changes (from baseline values) caused by S19014 (1, 3, 10 and 30 $\mu g.kg^{-1}$) in the animals treated with either prazosin or rauwolscine were compared with the corresponding doses in the control group using Student's unpaired t-test.

The concentration response curves obtained with S19014 and sumatriptan in the coronary artery rings were analysed using the GraphPad software (GraphPad software Inc., San Diego, CA, USA) to determine pEC_{50} values. In case that a concentration response curve did not reach a plateau, the contraction in response to the highest concentration was considered as E_{max}. E_{max} and pEC_{50} values of S19014 and sumatriptan were compared by paired t-test. Correlation coefficients were calculated according to Pearson (27). Experiments in the presence of U46619 were compared with paired experiments in the absence of U46619.

All data are presented as mean±SEM and differences were assumed to be significant when $P<0.05$.

6.2.4. Compounds

The following compounds were used: azaperone (Stresnil®; Janssen Pharmaceuticals, Beerse, Belgium), haemaccel® (Hoechst Marion Roussel b.v., Hoevelaken, The Netherlands), heparin sodium (to prevent blood clotting in catheters; Leo Pharmaceutical Products, Weesp, The Netherlands), midazolam hydrochloride (Dormicum®; Hoffmann La Roche b.v., Mijdrecht, The Netherlands), phenylephrine hydrochloride (Sigma-Aldrich Chemie b.v., Zwijndrecht, The Netherlands), prazosin hydrochloride (Bufa Chemie b.v., Castricum, The Netherlands), Prostaglandin $F_{2\alpha}$ tris salt (Sigma-Aldrich Chemie), rauwolscine dihydrochloride (RBI, Natick, USA), S19014 (Institut de Recherches Internationales Servier, Courbevoie Cedex, France), sodium pentobarbital (Sanofi Sante b.v., Maasluis, The Netherlands), substance P acetate (Sigma-Aldrich Chemie), sumatriptan hemisuccinate (Institut de Recherches Internationales Servier), and U46619 (Sigma-Aldrich Chemie).

Except U46619, all drugs were dissolved in distilled water (vehicle); however a short period of heating was needed to dissolve prazosin. U46619 was dissolved in ethanol and further diluted in distilled water. Solutions of S19014 and sumatriptan were freshly prepared for every experiment. The doses of the drugs refer to their respective salts.

6.2.5. Ethical approval

The local Ethics Committees dealing with the use of animals and humans in scientific experiments approved the protocol.

6.3. Results

6.3.1. Carotid blood flow distribution in anaesthetised pigs

6.3.1.1. Baseline values

Baseline values in the 27 anaesthetised pigs used for this protocol were: heart rate (104±2 beats.min^{-1}), mean arterial blood pressure (97±2 mmHg), total carotid blood flow (138±6 ml.min^{-1}) and total carotid vascular conductance (144±6 10^{-2} ml.min^{-1}mmHg^{-1}).

6.3.1.2. Effects of antagonists

Table 6.2 shows the values of systemic and carotid haemodynamics as well as the A-V SO_2 difference before and after pre-treatment with vehicle (control animals), prazosin (100 µg.kg^{-1}) or rauwolscine (300 µg.kg^{-1}). Whereas vehicle and prazosin did not produce any changes, rauwolscine elicited only a small, but significant, decrease in mean arterial blood pressure (9±2%).

Table 6.2 Absolute values of heart rate, mean arterial blood pressure and total carotid blood flow in anaesthetised pigs, before and after intravenous infusions of vehicle (control), prazosin or rauwolscine

Haemodynamic variables	**Treatment groups**					
	Vehicle (Control) (5 ml, n=6)		**Prazosin (100 µg.kg^{-1}, n=8)**		**Rauwolscine (300 µg.kg^{-1}, n=7)**	
	Before	*After*	*Before*	*After*	*Before*	*After*
Heart rate (beats.min^{-1})	102±2	101±3	114±3	112±3	98±4	99±5
MABP (mmHg)	97±3	95±3	98±3	91±4	108±2	99±2*
Total CBF (ml.min^{-1})	140±13	142±13	140±14	126±12	154±7	136±6
A-V SO_2 difference (%)	9±2	8±2	8±2	10±2	6±3	6±2

MABP, mean arterial blood pressure; Total CBF, total carotid artery blood flow; A-V SO_2 difference, difference between arterial and jugular venous oxygen saturations. *, P<0.05 vs. before treatment.

6.3.1.3. Systemic haemodynamics and A-V SO_2 difference

In control animals, S19014 (1, 3, 10 and 30 µg.kg^{-1}, i.v.) produced dose-dependent initial increases in mean arterial blood pressure that lasted for about 3 min (Figure 6.1). Prazosin neither affected the magnitude nor the duration of these pressor responses. On the other hand, in rauwolscine-treated animals mean arterial blood pressure increased only with the highest dose of S19014; the lowest two doses of S19014 caused a hypotension that lasted 10-15 min.

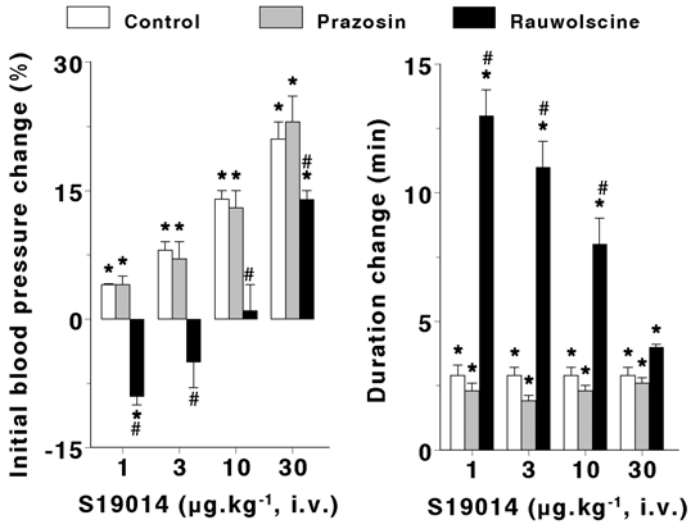


Figure 6.1. Magnitude (as percent change from baseline values; *left panel*) and duration (as min; *right panel*) of the initial change in mean arterial blood pressure following administration of S19014 (1, 3, 10 and 30 µg.kg^{-1}, i.v.) in control pigs (n=6) and in pigs treated with prazosin (n=8) or rauwolscine (n=7). All values are expressed as mean±s.e.mean. *, P<0.05 vs. baseline. #, P<0.05 vs. the corresponding response with S19014 in control animals.

Table 6.3 presents the values of heart rate, mean arterial blood pressure and A-V SO_2 differences measured before (baseline) and 15-min after vehicle (4 times 5 ml each) or S19014 (1, 3, 10 and 30 µg.kg^{-1}). In both vehicle and S19014 treated animals, similar small decreases in heart rate (maximum change: 4±1% and 3±1%, respectively) and mean arterial blood pressure (maximum change: 7±2% and 5±2%, respectively) were noticed. These small changes were probably time-related. However, S19014 caused a significant, but moderate, increase in the A-V SO_2 difference after the two highest doses of S19014 and this effect was attenuated by rauwolscine but not prazosin.

Table 6.3 Systemic haemodynamic effects of vehicle (n=6) per se and S19014 in the absence (n=6; control) or presence of either prazosin (100 µg.kg^{-1}; n=8) or rauwolscine (300 µg.kg^{-1}; n=7)

Treatment groups	**Vehicle (four times 5 ml, i.v.) or S19014 (µg.kg^{-1}, i.v.)**				
	Baseline	**1**	**3**	**10**	**30**
Heart rate (beats.min^{-1})					
Vehicle†	102±2	101±3	99±3*	98±3*	98±4*
Control	102±4	102±4	101±4*	100±4*	100±4*
Prazosin	112±3	110±3	110±3	109±3	108±2
Rauwolscine	99±5	98±5	98±5	98±6	98±6
Mean arterial blood pressure (mmHg)					
Vehicle†	97±3	95±3	96±3	95±3	90±3*
Control	101±3	99±3	96±4*	96±4*	96±3*
Prazosin	91±4	88±4	86±4*	84±4*	83±3*
Rauwolscine	98±2	94±2	93±3	93±3	91±3
A-V SO_2 difference (%)					
Vehicle†	9±2	8±2	8±2	8±2	10±3
Control	7±2	8±2	8±2	9±3*	10±2*
Prazosin	10±2	11±3	13±2*	12±2*	15±2*
Rauwolscine	6±2	6±2	6±1	7±2	8±2*#

A-V SO_2 difference, difference between arterial and jugular venous oxygen saturations. *, P<0.05 vs. baseline. #, P<0.05 vs. the corresponding change with S19014 in control animals. †, Four infusions of 5 ml distilled water were given after baseline measurements.

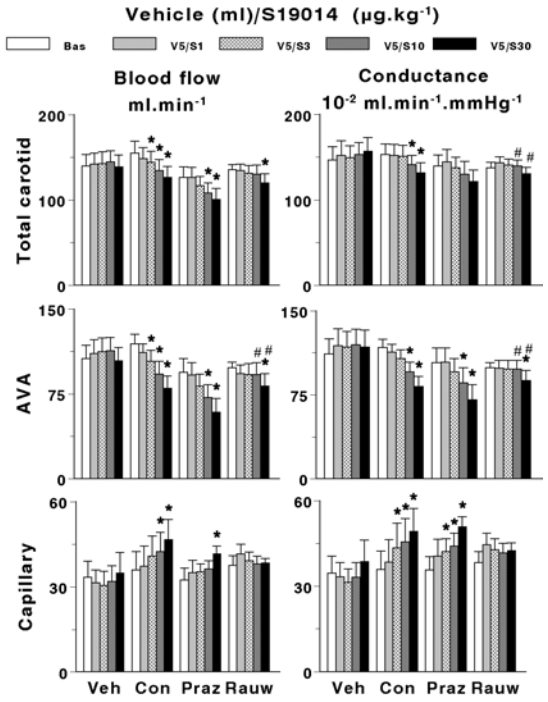


Figure 6.2. Total carotid, arteriovenous anastomotic (AVA) and capillary blood flows (*left panels*) and vascular conductances (*right panels*) measured in groups of pigs receiving either vehicle (Veh; n=6) or S19014; the latter were either untreated controls (Con; n=6) or treated with prazosin (Praz; 100 µg.kg^{-1}; n=8) or rauwolscine (Rauw; 300 µg.kg^{-1}; n=7). The measurements were made sequentially at baseline (Bas) and 15-min following infusions of the vehicle (four times each 5 ml, i.v., V5) or S19014 (1, 3, 10 and 30 µg.kg^{-1}, i.v. as indicated by S1, S3, S10 and S30, respectively). All values are expressed as mean±s.e.mean. *, P<0.05 vs. baseline; #, P<0.05 vs. the corresponding response with S19014 in control animals.

6.3.1.4. Carotid haemodynamic effects of S19014

Absolute values of total carotid, arteriovenous anastomotic and capillary blood flows and conductances in the different groups of animals are shown in Figure 6.2. Whereas vehicle was devoid of any carotid haemodynamic effects, S19014 (1, 3, 10 and 30 µg.kg^{-1}) produced dose-dependent decreases in total carotid and arteriovenous anastomotic blood flows (maximum change: 18±2% and 34±5%, respectively) and conductances (maximum change: 14±2% and 30±4%, respectively). In contrast, S19014 increased capillary blood flow and conductance (maximum change: 40±23% and 49±27%, respectively). These effects of S19014 remained largely unchanged in animals treated with prazosin, but were clearly attenuated by rauwolscine.

Figure 6.3 depicts the percent changes (from baseline values) in carotid arteriovenous anastomotic conductance by S19014 (1, 3, 10 and 30 µg.kg^{-1}) in control, and prazosin

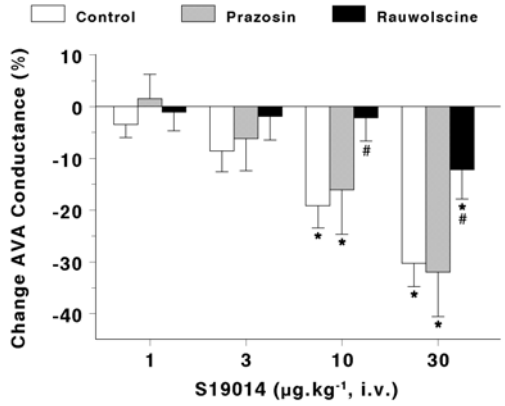


Figure 6.3. Percent changes (compared to baseline value) in porcine arteriovenous anastomotic (AVA) conductance 15-min following administration of S19014 (1, 3, 10 and 30 µg.kg^{-1}, i.v.) in control pigs (n=6) and in pigs treated with prazosin (n=8) or rauwolscine (n=7). All values are expressed as mean±s.e.mean. *, P<0.05 vs. baseline. #, P<0.05 vs. the corresponding response with S19014 in control animals.

(100 µg.kg^{-1})- or rauwolscine (300 µg.kg^{-1})-treated animals. While prazosin did not modify the constrictor effect of S19014 on arteriovenous anastomoses, rauwolscine clearly did.

The changes caused by vehicle (four doses) and S19014 (1, 3, 10 and 30 µg.kg^{-1}) in vascular conductance in the different cranial tissues are depicted in Figure 6.4. Whereas

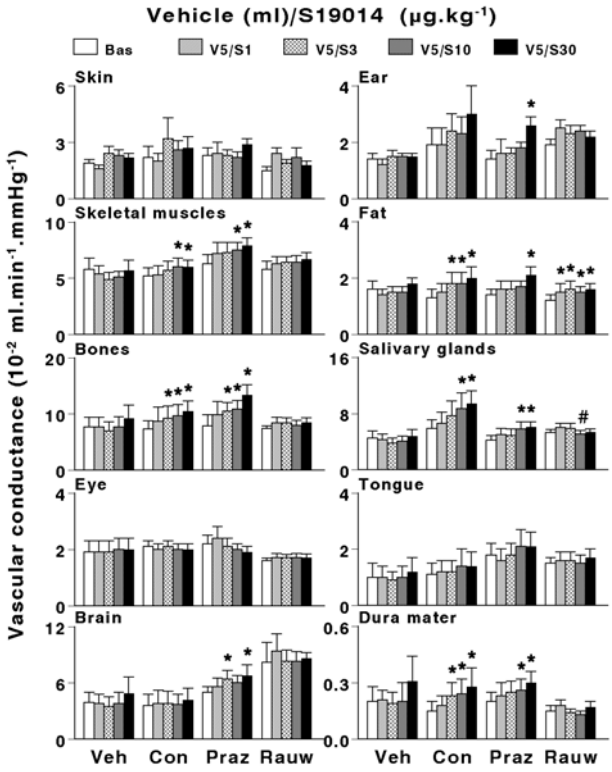


Figure 6.4. Carotid regional vascular conductances measured in groups of pigs receiving either vehicle (Veh; n=6) or S19014; the latter were either untreated controls (Con; n=6) or treated with prazosin (Praz; 100 µg.kg^{-1}; n=8) or rauwolscine (Rauw; 300 µg.kg^{-1}; n=7). The measurements were made sequentially at baseline (Bas) and 15-min following infusions of vehicle (four times each 5 ml, i.v., V5) or S19014 (1, 3, 10 and 30 µg.kg^{-1}, i.v. as indicated by S1, S3, S10 and S30, respectively). All values are expressed as mean±s.e.mean. *, P<0.05 vs. baseline; #, P<0.05 vs. the corresponding response with S19014 in control animals.

administration of the vehicle did not elicit any changes, S19014 moderately increased the vascular conductance in several tissues, including the skeletal muscle, bone, fat, salivary gland and dura mater, while those in the others (skin, eye, brain, ear or tongue) remained unchanged. These effects of S19014 on tissue vascular conductance were similar in animals treated with prazosin, but relatively less marked in animals treated with rauwolscine, suggesting that the vasodilator effects of S19014 may be partly passive following constriction of arteriovenous anastomoses.

6.3.2. Distribution of cardiac output

6.3.2.1. Baseline values

Baseline values in the 12 anaesthetised pigs used for this protocol before any treatment were: heart rate (112±3 beats.min^{-1}), mean arterial blood pressure (105±1 mmHg), cardiac output (1588±47 ml.min^{-1}), nutrient cardiac output (1354±49 ml.min^{-1}), stroke volume (14.3±0.5 ml) and systemic vascular conductance (1511±44 10^{-2} ml.min^{-1}mmHg^{-1}).

6.3.2.2. Systemic haemodynamics

The values of systemic haemodynamic variables in anaesthetised pigs collated at baseline and after i.v. treatments with vehicle or S19014 (1, 3, 10 and 30 µg.kg^{-1}) are presented in Figure 6.5. Mean arterial blood pressure did not change, but there were small decreases in other variables in both vehicle- and S19014-treated groups. The maximum changes, which did not significantly differ in the two group were, respectively: heart rate, 4±1% and 4±2%; cardiac output, 14±3% and 14±2%; stroke volume, 10±3% and 10±3%; and systemic vascular conductance, 10±2% and 11±3%. A small decrease in the nutrient cardiac output (maximal change: 14±3 %) was also observed in the vehicle subgroup.

6.3.2.3. Regional haemodynamics

As depicted in Figure 6.6, there were no significant changes in regional vascular conductances in the vehicle group other than a decrease in vascular conductance in the

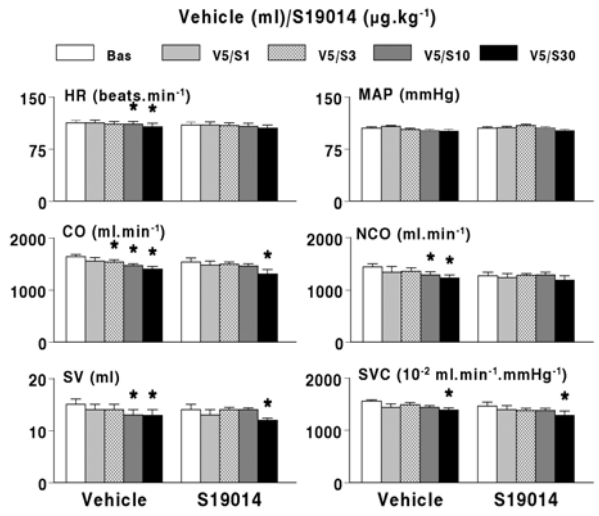


Figure 6.5. Systemic haemodynamic values of heart rate (HR), mean arterial pressure (MAP), cardiac output (CO), nutrient cardiac output (NCO), stroke volume (SV) and systemic vascular conductance (SVC) measured in groups of pigs receiving either vehicle (n=6) or S19014 (n=6). The measurements were made sequentially at baseline (Bas) and 15-min following infusions of vehicle (four times each 5 ml, i.v., V5) or S19014 (1, 3, 10 and 30 µg.kg^{-1}, i.v. as indicated by S1, S3, S10 and S30, respectively). All values are expressed as mean±s.e.mean. *, P<0.05 vs. baseline; #, P<0.05 vs. the corresponding response with S19014 in control animals (none were significant).

adrenals (maximal change: -30±11%), and increases in the brain with the highest dose (25±9%) and skin with the last two doses (maximal change: 54±16%); this latter effect was also observed in the animals receiving S19014. In addition, S19014 decreased vascular conductances in lungs (mainly peripheral arteriovenous anastomoses; maximal change: 50±5%), skeletal muscles (maximal change: -16±4%) and liver (maximal change: 50±5%).

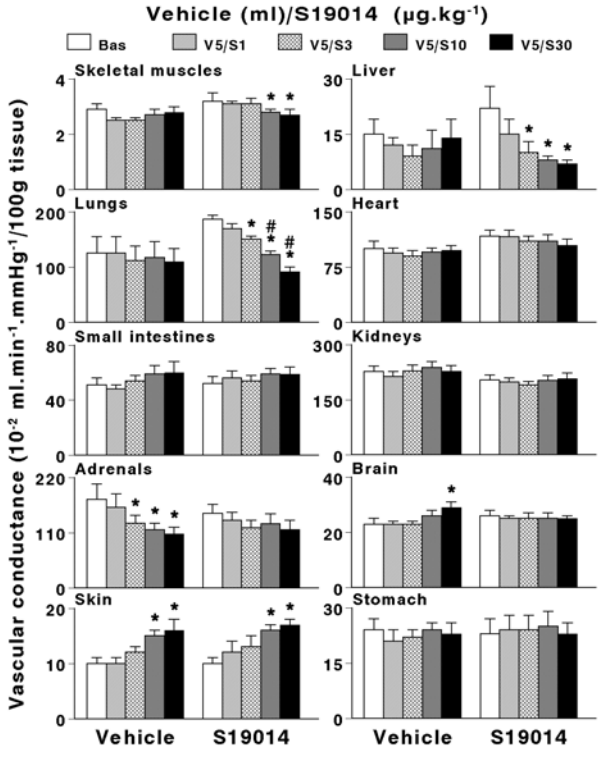


Figure 6.6. Regional vascular conductances measured in groups of pigs receiving either vehicle (n=6) or S19014 (n=6). The measurements were made sequentially at baseline (Bas) and 15-min following infusions of vehicle (four times each 5 ml, i.v., V5) or S19014 (1, 3, 10 and 30 µg.kg^{-1}, i.v. as indicated by S1, S3, S10 and S30, respectively). All values are expressed as mean±s.e.mean. *, P<0.05 vs. baseline; #, P<0.05 vs. the corresponding response with S19014 in control animals.

Except in the lungs, the changes with S19014 were not significantly different from those in the vehicle group.

6.3.3. Human isolated coronary artery

6.3.3.1. Basic contractile properties

The coronary artery contraction to 100 mM K^+ was 52±6 mN. Relaxation to substance P (1 nM) was 40±10% of the precontraction (30±5 mN) induced by 1 µM prostaglandin $F_{2\alpha}$ (*n*=8).

6.3.3.2. Contractile responses to S19014 and sumatriptan

Both S19014 and sumatriptan induced a concentration-dependent contraction of the human isolated coronary artery (Figure 6.7, left panel). Whereas the maximal contraction to S19014 was considerable smaller than that to sumatriptan (3±1% and 19±10% of the contraction to K^+, respectively), the difference was not statistically significant (p=0.13). This lack of

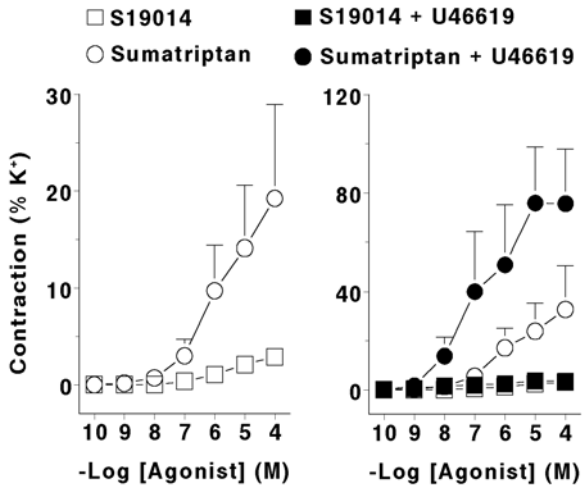


Figure 6.7. Human isolated coronary artery contractions to sumatriptan (circles) and S19014 (squares), expressed as % of the response to 100 mM K^+, observed in the absence (open symbols) or presence of the thromboxane A_2 analogue U46619 (30-100 nM; closed symbols). The two panels (*left,* n=8; *right*, n=4) present data obtained in vessel segments studied in parallel. Data are mean±SEM.

significance may be explained by the large variability of contraction to sumatriptan (E_{max}: 3-77% of contraction to K^+), which is in accordance with our previous findings (28). The pEC_{50} values for S19014 (5.55±0.24) and sumatriptan (5.99±0.14 respectively) were similar. The E_{max} of S19014 and sumatriptan did not correlate to the endothelial quality of the blood vessel segments as assessed with the relaxation to 1 nM substance P after precontraction with 1 μM prostaglandin $F_{2\alpha}$ (Pearson r_S: -0.243 and 0.364, respectively, $p>0.05$).

As depicted in Figure 6.7 (right panel), the contractions to S19014 were not different in the presence of U46619 (E_{max}: 4±1% of the contraction to K^+: pEC_{50} 6.55±0.77) compared to that in quiescent blood vessel segments (E_{max}: 3±1%, pEC_{50}: 5.34±0.24). In contrast, the E_{max} of sumatriptan was significantly augmented in the presence of U46619 (76±22% vs. 33±1% of the contraction to K^+, $p<0.05$), while the pEC_{50} remained unaffected (6.77±0.49 vs. 6.14±0.23). In the presence of U46619, the maximal contraction to S19014 was significantly lower than that to sumatriptan ($p<0.05$).

6.4. Discussion

It is generally agreed that both α_1- and α_2-adrenoceptors play an important role in the regulation of the vascular resistance and blood pressure (29-33). Recently, we showed that both these receptors mediate canine (external) and porcine (arteriovenous anastomotic) carotid vasoconstriction (13, 34, 35). In this context, several lines of evidence demonstrate that vasoconstriction in the carotid vascular bed is predictive for antimigraine activity (2, 3). Therefore, the present study was designed to assess the antimigraine potential of the novel α-adrenoceptor agonist S19104 in anaesthetised pigs and human isolated coronary artery, as previously described (22, 36, 37).

6.4.1. Systemic haemodynamics

Other than a moderate initial pressor effect, intravenous administrations of S19014 did not cause significant changes in systemic haemodynamics. This initial pressor effect of S19014 was short-lasting (~3 min) and, being amenable to blockade by rauwolscine but not prazosin, involves α_2-adrenoceptors (see Figure 6.1). Although pressor responses can be elicited via both α_1- and α_2-adrenoceptor subtypes (33), the involvement of α_2-adrenoceptors in the action of S19014 is in accordance with its higher affinity at α_2-adrenoceptor (pK_i: 8.33-8.98) than at α_1-adrenoceptors (pK_i: 7.65-7.80) subtypes (Table 6.1). Further, it may be pointed out that in our previous experiments (13) no pressor changes were observed with either

phenylephrine (α_1-adrenoceptor agonist) or BHT933 (α_2-adrenoceptor agonist) in anaesthetised pigs. One of the reasons for this apparent discrepancy may be that in these experiments phenylephrine and BHT933 were slowly infused into the carotid artery (13), while we injected S19014 i.v.

6.4.2. Carotid haemodynamics

As reported previously (13), i.v. administration of vehicle, prazosin (100 $\mu g.kg^{-1}$) and rauwolscine (300 $\mu g.kg^{-1}$) did not produce major carotid haemodynamic changes (Table 6.2). On the other hand, S19014 produced a dose-dependent constriction within the porcine carotid vasculature, which was confined to arteriovenous anastomoses; the vascular conductance in the capillary fraction was increased. In accordance with the constriction of carotid arteriovenous anastomoses (1, 2), S19014 increased A-V SO_2 difference. Both effects of S19014 were antagonised by rauwolscine and not at all by prazosin, thus establishing the involvement of one or more α_2-adrenoceptor subtypes. As mentioned above, the involvement of α_2-adrenoceptors in the action of S19014 is in accordance with its higher affinity at α_2-adrenoceptor (pK_i: 8.33-8.98) than at α_1-adrenoceptors (pK_i: 7.65-7.80) subtypes (Table 6.1) and it is possible that S19014 lacks efficacy, being a partial agonist or antagonist action at α_1-adrenoceptors. Interestingly, it has been reported that the venoconstrictor responses to S19014 are variable in potency and efficacy (18), suggesting that the tissue distribution of α-adrenoceptor (most likely α_2-adrenoceptor) subtypes at which S19014 is efficacious may be uneven.

6.4.3. Cardiac output and regional haemodynamics

Acutely acting antimigraine drugs (triptans and ergot alkaloids) have been shown to decrease cardiac output and systemic vascular conductance in anaesthetised pigs (38). In the present study with S19014, no changes in cardiac output, systemic vascular conductance and vascular conductances in many body organs, including the heart, kidneys, intestines, stomach, adrenals, were observed when compared with the vehicle subgroup. Admittedly, S19014 produced a decrease in the vascular conductance of the liver, but it is difficult to predict whether this decrease in the hepatic blood flow would be important in the clinical setting. Interestingly, a transient decrease in hepatic blood flow has also been observed in humans after i.v. ergotamine, unlike its prolonged constrictor property on large arteries (39).

As found in the carotid artery experiments, S19014 decreased the 'lung' blood flow and vascular conductance. This is due to constriction of arteriovenous anastomoses, because the contribution via the bronchial artery to the 'lung' blood flow is rather limited (26, 40). Indeed, the nutrient part of cardiac output remained unchanged.

6.4.4. Human coronary artery contraction

To further predict the cardiovascular safety of S19014, we decided to analyse its effects on the human isolated coronary artery as compared to those to sumatriptan. Thus, in quiescent blood vessel segments, i.e. in the absence of U46619, the coronary artery contraction to S19014 and sumatriptan was not significantly different, although contraction to S19014 tended to be less than that to sumatriptan. In the presence of U46619, the contraction to sumatriptan was significantly augmented, while that to S19014 remained unaffected.

The pEC_{50} value of S19014 in the human coronary artery (5.55) was found to be 100 to 1000-fold lower than its pK_i values at either α_1- or α_2-adrenoceptor subtypes (Table 6.1). However, it must be emphasised that agonist pK_i values do not necessarily correlate to the potency of a compound in inducing a functional response (41). Secondly, the pEC_{50} values may have been overestimated because the contraction observed at the highest concentration

of the agonists was considered as E_{max} when, in some cases, a plateau had not been reached. However, when we calculated the pEC_{50} values with the curves extrapolated to estimate E_{max} (sumatriptan, 5.65±0.35; S19014, 5.66±0.44), these did not differ significantly from those reported in the Results section (5.99±0.14, and 5.55±0.24; p=0.19 and 0.53, respectively, paired t-test). Finally, the fact that the contraction to S19014 was very low in the coronary artery may have possibly affected the accuracy of estimation of the pEC_{50} values. Although further experiments with subtype selective agonists and antagonists might elucidate which α-adrenoceptor subtype(s) mediates the S19014-induced contraction of the human coronary artery, such experiments would be difficult to interpret because of the small contractions elicited by S19014.

In the present study, the E_{max} of contraction to S19014 or sumatriptan was not related to the endothelial quality of the blood vessel segments. This suggests that the contraction to S19014 and sumatriptan might not solely depend on blood vessel wall characteristics, such as the presence of atherosclerosis. However, our study sample was fairly small (n=8) for such an analysis. Moreover, we only included blood vessel segments without macroscopically visible atherosclerotic lesions in our study. Indeed, we have previously demonstrated in a large post-hoc study that the contraction to sumatriptan is larger in human coronary artery segments with intact endothelium than in blood vessel segments with functionally impaired endothelium (42). In contrast, it has been reported that constriction to α-adrenoceptor agonists is augmented in atherosclerotic human coronary arteries (43).

6.4.5. Possible clinical implications

Both *in vitro* (44, 45) and *in vivo* (2, 3, 7) experimental models demonstrating cranial vasoconstrictor properties have consistently shown their value in predicting therapeutic potential of drugs in the acute treatment of migraine. Therefore, the results obtained with S19014 in the present experiments suggest that this compound may well have antimigraine properties. Since S19014 had little systemic and regional haemodynamic effects, this compound appears to be well tolerated. However, as is the case with the currently available antimigraine agents (22, 23, 46), we are aware of the potential liability of α-adrenoceptor agonists in constricting coronary arteries (47, 48). Nevertheless, the distribution of cardiac output to various body organs, including the heart, was not affected by S19014. This is further reinforced in this study, where S19014 was clearly less effective than sumatriptan in contracting the human isolated coronary arteries and, contrary to sumatriptan, its effect was not augmented by the thromboxane A_2 analogue U46619. This may be clinically relevant, since the plasma concentration of thromboxane A_2 increases in patients with myocardial infarction and unstable angina (49-51). Although the augmentation between 5-HT_{1B} receptor agonists and thromboxane A_2 has been well characterised (28), it is not yet clear whether such a mechanism also operates for α-adrenoceptor agonists in the human coronary artery. However, the α_2-adrenoceptor-mediated contraction of the porcine isolated ear artery is enhanced by U46619 (52). Thus, the coronary side-effect potential of S19014 may be less than that of sumatriptan. However, considering that S19014 induces constriction of the coronary artery, albeit only to a small degree, the compound might remain contraindicated in patients with coronary artery disease.

References

1. Heyck H. Pathogenesis of migraine. Res Clin Stud Headache 1969;2:1-28.
2. Saxena PR. Cranial arteriovenous shunting, an *in vivo* animal model for migraine. In: Olesen J, Moskowitz MA, eds. Experimental headache models. Vol. 27. Philadelphia, USA: Lippincott-Raven Publishers, 1995:189-198.
3. De Vries P, Villalón CM, Saxena PR. Pharmacological aspects of experimental headache models in relation to acute antimigraine therapy. Eur J Pharmacol 1999;375:61-74.
4. Villalón CM, Centurión D, Valdivia LF, De Vries P, Saxena PR. An introduction to migraine: from ancient treatment to functional pharmacology and antimigraine therapy. Proc West Pharmacol Soc 2002;45:199-210.
5. Den Boer MO, Heiligers JP, Saxena PR. Carotid vascular effects of ergotamine and dihydroergotamine in the pig: no exclusive mediation via 5-HT_1-like receptors. Br J Pharmacol 1991;104:183-189.
6. De Vries P, Villalón CM, Heiligers JPC, Saxena PR. Characterization of 5-HT receptors mediating constriction of porcine carotid arteriovenous anastomoses; involvement of 5-$HT_{1B/1D}$ and novel receptors. Br J Pharmacol 1998;123:1561-1570.
7. Villalón CM, De Vries P, Rabelo G, Centurión D, Sánchez-López A, Saxena PR. Canine external carotid vasoconstriction to methysergide, ergotamine and dihydroergotamine: a role of 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors. Br J Pharmacol 1999;126:385-394.
8. Kohno Y, Saito H, Takita M, Kigoshi S, Muramatsu I. Heterogeneity of α_1-adrenoceptor subtypes involved in adrenergic contractions of dog blood vessels. Br J Pharmacol 1994;112:1167-1173.
9. Kawai Y, Kobayashi S, Ohhashi T. Existence of two types of postjunctional α-adrenoceptors in the isolated canine internal carotid artery. Can J Physiol Pharmacol 1988;66:655-659.
10. Muramatsu I, Kigoshi S, Oshita M. Two distinct α_1-adrenoceptor subtypes involved in noradrenaline contraction of the rabbit thoracic aorta. Br J Pharmacol 1990;101:662-666.
11. Muramatsu I, Kigoshi S, Ohmura T. Subtypes of α_1-adrenoceptors involved in noradrenaline-induced contractions of rat thoracic aorta and dog carotid artery. Jpn J Pharmacol 1991;57:535-544.
12. Ohgushi M, Yasue H, Kugiyama K, Murohara T, Sakaino N. Contraction and endothelium dependent relaxation via alpha adrenoceptors are variable in various pig arteries. Cardiovasc Res 1993;27:779-784.
13. Willems EW, Trion M, De Vries P, Heiligers JPC, Villalón CM, Saxena PR. Pharmacological evidence that α_1- and α_2-adrenoceptors mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetized pigs. Br J Pharmacol 1999;127:1263-1271.
14. Den Boer MO, Van Woerkens LJ, Somers JA, Duncker DJ, Lachmann B, Saxena PR, Verdouw PD. On the preservation and regulation of vascular tone in arteriovenous anastomoses during anesthesia. J Appl Physiol 1993;75:782-789.
15. Willems EW, Valdivia LF, Villalón CM, Saxena PR. Possible role of α-adrenoceptor subtypes in acute migraine therapy. Cephalalgia 2003;23:245-257.

16. Cordi AA, Lacoste JM, Descombes JJ, Vayssettes-Courchay C, Vanhoutte PM, Laubie M, Verbeuren TJ. Design, synthesis, and structure-activity relationships of a new series of alpha-adrenergic agonists: spiro[(1,3-diazacyclopent-1-ene)-5,2'-(1',2',3',4'-tetrahydronaphthalene)]. J Med Chem 1995;38:4056-4069.
17. Vayssettes-Courchay C, Lacoste J-M, Cordi AA, Laubi M, Verbeuren TJ. In vivo cardiovascular effects of the α–adrenoceptors agonist S18149 in the anaesthetised dog. Br J Pharmacol 1996;117:225P.
18. Descombes J-J, Menant Y, Barou A, Cordi A, Verbeuren TJ. S19014 is a partial agonist at alpha-adrenoceptors that selectively contracts the veins. Pharmacol Toxicol 1998;83 (Suppl. 1):92.
19. Den Boer MO, Villalón CM, Heiligers JP, Humphrey PP, Saxena PR. Role of 5-HT_1-like receptors in the reduction of porcine cranial arteriovenous anastomotic shunting by sumatriptan. Br J Pharmacol 1991;102:323-330.
20. Spierings EL, Saxena PR. Effect of isometheptene on the distribution and shunting of 15 μM microspheres throughout the cephalic circulation of the cat. Headache 1980;20:103-106.
21. Willems EW, Valdivia LF, Saxena PR, Villalón CM. Pharmacological profile of the mechanisms involved in the external carotid vascular effects of the antimigraine agent isometheptene in anaesthetised dogs. Naunyn Schmiedebergs Arch Pharmacol 2001;364:27-32.
22. MaassenVanDenBrink A, Reekers M, Bax WA, Ferrari MD, Saxena PR. Coronary side-effect potential of current and prospective antimigraine drugs. Circulation 1998;98:25-30.
23. MaassenVanDenBrink A, Van den Broek RWM, De Vries R, Bogers AJJC, Avezaat CJJ, Saxena PR. Craniovascular selectivity of eletriptan and sumatriptan in human isolated blood vessels. Neurology 2000;55:1524-1530.
24. Saxena PR, Schamhardt HC, Forsyth RP, Hoeve J. Computer programs for the radioactive microsphere technique. Determination of regional blood flows and other haemodynamic variables in different experimental circumstances. Comput Programs Biomed 1980;12:63-84.
25. Saxena PR, Verdouw PD. Redistribution by 5-hydroxytryptamine of carotid arterial blood at the expense of arteriovenous anastomotic blood flow. J Physiol (Lond) 1982;332:501-520.
26. Baile EM, Nelems JM, Schlzer M, Pare PD. Measurement of regional bronchial arterial blood flow and bronchovascular resistance in dogs. J Appl Physiol 1982;53:1044-1049.
27. Steel RGD, Torrie JH. Principles and procedures of statistics. A biomedical approach (2nd edition). Tokyo, Japan: McGraw-Hill Kogakusha Ltd, 1980
28. MaassenVanDenBrink A, Bax WA, Ferrari MD, Zijlstra FJ, Bos E, Saxena PR. Augmented contraction of the human isolated coronary artery by sumatriptan; a possible role for endogenous thromboxane. Br J Pharmacol 1996;119:855-862.
29. Brodde OE, Michel MC. Adrenergic and muscarinic receptors in the human heart. Pharmacol Rev 1999;51:651-690.
30. Hieble JP, Ruffolo RR, Jr. Subclassification and nomenclature of α_1- and α_2-adrenoceptors. Prog Drug Res 1996;47:81-130.
31. Hieble JP, Bylund DB, Clarke DE, Eikenburg DC, Langer SZ, Lefkowitz RJ, Minneman KP, Ruffolo RR, Jr. International Union of Pharmacology. X. Recommendation for nomenclature of α_1-adrenoceptors: consensus update. Pharmacol Rev 1995;47:267-270.
32. Langer SZ. History and nomenclature of α_1-adrenoceptors. Eur Urol 1999;36:2-6.

33. Docherty JR. Subtypes of functional α_1- and α_2-adrenoceptors. Eur J Pharmacol 1998;361:1-15.
34. Willems EW, Valdivia LF, Saxena PR, Villalón CM. The role of several α_1- and α_2-adrenoceptor subtypes mediating vasoconstriction in the canine external carotid circulation. Br J Pharmacol 2001;132:1292-1298.
35. Willems EW, Valdivia LF, Ramírez-San Juan E, Saxena PR, Villalón CM. Pharmacological identification of the major subtypes of adrenoceptors involved in the canine external carotid vasoconstrictor effects of adrenaline and noradrenaline. Life Sci 2001;69:143-153.
36. Saxena PR, MaassenVanDenBrink A, Heiligers JP, Scalbert E, Lemaitre BG. Effects of S20749, a close analogue of sumatriptan, on porcine carotid haemodynamics and human isolated coronary artery. Pharmacol Toxicol 1996;79:199-204.
37. Saxena PR, De Vries P, Heiligers JP, Bax WA, Maassen VanDenBrink A, Yocca FD. BMS-181885, a 5-$HT_{1B/1D}$ receptor ligand, in experimental models predictive of antimigraine activity and coronary side-effect potential. Eur J Pharmacol 1998;351:329-339.
38. Den Boer MO, Somers JA, Saxena PR. Comparative effects of the antimigraine drugs sumatriptan and ergotamine on the distribution of cardiac output in anaesthetized pigs. Cephalalgia 1992;12:206-213.
39. Tfelt-Hansen P, Saxena PR, Dahlof C, Pascual J, Lainez M, Henry P, Diener H, Schoenen J, Ferrari MD, Goadsby PJ. Ergotamine in the acute treatment of migraine: a review and European consensus. Brain 2000;123:9-18.
40. Wu CH, Lindsey DC, Traber DL, Cross CE, Herndon DN, Kramer GC. Measurement of bronchial blood flow with radioactive microspheres in awake sheep. J Appl Physiol 1988;65:1131-1139.
41. Kenakin T. Pharmacologic analysis of drug-receptor interaction. New York: Raven Press, 1993:483.
42. MaassenVanDenBrink A, Bax WA, Ramrattan NN, Ferrari MD, Saxena PR. Human isolated coronary artery contraction to sumatriptan; a post hoc analysis. Cephalalgia 1999;19:651-654.
43. Baumgart D, Naber C, Haude M, Oldenburg O, Erbel R, Heusch G, Siffert W. G protein beta3 subunit 825T allele and enhanced coronary vasoconstriction on alpha$_2$-adrenoceptor activation. Circ Res 1999;85:965-969.
44. Olesen IJ, Edvinsson L. Human cranial arteries as an in vitro model of migraine. In: Olesen J, Moskowitz MA, eds. Experimental headache models. Philadelphia: Lippincott-Raven Publishers, 1995:143-151.
45. Razzaque Z, Heald MA, Pickard JD, Maskell L, Beer MS, Hill RG, Longmore J. Vasoconstriction in human isolated middle meningeal arteries: determining the contribution of 5-HT_{1B}- and 5-HT_{1F}-receptor activation. Br J Clin Pharmacol 1999;47:75-82.
46. Tfelt-Hansen P, De Vries P, Saxena PR. Triptans in migraine: a comparative review of pharmacology, pharmacokinetics and efficacy. Drugs 2000;60:1259-1287.
47. Baumgart D, Haude M, Gorge G, Liu F, Ge J, Grosse-Eggebrecht C, Erbel R, Heusch G. Augmented alpha-adrenergic constriction of atherosclerotic human coronary arteries. Circulation 1999;99:2090-2097.
48. Heusch G, Baumgart D, Camici P, Chilian W, Gregorini L, Hess O, Indolfi C, Rimoldi O. Alpha-adrenergic coronary vasoconstriction and myocardial ischemia in humans. Circulation 2000;101:689-694.

49. De Boer AC, Turpie AGC, Butt RW, Johnston RV, Genton E. Platelet release and thromboxane synthesis in symptomatic coronary artery disease. Circulation 1982;66:327-333.
50. Rubanyi GM, Frye RL, Holmes DR, Vanhoutte PM. Vasoconstrictor activity of coronary sinus plasma from patients with coronary artery disease. J Am Coll Cardiol 1987;9:1243-1249.
51. Tada M, Kuzuya T, Inoue M, Kodama K, Mishima M, Yamada M, Inui M, Abe H. Elevation of thromboxane B_2 levels in patients with classic and variant angina pectoris. Circulation 1981;64:107-115.
52. Bhattacharya B, Roberts RE. Enhancement of alpha$_2$-adrenoceptor-mediated vasoconstriction by the thromboxane-mimetic U46619 in the porcine isolated ear artery: role of the ERK-MAP kinase signal transduction cascade. Br J Pharmacol 2003;139:156-162.

CHAPTER 7

Effects of donitriptan on carotid haemodynamics and cardiac output distribution in anaesthetised pig

Based on: Tom B, De Vries P, Heiligers JP, Willems EW, Kapoor K, John GW, Saxena PR. Effects of donitriptan on carotid haemodynamics and cardiac output distribution in anaesthetized pigs. Cephalalgia 2002;22:37-47.

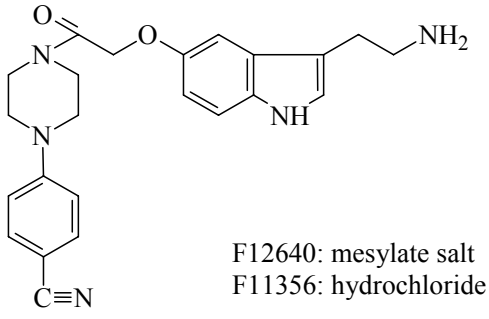
7. Effects of donitriptan on carotid haemodynamics and cardiac output distribution in anaesthetised pig

7.1. Introduction

The last decade has seen a tremendous progress in migraine therapy, with sumatriptan, belonging to a new class of drugs designated as 5-$HT_{1B/1D/1F}$ receptor agonists, providing the lead (1-3). Several studies have clearly established the therapeutic value of sumatriptan as well as of other triptans (4-7). Pharmacologically, triptans inhibit dural plasma protein extravasation, suppress action potentials in trigeminal nucleus caudalis, constrict isolated cranial blood vessels and decrease carotid arteriovenous anastomotic blood flow in anaesthetised animals (6, 8, 9). Although the trigeminal neural effects of triptans may be involved to some extent in their antimigraine action (7, 10, 11), the efficacy of triptans is primarily attributed to cranial vasoconstriction (6, 10). Using compounds selective at 5-HT_{1B}, 5-HT_{1D} or 5-HT_{1F} receptors, it is now well established that the vasoconstrictor effect of triptans is mediated via 5-HT_{1B} receptors (12-18).

Despite its success in the treatment of migraine headaches, sumatriptan has several shortcomings, such as low bioavailability, headache recurrence and propensity to elicit chest symptoms (6). Several newer triptans display better pharmacokinetic profile, but this has not been translated into any substantial improvement in either the efficacy or side-effect potential compared to sumatriptan (for reviews, see 6, 7). Recently, another triptan, donitriptan, (F11356 or F12640), has been synthesised by Centre de Recherche, Pierre Fabre, Castres, France; Figure 7.1). Donitriptan displays a very high affinity at recombinant human as well as porcine 5-$HT_{1B/1D}$ receptors with little affinity for the 5-HT_{1F} receptor and, more importantly, it has uniquely high intrinsic efficacy (inhibition of cAMP accumulation and enhancement of [^{35}S]GTPγS binding) at human 5-$HT_{1B/1D}$ receptors (Table 7.1, 19, 20-23). John et al. (19, 24) have hypothesised that the relatively low intrinsic activity of the currently

Donitriptan



F12640: mesylate salt
F11356: hydrochloride salt

Sumatriptan

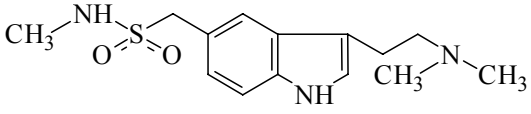


Figure 7.1. Chemical structures of donitriptan and sumatriptan.

available triptans at 5-$HT_{1B/1D}$ receptors may explain this ceiling effect in therapeutic response and, therefore, donitriptan may show a high therapeutic efficacy.

In the present study, we report on the effects of donitriptan on the distribution of carotid blood flow into nutrient (capillary) and non-nutrient (arteriovenous anastomotic) fractions in the anaesthetised pig. Previous investigations from our laboratory have established that

Table 7.1. Affinity and efficacy of donitriptan, sumatriptan and 5-HT at human (h) and porcine (p) 5-HT receptors.

Receptors	Donitriptan[a]	Sumatriptan[b]	5-HT[b]
Ligand binding affinity (pK_i)			
h5-HT_{1A}	7.6	6.0	7.9
h5-HT_{1B}	9.4-10.1	7.4	8.0
p5-HT_{1B}	9.0[c]	7.3[d]	7.8[e]
h5-HT_{1D}	9.3-10.2	8.3	8.4
p5-HT_{1D}	9.7[c]	8.1[d]	8.4[e]
h5-ht_{1E}	5.9	5.7	8.2
h5-ht_{1F}	5.5	7.6	8.0
p5-ht_{1F}	5.5[g]	5.4[g]	7.8[g]
h5-HT_{2A}	6.7	<5.0	6.5
h5-ht_6	5.6	<5.5	7.2
h5-HT_7	6.4	6.0	8.1
Inhibition of forskolin-evoked cAMP accumulation (pEC_{50})			
h5-HT_{1B}	8.91	7.16[f]	7.81[a]
h5-HT_{1D}	9.57	8.79[f]	8.62[a]
Enhancement of [^{35}S]GTPγS binding (pEC_{50})			
h5-HT_{1B}	8.74	6.63[f]	7.04[a]
h5-HT_{1D}	9.08	7.75[f]	7.84[a]

pK_i, Negative logarithm of the dissociation equilibrium constant; pEC_{50}, negative logarithm of the molar concentration that elicits 50% of its maximum effect. [a], John et al. (19); [b], IUPHAR Compendium (20); [c], Pauwels et al., Unpublished; [d], Bhalla et al. (21); [e], Bhalla et al. (22); [f], Pauwels et al. (23); [g], Bhalla et al., Unpublished.

constriction of carotid arteriovenous anastomoses in the anaesthetised pig serves as a predictive model for the antimigraine efficacy of 5-HT-based drugs (10, 25). Moreover, to establish the cardiovascular safety, we studied the effects of donitriptan on cardiac output and its regional distribution to vital organs.

7.2. Materials and methods

7.2.1. General

After an overnight fast, 36 pigs (Yorkshire x Landrace; 10-15 kg) were anaesthetised with azaperone (140 mg, i.m.), midazolam hydrochloride (7.5 mg, i.m.) and pentobarbitone sodium (600 mg, i.v.). The animals were intubated and connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; pO_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of pentobarbitone sodium at 12-20 $mg.kg^{-1}.h^{-1}$. It may be noted that with this anaesthetic regimen, arteriovenous anastomotic blood flow is considerably higher than that in pigs in a conscious state or under thiopentone anaesthesia (26), thereby producing one of the putative features of migraine, i.e. dilatation of carotid arteriovenous anastomoses (25, 27). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The

Netherlands) triggered by electrocardiographic signals. Catheters were placed in the inferior vena cava via the left femoral vein for the administration of drugs and fluids, and in the aortic arch via the left femoral artery for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) and the withdrawal of arterial blood for determining blood gases (ABL-510, Radiometer, Copenhagen, Denmark). Body temperature was kept at about 37ºC and the animals were continuously infused with saline to compensate for fluid losses during the experiment.

In the case of the carotid artery experiments, both common carotid arteries, external jugular veins and vagosympathetic trunks were identified. The vagosympathetic trunks were cut between two ligatures in order to avoid reflex-mediated cardiovascular changes. Subsequently, the right common carotid artery was dissected free and a needle was inserted against the direction of blood flow for the administration and uniform mixing of radioactive microspheres. Another catheter was placed in the right external jugular vein for the withdrawal of venous blood samples. Blood flow was measured in the right common carotid artery with a flow probe (internal diameter: 2.5 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands).
In the case of the cardiac output experiments, a 6F Swan-Ganz thermodilution catheter (Braun Melsungen AG, Melsungen, Germany) was introduced into the pulmonary artery via the right femoral vein to measure cardiac output. Another catheter, connected to a pressure transducer (Combitrans disposable pressure transducer; Braun, Melsungen, Germany), was guided through the left carotid artery into the left ventricle for the injection of radioactive microspheres. The presence of the tip of the catheter in the left ventricle was confirmed by the observation of the sudden switch from an arterial to a ventricular pressure profile. Lastly, a catheter was placed into the right femoral artery and connected to a Harvard pump for the withdrawal of reference blood samples during the injection of radioactive microspheres.

Heart rate, systolic, diastolic and mean arterial blood pressure as well as carotid blood flow were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). Vascular conductance was calculated by dividing blood flow or cardiac output ($ml.min^{-1}$) by mean arterial blood pressure (mmHg), multiplied by hundred and expressed as 10^{-2} $ml.min^{-1}.mmHg^{-1}$. Stroke volume (cardiac output dividing by heart rate) and nutrient cardiac output (cardiac output minus lung blood flow, i.e. mainly total arteriovenous shunting) were calculated.

7.2.2. Distribution of carotid blood flow

The distribution of common carotid blood flow was determined with 15.5±0.1 (S.D.) µm diameter microspheres labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, USA). For each measurement, a suspension of about 200,000 microspheres, labelled with one of the isotopes, was mixed and injected into the carotid artery. At the end of the experiment, the animal was killed, using an overdose of pentobarbital, and the heart, kidneys, lungs and the different cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 5 min in a γ-scintillation counter (Minaxi autogamma 5000; Packard Instruments, Downers Grove, IL, USA), using suitable windows for discriminating the different isotopes (^{141}Ce: 120-167, KeV, ^{113}Sn: 355-435 KeV, ^{103}Ru: 450-548 KeV, ^{95}Nb: 706-829 KeV and ^{46}Sc: 830-965 KeV). All data were processed by a set of specially designed programs (28), using a personal computer.

The fraction of carotid blood flow distributed to different tissues was calculated by multiplying the ratio of tissue and total radioactivities by the total common carotid blood flow at the time of the injection of microspheres. Since little or no radioactivity is detected in the heart or kidneys, all microspheres trapped in lungs reach lungs from the venous side after escaping via carotid arteriovenous anastomoses. Therefore, the amount of radioactivity in the

lungs can be used as an *index* of the arteriovenous anastomotic fraction of carotid blood flow (28, 29).

7.2.3. Distribution of cardiac output

The distribution of cardiac output was also determined with radioactive microspheres (see above). For each measurement, about 1,000,000 microspheres, labelled with one of the isotopes, were injected into the left ventricle. Starting 15 s before microsphere injection and lasting 70 s, a reference arterial blood sample was drawn at the rate of 6 $ml.min^{-1}$. Blood loss during this procedure was compensated by infusing the corresponding volume of haemaccel. At the end of the experiments, the animals were killed as described above and a number of tissues (lungs, kidneys, heart, stomach, small intestine, spleen, liver, adrenals, brain, skin and skeletal muscles) were dissected out, weighed, put into vials for counting radioactivity (see above). As described by Saxena et al. (28), tissue blood flow was calculated by multiplying the ratio of tissue and reference blood sample radioactivity by the blood withdrawal rate (6 $ml.min^{-1}$). Radioactivity in the lungs mainly represents peripheral arteriovenous anastomotic blood flow (non-nutrient part of the cardiac output), although a small part (1-1.5% of cardiac output) is derived from the bronchial arteries (30).

7.2.4. Experimental protocol

After a stabilisation period of about 1 h, the animals were divided into two groups (n=18 each) used for either carotid blood flow or cardiac output experiments. The two experimental groups, where an identical experimental protocol was followed, were subdivided into three subgroups (n=6 each). The first and second subgroups received physiological saline, whereas the third subgroup was pretreated with 0.5 $mg.kg^{-1}$ of the 5-$HT_{1B/1D}$ receptor antagonist GR127935 (31-33); both were given intravenously over a period of 10 min at a rate of 0.5 $ml.min^{-1}$. Ten min after the end of these infusions, baseline values of heart rate, mean arterial blood pressure, carotid blood flow or cardiac output and their distributions, as well as arterial and jugular venous blood gases were measured. Then, the first group received four consecutive infusions of vehicle (5 ml of distilled water, containing 40% polyethylene glycol, $v.v^{-1}$), whereas the second and third groups received consecutive doses of donitriptan (0.16, 2.5, 40 and 100 $\mu g.kg^{-1}$). Both vehicle and donitriptan were administered intravenously over a period of 10 min at a rate of 0.5 $ml.min^{-1}$, given every 20 min. Ten min after the end of each infused dose of vehicle or donitriptan, all haemodynamic variables were again collated.

The Ethics Committee of the Erasmus University Rotterdam dealing with the use of animals in scientific experiments approved the protocol for this investigation.

7.2.5. Data presentation and statistical analysis

All data have been expressed as the mean±SE mean. The significance of changes within one group was evaluated with Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations (34). The percent changes from baseline values caused by donitriptan in the GR127935 pretreated group were compared to those with the corresponding doses of donitriptan in the saline-pretreated group by using Student's unpaired *t*-test. Statistical significance was accepted at $P<0.05$ (two-tailed).

In the saline pretreated group, the dose of donitriptan needed to decrease baseline values of carotid arteriovenous anastomotic blood flow or vascular conductance by 50% ($ED_{50\%}$) was calculated using linear regression analysis.

7.2.6. Drugs

Apart from the anaesthetics, azaperone (Janssen Pharmaceutica, Beerse, Belgium), midazolam hydrochloride (Hoffmann La Roche b.v., Mijdrecht, The Netherlands) and pentobarbitone sodium (Apharma, Arnhem, The Netherlands), the compounds used in this study were: donitriptan (4-[4-[2-[3-(2-aminoethyl)-1H-indol-5-yloxy]-acetyl]-piperazin-1-yl] benzonitrile mesylate; F12640) and GR127935 (N-[methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide hydrochloride; both from Centre de Recherche Pierre Fabre, Castres, France), haemaccel® (Hoechst Marion Roussel b.v., Hoevelaken, The Netherlands) and heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) for preventing clotting of the catheters. Donitriptan was dissolved in distilled water, containing 40% polyethylene glycol ($v.v^{-1}$).

7.3. Results

7.3.1. Carotid blood flow experiments

7.3.1.1. Systemic haemodynamics and arterio-jugular venous oxygen saturation difference
As shown in Table 7.2, donitriptan as well as its vehicle did not change heart rate. There was a slight decrease in mean arterial blood pressure after second and third infusions of vehicle.

Table 7.2. Values of heart rate (HR), mean arterial blood pressure (MAP) and arterio-jugular venous oxygen saturation difference (A-V SO_2) at baseline and after 10 min intravenous infusions (0.5 $ml.min^{-1}$) of either donitriptan or the corresponding volumes of vehicle (n=6). The effects of donitriptan were analysed in animals pretreated with saline (n=6) or the 5-$HT_{1B/1D}$ receptor antagonist GR127935 (0.5 $mg.kg^{-1}$; n=6).

Pretreatment		HR ($beats.min^{-1}$)	MAP (mmHg)	A-V SO_2 (%)
		Vehicle (ml)		
Saline	Baseline	92±5	93±4	8±4
	5	91±6	87±4	9±3
	5	90±6	84±5*	10±4
	5	89±5	84±6*	10±4
	5	89±5	89±6	10±5
		Donitriptan ($\mu g.kg^{-1}$)		
Saline	Baseline	100±3	98±3	6±2
	0.16	99±3	98±3	6±1
	2.5	99±3	98±5	8±2
	40	99±3	93±6	17±4*
	100	99±4	88±7	18±3*
		Donitriptan ($\mu g.kg^{-1}$)		
GR127935	Baseline	103±1	96±3	6±3
	0.16	103±2	97±5	6±3
	2.5	103±2	97±4	7±3
	40	103±2	92±4	10±3*
	100	104±1	88±3*	13±4*

All values have been presented as the mean±SE mean. *, $P<0.05$ vs baseline.

In the saline pretreated group donitriptan did not change mean arterial blood pressure, but there was a moderate hypotensive response (-7.4±1.9%) following the highest dose of the drug in the GR127935 pretreated animals. Donitriptan produced a dose-dependent increase in arterio-jugular venous oxygen saturation difference in both saline and GR127935 pretreated animals; vehicle was devoid of this effect.

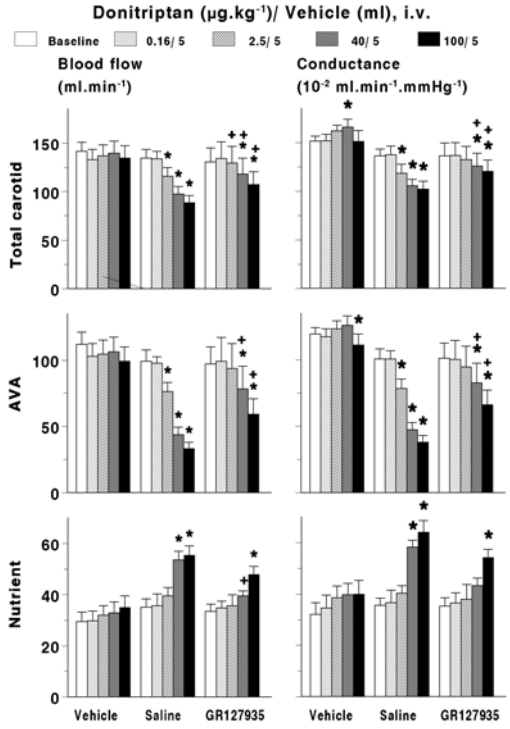


Figure 7.2. Values of total carotid, arteriovenous anastomotic (AVA) and nutrient blood flow (*left panels*) and conductance (*right panels*) at baseline and after 10 min intravenous infusions (0.5 ml.min^{-1}) of either donitriptan (0.16, 2.5, 40 and 100 µg.kg^{-1}) or the corresponding volumes of vehicle (5 ml; n=6). The effects of donitriptan were analysed in animals pretreated with either saline (n=6) or GR127935 (0.5 mg.kg^{-1}; n=6). All values are presented as the mean±SE mean. *, P<0.05 vs baseline. $^{+}$, P<0.05 vs response (% change from baseline) by corresponding dose of donitriptan in animals pretreated with saline.

7.3.1.2. Carotid haemodynamics

As shown in Figures 7.2 (absolute values) and 7.3 (percent changes from baseline), except for an increase in total carotid vascular conductance after the third infusion and a decrease in arteriovenous anastomotic conductance after the last infusion, the vehicle did not produce any changes in carotid haemodynamics. On the other hand, donitriptan dose-dependently decreased total carotid and arteriovenous anastomotic blood flow and concomitant

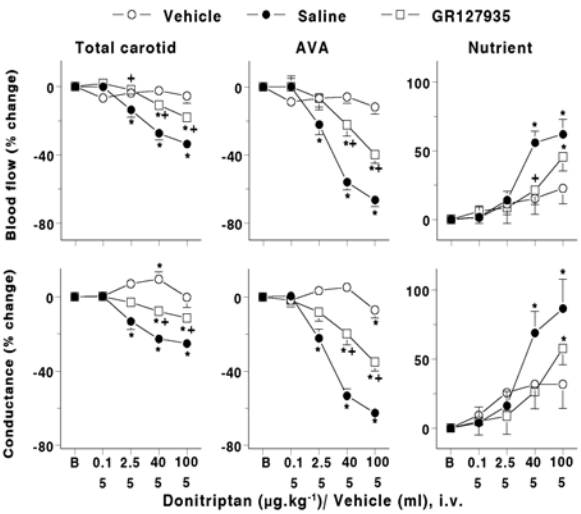


Figure 7.3. Percent changes from baseline values in total carotid, arteriovenous anastomotic (AVA) and nutrient blood flow (*upper panels*) and conductance (*lower panels*) after 10 min intravenous infusions (0.5 ml.min^{-1}) of either donitriptan (0.16, 2.5, 40 and 100 µg.kg^{-1}) or the corresponding volumes of vehicle (5 ml; n=6). The effects of donitriptan were analysed in animals pretreated with either saline (n=6) or GR127935 (0.5 mg.kg^{-1}; n=6). All values are presented as the mean± SE mean. *, P<0.05 vs baseline. $^{+}$, P<0.05 vs response (% change from baseline) by corresponding dose of donitriptan in animals pretreated with saline.

conductances; nutrient blood flow and conductance increased. The dose of donitriptan that was needed to decrease baseline values of arteriovenous anastomotic blood flow and conductance by 50% ($ED_{50\%}$) was found to be 58±27 $\mu g.kg^{-1}$ (113±52 $nmol.kg^{-1}$) and 47±16 $\mu g.kg^{-1}$ (92±31 $nmol.kg^{-1}$), respectively.

The maximum changes observed in total carotid, arteriovenous anastomotic and nutrient vascular conductances with the highest dose of donitriptan were -25±3%, -63±3% and +87±21%, respectively. After treatment with GR127935, donitriptan-induced decreases in the total carotid and arteriovenous anastomotic blood flows and vascular conductances were significantly less; the maximum decreases in total carotid and arteriovenous anastomotic vascular conductances were -12±2% and -35±5%, respectively (see Figures 7.2 and 7.3). Compared to saline-treated animals, GR127935 appeared to reduce donitriptan-induced increases in nutrient blood flow and vascular conductance, but statistical significance (based on percentage changes from the baseline in the two groups) was achieved only in the case of blood flow increase observed with 40 $\mu g.kg^{-1}$ of donitriptan.

The distribution of carotid blood flow to the head tissues in the three groups of animals is shown in Figure 7.4. Donitriptan produced significant increases in vascular conductance to the skin, ear, skeletal muscle, tongue, bone, fat, salivary gland, dura mater and brain; no change was observed in the eyes. In animals treated with GR127935, these increases were attenuated, but statistical significance (based on percentage changes from the baseline in the two groups) was reached only in skeletal muscle and fat. The corresponding volumes of the vehicle did not produce changes in vascular conductance values.

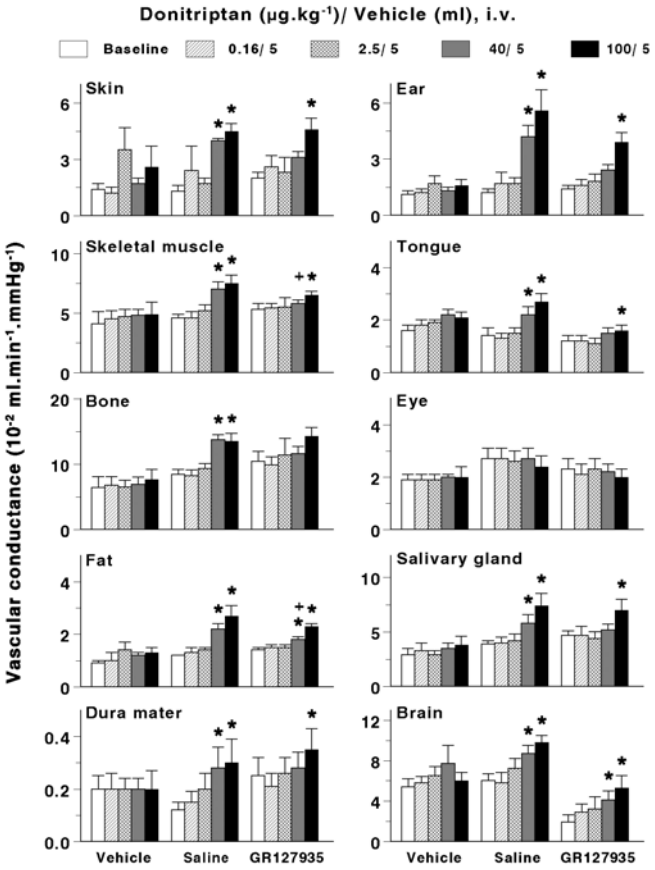


Figure 7.4. Values of carotid vascular conductance in different cranial tissues at baseline and after 10 min intravenous infusions (0.5 $ml.min^{-1}$) of either donitriptan (0.16, 2.5, 40 and 100 $\mu g.kg^{-1}$) or the corresponding volumes of vehicle (5 ml; n=6). The effects of donitriptan were analysed in animals pretreated with either saline (n=6) or GR127935 (0.5 $mg.kg^{-1}$; n=6). All values are presented as the mean± SE mean. *, $P<0.05$ vs baseline. $^{+}$, $P<0.05$ vs response (% change from baseline) by corresponding dose of donitriptan in animals pretreated with saline.

7.3.2. Cardiac output experiments

7.3.2.1. Systemic haemodynamics

The effects of vehicle and donitriptan on systemic haemodynamic variables are presented in Figure 7.5. No changes were observed with vehicle. In animals pretreated with saline, donitriptan did not change heart rate, mean arterial blood pressure, nutrient cardiac output or systemic vascular conductance, but it moderately decreased cardiac output (maximum change: -10±6%) and stroke volume (maximum change: -12±6%). These changes in cardiac output and stroke volume were absent in animals pretreated with GR127935, where donitriptan did decrease blood pressure by up to 18±3%.

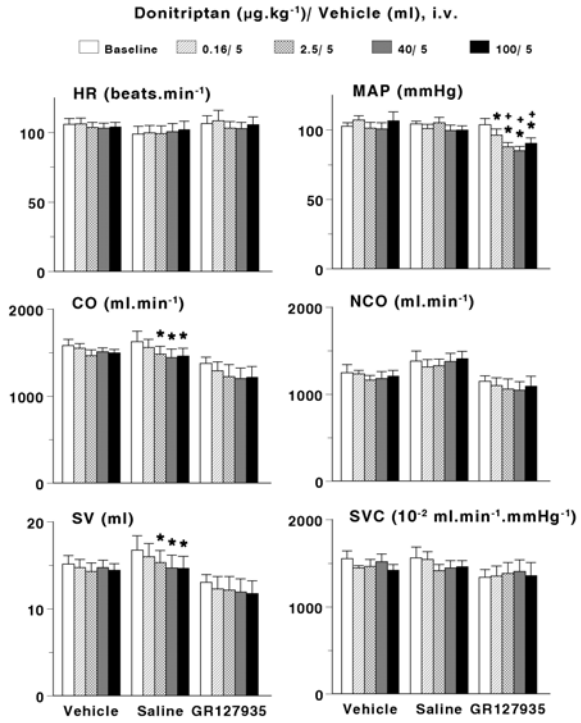


Figure 7.5. Systemic haemodynamic values of heart rate (HR), mean arterial blood pressure (MAP), cardiac output (CO), nutrient cardiac output (NCO), stroke volume (SV) and systemic vascular conductance (SVC) at baseline and after 10 min intravenous infusions (0.5 ml.min^{-1}) of either donitriptan (0.16, 2.5, 40 and 100 μg.kg^{-1}) or the corresponding volumes of vehicle (5 ml; n=6). The effects of donitriptan were analysed in animals pretreated with either saline (n=6) or GR127935 (0.5 mg.kg^{-1}; n=6). All values are presented as the mean± SE mean. *, P<0.05 vs baseline; $^{+}$, P<0.05 vs response (% change from baseline) by corresponding dose of donitriptan in animals pretreated with saline.

7.3.2.2. Regional haemodynamics

Apart from a slight decrease in skeletal muscle vascular conductance, vehicle did not change tissue vascular conductances (Table 7.3). Donitriptan decreased vascular conductance in lungs (maximum change: -75±6%), which was significantly less in GR127935 pretreated animals (maximum change: -32±13%). Whereas no changes were observed in vascular conductances in the kidneys, heart and portal tissues (liver, spleen, stomach, adrenals and small intestines), donitriptan slightly increased brain, skin and skeletal muscle vascular conductances.

7.4. Discussion

John et al. (19, 24) have reported that donitriptan equals 5-HT in potency and efficacy in contracting rabbit isolated saphenous vein and in increasing outward K^{+}-current in guinea-pig trigeminal ganglion cells. Donitriptan also decreases carotid blood flow in anaesthetised pigs and conscious dogs. In both *in vitro* and *in vivo* experiments, the responses to donitriptan can be antagonised by GR127935 and, thus, donitriptan shows a high affinity as well as efficacy at 5-HT$_{1B/1D}$ receptors (Table 7.1).

Table 7.3. Effects of 10 min intravenous infusions (0.5 $ml.min^{-1}$) of either donitriptan or the corresponding volumes of vehicle (n=6) on regional vascular conductance in anaesthetised pigs. The effects of donitriptan were analysed in animals pretreated with either saline (n=6) or the 5-$HT_{1B/1D}$ receptor antagonist GR127935 (0.5 $mg.kg^{-1}$; n=6).

Pretreat-ment		Vascular Conductance (10^{-2} $ml.min^{-1}.mmHg^{-1}.100\ g^{-1}$)						
		Lungs	**Kidneys**	**Heart**	**Portal**	**Brain**	**Skin**	**Muscles**
Saline	*Vehicle (ml)*							
	Baseline	236±72	234±18	98±12	44±3	34±4	15±4	4±1
	5	202±50	223±20	94±15	41±3	31±6	12±4	3±1
	5	201±45	248±17	98±18	45±3	35±6	15±5	4±1
	5	218±61	254±15	107±18	47±4	38±5	16±4	4±1
	5	173±39	215±21	93±13	43±4	33±4	12±3	3±0*
Saline	*Donitriptan ($\mu g.kg^{-1}$)*							
	Baseline	175±31	231±13	100±8	52±4	31±3	12±3	3±0
	0.16	176±28	247±4.2	111±8	56±5	35±2	11±3	4±0*
	2.5	108±18*	225±12	111±10	49±5	36±1*	12±3	3±0
	40	46±7*	241±11	112±9	57±5	41±2*	17±3*	4±0*
	100	39±5*	223±5	112±12	57±4	40±2*	18±4*	3±0
GR127935	*Donitriptan ($\mu g.kg^{-1}$)*							
	Baseline	161±29	266±30	117±17	40±4	30±2	25±5	3±0
	0.16	149±34	278±29	115±8	44±5	35±2*	26±7	4±0
	2.5	137±38	268±21	106±6	46±5*	38±2*	25±6	4±0*
	40	131±34^{+}	265±23	105±7	48±5*	42±3*	27±6	4±0*
	100	101±22*$^{+}$	233±23*	106±5	46±5*	39±2*	27±5	4±0*

All values have been presented as the mean±SE mean. *, $P<0.05$ vs baseline. $^{+}$, $P<0.05$ vs response by corresponding dose of donitriptan in animals pretreated with saline.

7.4.1. Heart rate and blood pressure

In contrast to the clinical experience (for references, see 6), sumatriptan as well as other triptans exert small bradycardic and hypotensive responses in anaesthetised pigs (17, 35). Donitriptan was devoid of these systemic effects in saline-treated animals. The exact reason for the difference between donitriptan and other triptans is not clear. However, this difference may be partly related to the slightly higher affinity of donitriptan at the 5-HT_{2A} receptor (Table 7.1), which, by virtue of its capacity to mediate hypertension, could annul a potential hypotensive effect of donitriptan. This view seems to be supported by the appearance of hypotension with donitriptan in animals treated with GR127935 (see Table 7.2; Figure 7.5), which can moderately attenuate 5-HT_{2A} receptor-mediated hypertension in rats (33).

7.4.2. Carotid haemodynamics

In agreement with earlier findings (19, 24), donitriptan dose-dependently decreased total porcine carotid blood flow and conductance in the present experiments. In addition, we observed that this vasoconstrictor effect of donitriptan, as is the case with other triptans, including sumatriptan, avitriptan and eletriptan (see 36), was exclusively confined to cephalic

arteriovenous anastomoses; the total nutrient fraction distributed to the head tissues increased. In this respect donitriptan exhibited the highest potency amongst several triptans that we have examined in this porcine model; the mean±SE mean $ED_{50\%}$ ($nmol.kg^{-1}$) of donitriptan in decreasing arteriovenous anastomotic vascular conductance (92±31) was lower than that of avitriptan (150±37), sumatriptan (156±54), eletriptan (400±91) or GMC2021 (2317±734) (36). The increase in the arterio-jugular venous oxygen saturation difference with donitriptan confirmed its constriction action on arteriovenous anastomoses.

It is now well recognised that the vasoconstrictor effect of triptans is mediated via $5\text{-}HT_{1B}$ receptors (12, 13, 15-18). Accordingly, in the present experiments, GR127935 ($0.5\ mg.kg^{-1}$) antagonised the constriction of cephalic arteriovenous anastomoses elicited by donitriptan. However, unlike sumatriptan, but as observed with some other triptans, for example eletriptan and GMC2021 (36), this dose of GR127935 did not completely abolish the donitriptan-induced arteriovenous constriction, suggesting the involvement of another, yet uncharacterised, receptor to some extent. Indeed, this is also the case for the arteriovenous anastomotic constriction elicited by ergotamine, dihydroergotamine and 5-HT (36, 37). On the other hand, it is possible that higher doses of GR127935 may be needed for a complete blockade of the effects of donitriptan. Unfortunately, the partial agonist property of GR127935 precludes the use of much higher doses of this antagonist in our experiments (33).

Similar to other $5\text{-}HT_{1B/1D}$ agonists, donitriptan produced a dilatation of carotid arterioles (nutrient vascular bed) and, as a result, blood flow and vascular conductance in many cranial tissues increased (see Figure 7.4). In view of the partial antagonism by GR127935, this dilatation seems to be at least partly due to the activation of the $5\text{-}HT_{1B}$ receptor, probably mediating endothelium-dependent relaxation in different vascular preparations. Indeed, the $5\text{-}HT_{1B}$ receptor has been located on vascular endothelium (12) and there is some evidence $5\text{-}HT_{1B/D}$ receptors may mediate endothelium-dependent vasodilatation (38-40). Alternatively or in addition, it may be due to an indirect consequence of the closure of arteriovenous anastomoses.

7.4.3. Cardiac output and regional haemodynamics

In previous studies in anaesthetised pigs, it has been shown that sumatriptan reduces total cardiac output and systemic vascular conductance, including decreases in vascular conductances in the kidneys and spleen (41). A decrease in renal vascular conductance by sumatriptan has also been reported in anaesthetised dogs (42). In our study, no decrease in blood flows to or vascular conductances in vital organs (brain, heart, kidneys and portal tissues) were observed with donitriptan; in fact there was a small increase in vascular conductance in the skin, skeletal muscle and brain. Donitriptan was also devoid of any effect on the total systemic vascular conductance, but cardiac output decreased. However, as was the case with carotid blood flow, the decrease in cardiac output was also entirely in the non-nutrient part, i.e. lung blood flow, which mainly reflects peripheral arteriovenous shunting (29). Indeed, the nutrient fraction of cardiac output (blood distributed to peripheral tissues) remained unchanged (Figure 7.5).

7.4.4. Therapeutic implications

Our study shows that donitriptan selectively constricts porcine carotid arteriovenous anastomoses, with a higher potency compared to several other triptans. Since constriction of cephalic arteriovenous anastomoses is of high predictive value for antimigraine activity (6), donitriptan should be effective in the treatment of migraine. The drug does not decrease blood flows to vital organs, including the heart, brain and kidneys; sumatriptan can decrease renal blood flow in dogs (42). Lastly, it may be mentioned here that donitriptan has little

affinity at the 5-HT_{1F} receptor, which, in view of the effects of the 5-HT_{1F} receptor agonist LY334370 (43, 44), may be responsible for side-effects, such as somnolence, asthenia, numbness and paresthesia.

References

1. Humphrey PPA, Feniuk W, Perren MJ, Connor HE, Oxford AW, Coates LH, Butina D. GR43175, a selective agonist for the 5-HT_1-like receptor in dog isolated saphenous vein. Br J Pharmacol 1988;94:1123-1132.
2. Humphrey PPA, Apperley E, Feniuk W, Perren MJ. A rational approach to identifying a fundamentally new drug for the treatment of migraine. In: Saxena PR, Wallis DI, Wouters W, Bevan P, eds. Cardiovascular pharmacology of 5-hydroxytryptamine: prospective therapeutic applications. Dordrecht: Kluwer academic publishers, 1990:416-431.
3. Saxena PR, Ferrari MD. From serotonin receptor classification to the antimigraine drug sumatriptan. Cephalalgia 1992;12:187-196.
4. The Subcutaneous Sumatriptan International Study Group. Treatment of migraine attacks with sumatriptan. New Engl J Med 1991;325:316-321.
5. Visser WH, De Vriend RH, Jaspers MW, Ferrari MD. Sumatriptan in clinical practice: a 2-year review of 453 migraine patients. Neurology 1996;47:46-51.
6. Tfelt-Hansen P, De Vries P, Saxena PR. Triptans in migraine: a comparative review of pharmacology, pharmacokinetics and efficacy. Drugs 2000;60:1259-1287.
7. Goadsby PJ. Serotonin 5-$HT_{1B/1D}$ receptor agonists in migraine. Comparative pharmacology and its therapeuthic implications. CNS Drugs 1998;10:271-286.
8. Moskowitz MA. Neurogenic versus vascular mechanisms of action of sumatriptan and ergot alkaloids in migraine. Trends Pharmacol Sci 1992;13:307-311.
9. Goadsby PJ, Knight Y. Inhibition of trigeminal neurones after intravenous administration of naratriptan through an action at 5-hydroxytryptamine (5-$HT_{1B/1D}$) receptors. Br J Pharmacol 1997;122:918-922.
10. De Vries P, Villalón CM, Saxena PR. Pharmacological aspects of experimental headache models in relation to acute antimigraine therapy. Eur J Pharmacol 1999;375:61-74.
11. Hargreaves RJ, Shepheard SL. Pathophysiology of migraine - new insights. Can J Neurol Sci 1999;26:S12-19.
12. Nilsson T, Longmore J, Shaw D, Olesen IJ, Edvinsson L. Contractile 5-HT_{1B} receptors in human cerebral arteries: pharmacological characterization and localization with immunocytochemistry. Br J Pharmacol 1999;128:1133-1140.
13. Razzaque Z, Heald MA, Pickard JD, Maskell L, Beer MS, Hill RG, Longmore J. Vasoconstriction in human isolated middle meningeal arteries: determining the contribution of 5-HT_{1B}- and 5-HT_{1F}-receptor activation. Br J Clin Pharmacol 1999;47:75-82.
14. Shepheard S, Edvinsson L, Cumberbatch M, Williamson D, Mason G, Webb J, Boyce S, Hill R, Hargreaves R. Possible antimigraine mechanisms of action of the $5HT_{1F}$ receptor agonist LY334370. Cephalalgia 1999;19:851-858.
15. Bouchelet I, Case B, Olivier A, Hamel E. No contractile effect for 5-HT_{1D} and 5-HT_{1F} receptor agonists in human and bovine cerebral arteries: similarity with human coronary artery. Br J Pharmacol 2000;129:501-508.
16. Verheggen R, Hundeshagen AG, Brown AM, Schindler M, Kaumann AJ. 5-HT_{1B} receptor-mediated contractions in human temporal artery: evidence from selective

antagonists and 5-HT receptor mRNA expression. Br J Pharmacol 1998;124:1345-1354.
17. De Vries P, Willems EW, Heiligers JPC, Villalón CM, Saxena PR. Investigation of the role of 5-HT_{1B} and 5-HT_{1D} receptors in the sumatriptan-induced constriction of porcine carotid arteriovenous anastomoses. Br J Pharmacol 1999;127:405-412.
18. Van den Broek RW, MaassenVanDenBrink A, de Vries R, Bogers AJ, Stegmann AP, Avezaat CJ, Saxena PR. Pharmacological analysis of contractile effects of eletriptan and sumatriptan on human isolated blood vessels. Eur J Pharmacol 2000;407:165-173.
19. John GW, Pauwels PJ, Perez M, Halazy S, Le Grand B, Verscheure Y, Valentin JP, Palmier C, Wurch T, Chopin P, Marien M, Kleven MS, Koek W, Assi MB, Carilla-Durand E, Tarayre JP, Colpaert FC. F 11356, a novel 5-hydroxytryptamine (5-HT) derivative with potent, selective, and unique high intrinsic activity at 5-$HT_{1B/1D}$ receptors in models relevant to migraine. J Pharmacol Exp Ther 1999;290:83-95.
20. Martin GR. 5-Hydroxytryptamine receptors. In: IUPHAR, ed. The IUPHAR compendium of receptor characterization and classification. London, UK: IUPHAR Media, 1998:169-185.
21. Bhalla P, Sharma HS, Wurch T, Pauwels PJ, Saxena PR. Molecular Cloning, Sequence analysis and pharmacological properties of the porcine 5-HT_{1D} receptor. Br J Pharmacol 2000;131:949-957.
22. Bhalla P, Sharma HS, Ma X, Wurch T, Pauwels PJ, Saxena PR. Molecular cloning, pharmacological properties and tissue distribution of the porcine 5-HT_{1B} receptor. Br J Pharmacol 2001;133:891-901.
23. Pauwels PJ, Tardif S, Palmier C, Wurch T, Colpaert FC. How efficacious are 5-$HT_{1B/D}$ receptor ligands: an answer from GTP gamma S binding studies with stably transfected C6-glial cell lines. Neuropharmacology 1997;36:499-512.
24. John GW, Perez M, Pauwels PJ, Le Grand B, Verscheure Y, Colpaert FC. Donitriptan, a unique high efficacy 5-$HT_{1B/1D}$ agonist. Key features and acute antimigraine potential. CNS Drug Reviews 2000;6:278-289.
25. Saxena PR. Cranial arteriovenous shunting, an *in vivo* animal model for migraine. In: Olesen J, Moskowitz MA, eds. Experimental headache models. Vol. 27. Philadelphia: Lippincott-Raven Publishers, 1995:189-198.
26. Den Boer MO, Van Woerkens LJ, Somers JA, Duncker DJ, Lachmann B, Saxena PR, Verdouw PD. On the preservation and regulation of vascular tone in arteriovenous anastomoses during anesthesia. J Appl Physiol 1993;75:782-789.
27. Heyck H. Pathogenesis of migraine. Res Clin Stud Headache 1969;2:1-28.
28. Saxena PR, Schamhardt HC, Forsyth RP, Loeve J. Computer programs for the radioactive microsphere technique. Determination of regional blood flows and other haemodynamic variables in different experimental circumstances. Comput Programs Biomed 1980;12:63-84.
29. Saxena PR, Verdouw PD. Redistribution by 5-hydroxytryptamine of carotid arterial blood at the expense of arteriovenous anastomotic blood flow. J Physiol (Lond) 1982;332:501-520.
30. Baile EM, Nelems JM, Schulzer M, Pare PD. Measurement of regional bronchial arterial blood flow and bronchovascular resistance in dogs. J Appl Physiol 1982;53:1044-1049.
31. Pauwels PJ. Pharmacological properties of a putative 5-$HT_{1B/D}$ receptor antagonist GR127935. CNS Drug Rev 1996;2:415-428.
32. Skingle M, Beattie DT, Scopes DIT, Starkey SJ, Connor HE, Feniuk W, Tyers MB. GR127935: a potent and selective 5-HT_{1D} receptor antagonist. Behav Brain Res 1996;73:157-161.

33. De Vries P, Apaydin S, Villalón CM, Heiligers JPC, Saxena PR. Interactions of GR127935, a 5-$HT_{1B/D}$ receptor ligand, with functional 5-HT receptors. Naunyn-Schmiedeberg's Arch Pharmacol 1997;355:423-430.
34. Steel RGD, Torrie JH. Principles and procedures of statistics. A biomedical approach (2nd edition). Tokyo: McGraw-Hill Kogakusha Ltd, 1980
35. Willems E, De Vries P, Heiligers JPC, Saxena PR. Porcine carotid vascular effects of eletriptan (UK-116,044): a new 5-$HT_{1B/1D}$ receptor agonist with anti-migraine activity. Naunyn Schmiedebergs Arch Pharmacol 1998;358:212-219.
36. De Vries P, Willems EW, Heiligers JPC, Villalón CM, Saxena PR. Constriction of porcine carotid arteriovenous anastomoses as indicator of antimigraine activity: the role of 5-$HT_{1B/1D}$ receptors as well as unidentified receptors. In: Edvinsson L, ed. Migraine & headache pathophysiology. London, UK: Martin Dunitz, 1998:119-132.
37. De Vries P, Villalón CM, Heiligers JPC, Saxena PR. Characterisation of 5-HT receptors mediating constriction of porcine carotid arteriovenous anastomoses; involvement of 5-$HT_{1B/1D}$ and novel receptors. Br J Pharmacol 1998;123:1561-1570.
38. Schoeffter P, Hoyer D. 5-Hydroxytryptamine (5-HT)-induced endothelium-dependent relaxation of pig coronary arteries is mediated by 5-HT receptors similar to the 5-HT_{1D} receptor subtype. J Pharmacol Exp Ther 1990;252:387-395.
39. Gupta P. An endothelial 5-HT receptor that mediates relaxation in guinea-pig isolated jugular vein resembles the 5-HT_{1D} subtype. Br J Pharmacol 1992;106:703-709.
40. Glusa E, Richter M. Endothelium-dependent relaxation of porcine pulmonary arteries via 5-HT_{1C}-like receptors. Naunyn-Schmiedeberg's Arch Pharmacol 1993;347:471-477.
41. Den Boer MO, Somers JEA, Saxena PR. Comparative effects of the antimigraine drugs sumatriptan and ergotamine on the distribution of cardiac output in anaesthetised pigs. Cephalalgia 1992;12:205-213.
42. Cambridge D, Whiting MV, Butterfield LJ. 5-Carboxamidotryptamine induced renal vasoconstriction in the dog. In: Fozard JR, Saxena PR, eds. Serotonin: Molecular biology, receptors, and functional effects. Basel: Birkhauser Verlag, 1991:282-288.
43. Granier LA, Gossen D, Vandenhende F. Safety, pharmacokinatics and efficacy of intravenous LY334370. Cephalalgia 2000;20:351-352.
44. Roon KI. New 5-HT_1 agonists in migraine, Clinical efficacy and mechanisms. Thesis, Department of Neurology. Leiden: Leiden University Medical Centre, 2000:174.

CHAPTER 8

General discussion

8. General discussion

8.1. Newer agents in migraine treatment

8.1.1. 5-$HT_{1B/1D}$ receptors

It has previously been demonstrated that 5-HT is able to induce a vasoconstriction within the external carotid vascular bed of the dog (1, 2). Similarly, 5-HT constricts the porcine carotid vasculature (3). The decrease in the carotid blood flow is exclusively confined to the arteriovenous anastomotic fraction (4-6). This 5-HT-induced cranial vasoconstriction has been demonstrated to be mediated primarily by sumatriptan-sensitive 5-HT_1 like receptors (1, 7-9). Due to development of 5-$HT_{1B/1D}$ receptor antagonists, particularly GR127935 (10-12), 5-HT_1 like receptors showed pharmacological resemblance to 5-$HT_{1B/1D}$ receptors. The launch of the first 5-$HT_{1B/1D}$-receptor agonist sumatriptan (13) has been hailed as the most significant advance in the acute treatment of migraine. The precise mechanisms by which it alleviates migraine are still not fully elucidated, but three distinct pharmacological actions on the vasculature and neurones have been invoked viz. vasoconstriction of cranial blood vessels (13-15), inhibition of neurogenic inflammation due to reduced vasodilator neuropeptide release from the sensory trigeminal sensory neurones (16, 17) and/or inhibition of firing of trigeminal neurones (18). Major limitations associated with sumatriptan include low oral bioavailability (19), low responders after 2 hours post drug administration and headache recurrence within 48 hours (20) and cardiovascular side effects (21-23). The room for improvement over the clinical effectiveness of sumatriptan is therefore substantial. Thus, newer triptans (tryptamine derivatives) though having improved oral bioavailability (19), have not superseded sumatriptan in terms of therapeutic effectiveness (19). Hence, donitriptan (F11356; F12650) has been developed (24, 25) as a selective 5-$HT_{1B/1D}$ agonist with some higher intrinsic activity in comparison to the well described tryptamine derivatives, naratriptan, zolmitriptan and sumatriptan (26).

As described in Chapter 7, donitriptan is a new triptan that possesses a uniquely high affinity as well as efficacy at 5-$HT_{1B/1D}$ receptors. We investigated the effects of donitriptan on carotid haemodynamics and complete distribution of cardiac output in anaesthetised pigs (27). Donitriptan dose-dependently decreased total carotid blood flow and vascular conductance. This effect was entirely due to a selective reduction in the cephalic arteriovenous anastomotic fraction. Donitriptan did not decrease vascular conductances or blood flows to a number of organs, including the heart and kidneys; in fact vascular conductances in the skin, brain and skeletal muscles increased. Cardiac output was slightly decreased by donitriptan, but this effect was also confined to the non-nutrient part (peripheral arteriovenous anastomoses). The 5-$HT_{1B/1D}$ receptor antagonist GR127935 substantially reduced the haemodynamic effects of donitriptan. These results show that donitriptan selectively constricts arteriovenous anastomoses via 5-HT_{1B} receptor activation (27). Additionally, *in vitro* studies on human coronary arteries demonstrated a similar coronary side-effect profile as sumatriptan. It was, therefore, thought to explore novel receptor areas for migraine treatment apart from 5-HT receptors.

8.1.2. α-Adrenoceptors

Role of α-adrenoceptors in migraine has seldom been explored despite the fact that therapeutic efficacy of ergot alkaloids in moderate to severe migraine is due to their 5-HT and α-adrenergic agonistic activity (28). Some initial reports suggest a deranged central

noradrenergic activity in migraine as evidenced by disinhibition of the hypothalamopituitary-adrenal axis (29). Interestingly, a lack of hormonal response to clonidine in menstrual migraine may suggest a postsynaptic α_2-adrenoreceptor hyposensitivity during the premenstrual period, which demonstrates a transient vulnerability of the neuroendocrine/neurovegetative systems (30). However, he question remains open, whether additional receptors and/or mechanisms play a role in antimigraine effects. It is suggested that sensitisation of blood vessels, reinforced by a direct vasoconstriction through activation of smooth muscle α-adrenoceptors, may contribute to the mechanism of action of clonidine and methysergide in migraine (31). Additionally, the inhibitory effect of clonidine in neurosympathetic transmission (32) might also explain the efficacy of the drug in the treatment of migraine. Many clinical studies have been done to elucidate possible role of α-adrenoceptors in migraine. For this reason recently, it has been shown that both α_1- and α_2-adrenoceptors mediate constriction of arteriovenous anastomoses within the carotid vascular bed in anaesthetised pigs (33). Additionally, a role of α_1-adrenoceptor subtypes in mediating constriction in the carotid circulation of anaesthetised pigs was further explored (34). As mentioned in Chapter 4, α_{1A}- and α_{1B}-adrenoceptors mediate constriction of carotid arteriovenous anastomoses in anaesthetised pigs. This conclusion is based on the findings that intracarotid infusions of phenylephrine induced a dose-dependent decrease in total carotid and arteriovenous anastomotic conductance, accompanied by a small tachycardia. These carotid vascular effects were abolished by L-765,314 (α_{1B}-antagonist), while these responses were only attenuated by 5-methylurapidil (α_{1A}-antagonist) as well as BMY 7378 (α_{1D}-antagonist). Furthermore, intravenous bolus injections of phenylephrine produced a dose-dependent vasopressor response, which was only affected by 5-methylurapidil, while the other antagonists were ineffective. Of the two α_1-adrenoceptor subtypes, the α_{1B}-adrenoceptor is an interesting target for future antimigraine drugs, especially when considering that this receptor, unlike the α_{1A}-adrenoceptor, does not seem to be much involved in the constriction of the peripheral blood vessels leading to an increase in blood pressure (35). Interestingly, the hypertensive effect produced by intravenous administration of phenylephrine is predominantly mediated via the α_{1A}-, but not α_{1B}-adrenoceptor.

To further confirm any involvement of α_{1A}-adrenoceptors in migraine, we studied the effects of a potent and selective α_{1A}-adrenoceptor agonist, A61603 (N-[5-(4,5-dihydro-1H-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl] methane sulphonamide) (36, 37), on regional carotid blood flow in anaesthetised pigs (Chapter 5). The response to A61603 was characterised by using selective α-adrenoceptor antagonists, 5-methylurapidil (α_{1A}), prazosin (α_1) and a combination of prazosin (α_1) and rauwolscine (α_2). Similarly, the effects of GR127935 (N-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2'-methyl-4' ($5\text{-HT}_{1B/1D}$), ketanserin (5-HT_2, α_1) and methiothepin ($5\text{-HT}_{1/2}$), in doses sufficient to block their respective receptors were also investigated. It is known that a relatively high dose of methiothepin is required to abolish sumatriptan-induced carotid vasoconstriction in anaesthetised dogs and pigs (7, 38), while a lower dose is ineffective. In our study too, a higher dose of methiothepin markedly attenuated the A61603-induced vasoconstriction in the porcine carotid vascular bed. Since all currently known vasoconstrictor receptors/ mechanisms ($\alpha_{1/2}$-adrenoceptors, $5\text{-HT}_{1B/1D}$, 5-HT_2 and eicosanoid receptors) had already been excluded in this study (Chapter 5), it points to some novel receptor mechanisms being involved in the mediation of constriction of carotid arteriovenous anastomoses. Because this *in vivo* porcine model is predictive for antimigraine activity (39), the above-mentioned novel mechanism could be a potential target for the development of antimigraine agents in the future. Admittedly, as an antimigraine drug, such an agonist must be devoid of systemic vasoconstrictor properties leading to an increase in arterial blood pressure.

Further, we explored another α-adrenoceptor agonist compound, S19014, for its antimigraine potential. As described in Chapter 6, S19014 causes a dose-dependent vasoconstriction in the carotid vasculature of anaesthetised pigs, an effect exclusively caused by vasoconstriction of carotid arteriovenous anastomoses; the vascular conductance in the capillary fraction was increased. In accordance with the constriction of carotid arteriovenous anastomoses (39, 40), S19014 produces an increase in arteriovenous oxygen saturation difference. Although S19014 has only a little less affinity at the three α_1-adrenoceptor subtypes than at the three α_2-adrenoceptor subtypes, the constriction of carotid arteriovenous anastomoses by S19014 was antagonised by rauwolscine and not at all by prazosin. This shows a possible involvement of α_2-adrenoceptors in S19014 mediated effects. Additionally, the vasoconstrictor responses to S19014 are variable in potency and efficacy (41), and this suggests that α-adrenoceptor subtypes at which S19014 is efficacious may be unevenly distributed.

In this connection, it is interesting to compare the efficacy of S19014 with that of triptans and ergot alkaloids in the present porcine model. As can be observed in Figure 8.1 (42), S19014 (30 $\mu g.kg^{-1}$) and sumatriptan (30 $\mu g.kg^{-1}$) were equi-effective in constricting porcine carotid arteriovenous anastomoses, but we do not know if higher doses of S19014

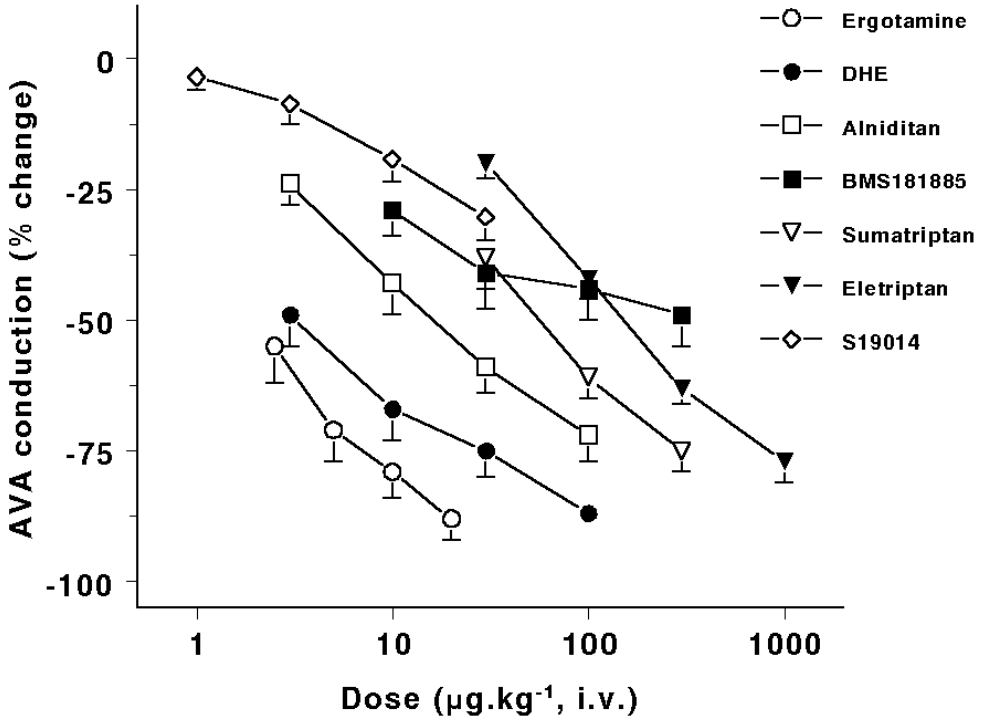


Figure 8.1. Comparison of the contractile effect of S19014 and some antimigraine drugs on carotid arteriovenous anastomoses in anaesthetised pigs.

will exhibit higher efficacy with relatively little systemic haemodynamic effects. Our experience with this porcine model is largely limited to 5-HT_{1B} receptor agonists and, in view of the fact that porcine α-adrenoceptors have not yet been cloned and compared with the human receptors, we do not know how the porcine carotid vascular responses mediated via α-adrenoceptors would be predictive of antimigraine efficacy in humans. Nevertheless, it will be worthwhile to explore this aspect with S19014, which mainly acts via

α_2-adrenoceptors that are less ubiquitous than α_1-adrenoceptors. It may be pointed out that S19014 causes mild hypotension after an initial immediate short lasting vasopressor response when given intravenously. It would be interesting to observe any pressor effect after oral doses of S19014 in a clinical set up.

As with the currently available antimigraine agents (43, 44), we are aware of the potential liability of α-adrenoceptor agonists in constricting coronary arteries. However, S19014 was clearly less effective than sumatriptan in contracting human isolated coronary arteries and that, contrary to sumatriptan, its effect is not potentiated upon pre-contraction of the vessels with thromboxane A_2 (Chapter 7). This is further re-enforced in this study, where the distribution of cardiac output to various body organs was not effected by S19014.

8.1.3. Calcitonin gene related peptide (CGRP) receptors

Migraine involves dysfunction of brain stem pathways that normally modulate sensory input. Involvement of CGRP in migraine pathology is supported by both clinical and experimental evidence. Release of CGRP and other neuropeptides from trigeminal nerves is thought to mediate neurogenic inflammation within the meninges that contributes to generation of the severe cerebral pain experienced during migraine attacks. Although other neuropeptides, such as substance P and neurokinin A, are involved in regulation of the cerebral vasculature, their role in migraine is not clear. Concentrations of CGRP in blood obtained from the external jugular vein are elevated in patients during all forms of vascular headaches, including migraine with and without aura and cluster headaches. Further evidence for a role of CGRP in migraine comes from clinical studies in which sumatriptan was shown to decrease elevated CGRP levels in migraine patients, coincident with relief of headache pain (45, 46). In a number of animal studies involving cats and rats (47, 48), CGRP levels have also been shown to be elevated in the sagittal sinus following chemical or electrical stimulation of the trigeminal ganglion. Recently, chemical and electrical stimulation of dural afferents caused a significant increase in the amount of CGRP, but not substance P, released from trigeminal nerves (49). Based on these data, CGRP is believed to play a central role in migraine pathophysiology, due to its ability to regulate cerebral blood flow and mediate neurogenic inflammation within the dura. The key pathways of pain are the trigeminovascular input from the meningeal vessels, which passes through the trigeminal ganglion and synapses on second order neurons in the trigeminovascular complex. This in turn finally synapses with the neurons in the thalamus. This trigemino-autonomic reflex is present in normal persons (50) and is expressed most strongly in patients with trigeminal-autonomic cephalgias, including migraine. Peripheral trigeminal activation in migraine is evident by the release of CGRP, a vasodilator (51), but the mechanism of generation of pain is still not clear. Studies in animals suggest that pain may be caused by a sterile neurogenic inflammatory process in the dura mater (52). The pain may be a combination of altered perception as a result of peripheral or central sensitisation of craniovascular input that is not usually painful (50) and the activation of neurovascular vasodilator mechanism that is specific for the first division of the trigeminal nerve (53). To address this issue, we attempted to explore the role of BIBN4096BS, a novel CGRP antagonist, in the porcine model of migraine. This hypothesis is based on the findings that increased concentrations of CGRP have been observed in migraineurs during the headache phase (54, 55). Chapter 3 describes the effect of BIBN4096BS in capsaicin-induced release of CGRP in porcine model of migraine. Intracarotid capsaicin infusion caused an increase in mean blood pressure along with dose-dependent increases in total carotid, arteriovenous anastomotic and capillary blood flows and carotid pulsations and decreased the difference between arterial and jugular venous oxygen saturations (A-V SO_2 difference). BIBN4096BS significantly abolished the carotid haemodynamic effects. Capsaicin infusion (10 $\mu g\ kg^{-1}\ min^{-1}$, i.c.) more than doubled jugular

venous plasma concentration of CGRP. This effect was not blocked, but rather increased by BIBN4096BS. This is an interesting observation, based on which we hypothesise a possible prejunctional positive feedback regulatory mechanism of action of BIBN4096BS leading to CGRP release as seen with sympathetic neurotransmission involving prejunctional α-adrenoceptors.

To further endorse this view that the vasodilator effects of capsaicin-induced CGRP release are responsible for the haemodynamic changes observed in the carotid vasculature, we employed intracarotid infusions of human α-CGRP, as described in Chapter 2. A similar dose dependent increase in the carotid blood flow and conductance was observed which were blocked by BIBN4096BS. Since we observed a decrease in the CGRP induced mean blood pressure, it can be concluded that CGRP does not play any important role in regulating basal vascular tone. Furthermore, BIBN4096BS did not affect cardiac output, which could be an advantage if found effective in the clinical trials on patients of migraine. In a recent review, CGRP receptor antagonist BIBN4096BS has been addressed as one of the exciting lead molecules presently in clinical trials for migraine patients (56).

8.1.4. Other receptors

Another lead molecule described is ethyl(3*S*,4a*R*,6*S*,8a*R*)-6-(4-ethoxycar-bonylimidazol-1-ylmethyl) decahydro-isoquinoline-3-carboxylic ester. This compound, which is a prodrug of a GluR5 kainate receptor antagonist, has been found active in two animal models of acute migraine viz. neurogenic dural plasma protein extravasation model and the nucleus caudalis c-fos expression model (57). The validity of this concept was tested with LY293558, a non-selective AMPA/Kainate glutamate receptor antagonist, which showed similar efficacy to sumatriptan 6 mg in a small placebo-controlled parallel-group study (58).

An additional avenue that may show promise in antimigraine drug development is regulation of Ca^{2+} levels. Recently, 5-HT_1 receptor activation by sumatriptan was shown to couple to a prolonged elevation in intracellular Ca^{2+} in trigeminal ganglia neurons (46). This increase in Ca^{2+} is likely responsible for mediating the inhibitory effects of this drug on CGRP gene expression. Although the signaling mechanisms of how elevated Ca^{2+} levels inhibit CGRP synthesis and release from neuronal cells are just beginning to be elucidated, it is highly probable that Ca^{2+}-sensitive phosphatases are involved. Prolonged elevations of intracellular Ca^{2+} have been reported to inhibit neuropeptide expression (59) and the activity of membrane-localised voltage-dependent Ca^{2+} channels through changes in the phosphorylation state of the cell. Furthermore, α-eudesmol, a P/Q-type Ca^{2+}-channel blocker, was shown to inhibit neurogenic vasodilation, plasma extravasation in the dura mater, and depolarisation-evoked CGRP and SP release from sensory nerves (60).

8.2. Future prospects in migraine management

8.2.1. Role of botulinum toxin A

The mechanism by which botulinum toxin A (BTX-A) acts in migraine is probably unrelated to its effect on muscle relaxation. BTX-A may have a distinct antinociceptive mechanism, either through action on the muscle spindles or through a direct effect on the central nervous system. Several trials and case reports have demonstrated the safety and efficacy of BTX-A in migraine headache (61).

In the first case-report of its use, BTX-A was found effective in aborting migraine headache without any recurrence over two months (62), which has been supported in other clinical trials (63). In a recent review, gabapentin, magnesium, lisinopril and botulinum

toxin A have been suggested to be effective; however, at present, there are insufficient rigorous and reliable controlled data on these drugs for them to be indicated for such use in migraine.

8.2.2. Selective adenosine A_1 receptor agonists

In a recent study selective adenosine A_1 receptor agonist, GR79236 caused a dose-dependent inhibition of neurogenic vasodilatation, but had no significant effect on dural vasodilation caused by CGRP. This was associated with mild bradycardia and hypotension (64). These data suggest that the inhibition of neurogenic vasodilatation by GR79236 is mediated via the activation of prejunctional adenosine A_1 receptors. In another study in cats, intravenous administration of the highly selective adenosine A_1 receptor agonist, GR79236 had a dose-dependent inhibitory effect on superior sagittal sinus-evoked trigeminal activity which could be inhibited by the selective adenosine A_1 receptor antagonist DPCPX. Superior sagittal sinus stimulation increased cranial CGRP levels (65). In this model of trigeminovascular nociception, adenosine A_1 receptor activation leads to neuronal inhibition without concomitant vasoconstriction, suggesting a novel avenue for the treatment of migraine and cluster headache.

8.2.3. eNOS inhibitors in migraine

Nitric oxide (NO) can trigger a delayed migraine. The initial headache is thought to be caused via a direct action of the NO-cGMP pathway that causes vasodilatation by vascular smooth muscle relaxation, while the delayed headache is likely to be a result of triggering trigeminovascular activation (66). Nitric oxide synthase (NOS) inhibitors are effective in the treatment of acute migraine. Additionally, non-specific and neuronal NOS (nNOS) inhibitors are able to partially inhibit neurogenic dural vasodilatation, while the non-specific and endothelial NOS (eNOS) inhibitors were able to partially inhibit the CGRP induced dilation. This suggests a role of eNOS and nNOS in the generation of initial and delayed headache response in migraine (66).

8.2.4. Selective 5-HT_{1F} receptor agonists in migraine

Triptans (5-$HT_{1B/1D}$ receptor agonists) are effective drugs for acute migraine, but the side effect of coronary vasoconstriction restricts their use in patients who are at risk of coronary artery disease. LY334370, a selective 5-HT_{1F} receptor agonist, showed preclinical efficacy and no vasoconstriction, for migraine relief possibly through selective trigeminovascular neuronal inhibition (67).

8.2.5. Upregulation of 5-HT_{2A} receptor in migraine

Recently, it has been demonstrated that activation of the 5-HT_{2A} receptor leads to an enhancement of NO (nitric oxide) production in trigeminovascular pathway. NO may trigger migraine attacks by inducing cerebral vasodilatation and sensitizing the perivascular nociceptors and central nociceptive neurons in trigeminovascular system. Up-regulation of this pronociceptive receptor can increase headache attacks and contributes to the development of chronic daily headache (68). An explanation for this above hypothesis stems from the fact that during the migraine-free period, 5-HT at its optimum concentration, will bind to the 5-$HT_{1B/1D}$ receptor. The occupation of these receptors results in vasoconstriction and stabilisation of perivascular nociceptors and central nociceptive neurons. On the other hand, excessive amounts of 5-HT released during migraine initiation may couple to the 5-HT_{2A} receptor and increase NO production. NO will then induce vasodilatation, sensitise

perivascular and myofascial nociceptors, and sensitise central nociceptive neurons precipitating migraine (68).

To conclude, one may admit that despite some exciting future prospects for migraine management still is wide open. Migraine patients worldwide are not receiving adequate treatment and there remains a significant unmet need in migraine care. According to a recent MAZE survey conducted in Europe and United States the average prevalence of migraine reported was 9% (69). Less than one-third of patients reported that their current medication was consistently effective and only 36% were 'very satisfied' with their current therapy (69). Hence, the challenge for the future is to diagnose migraine early and offer patients effective migraine-specific therapies. Physicians particularly need to reach patients who do not realise they have migraine and those who have lapsed from care.

References

1. Villalón CM, Terrón JA, Hong E. Role of 5-HT_1-like receptors in the increase in external carotid blood flow induced by 5-hydroxytryptamine in the dog. Eur J Pharmacol 1993;240:9-20.
2. Saxena PR, Van Houwelingen P, Bonta IL. The effects of mianserin hydrochloride on the vascular responses evoked by 5-hydroxytryptamine and related vasoactive substances. Eur J Pharmacol 1971;13:295-305.
3. Saxena PR, Duncker DJ, Bom AH, Heiligers J, Verdouw PD. Effects of MDL72222 and methiothepin on carotid vascular responses to 5-hydroxytryptamine in the pig: evidence for the presence of "5-hydroxytryptamine$_1$-like" receptors. Naunyn-Schmiedeberg's Arch Pharmacol 1986;333:198-204.
4. Verdouw PD, Jennewein HM, Heiligers JPC, Duncker DJ, Saxena PR. Redistribution of carotid artery blood flow by 5-HT: effects of the 5-HT_2 receptor antagonists ketanserin and WAL1307. Eur J Pharmacol 1984;102:499-509.
5. Saxena PR. Arteriovenous shunting and migraine. Res Clin Stud Headache 1978;6:89-102.
6. Saxena PR, Verdouw PD. Redistribution by 5-hydroxytryptamine of carotid arterial blood at the expense of arteriovenous anastomotic blood flow. J Physiol (Lond) 1982;332:501-520.
7. Den Boer MO, Villalón CM, Heiligers JP, Humphrey PP, Saxena PR. Role of 5-HT_1-like receptors in the reduction of porcine cranial arteriovenous anastomotic shunting by sumatriptan. Br J Pharmacol 1991;102:323-330.
8. Villalón CM, Terrón JA. The 5-HT_1-like receptor mediating the increase in canine external carotid blood flow: close resemblance to the 5-HT_{1D} subtype. Br J Pharmacol 1994;113:13-20.
9. Saxena P, Ferrari M. 5-HT_1-like receptor agonists and the pathophysiology of migraine. Trends Pharmacol Sci 1989;10:200-204.
10. De Vries P, Apaydin S, Villalón CM, Heiligers JPC, Saxena PR. Interactions of GR127935, a 5-$HT_{1B/D}$ receptor ligand, with functional 5-HT receptors. Naunyn-Schmiedeberg's Arch Pharmacol 1997;355:423-430.
11. De Vries P, Heiligers JPC, Villalón CM, Saxena PR. Blockade of porcine carotid vascular response to sumatriptan by GR127935, a selective 5-HT_{1D} receptor antagonist. Br J Pharmacol 1996;118:85-92.
12. Villalon CM, Centurion D, Lujan-Estrada M, Terron JA, Sanchez-Lopez A. Mediation of 5-HT-induced external carotid vasodilatation in GR 127935-pretreated

vagosympathectomized dogs by the putative 5-HT7 receptor. Br J Pharmacol 1997;120:1319-1327.
13. Humphrey PPA, Feniuk W. Mode of action of the anti-migraine drug sumatriptan. Trends Pharmacol Sci 1991;12:444-446.
14. Ferrari MD. Sumatriptan in the treatment of migraine. Neurology 1993;43:S43-47.
15. Ferrari MD, Saxena PR. Clinical and experimental effects of sumatriptan in humans. Trends Pharmacol Sci 1993;14:129-133.
16. Moskowitz MA. Neurogenic inflammation in the pathophysiology and treatment of migraine. Neurology 1993;43:S16-20.
17. Moskowitz MA. Neurogenic versus vascular mechanisms of sumatriptan and ergot alkaloids in migraine. Trends Pharmacol Sci 1992;13:307-311.
18. Hoskin KL, Kaube H, Goadsby PJ. Sumatriptan can inhibit trigeminal afferents by an exclusively neural mechanism. Brain 1996;119:1419-1428.
19. Goadsby PJ. Serotonin 5-$HT_{1B/1D}$ receptor agonists in migraine. Comparative pharmacology and its therapeuthic implications. CNS Drugs 1998;10:271-286.
20. Ferrari MD. Migraine. Lancet 1998;351:1043-1051.
21. Hood S, Birnie D, MacIntyre PD, Hillis WS. Sumatriptan-induced chest pain. Lancet 1994;344:1500-1501.
22. MaassenVanDenBrink A, Bax WA, Ferrari MD, Zijlstra FJ, Bos E, Saxena PR. Augmented contraction of the human isolated coronary artery by sumatriptan; a possible role for endogenous thromboxane. Br J Pharmacol 1996;119:855-862.
23. MaassenVanDenBrink A, Ramrattan NR, Bax WA, Ferrari MD, Saxena PR. Human isolated coronary artery contraction to sumatriptan is inversely related to age and positively related to endothelial functional integrity. Cephalalgia 1997;17:244.
24. John GW, Pauwels PJ, Perez M, Halazy S, Le Grand B, Verscheure Y, Valentin JP, Palmier C, Wurch T, Chopin P, Marien M, Kleven MS, Koek W, Assi MB, Carilla-Durand E, Tarayre JP, Colpaert FC. F 11356, a novel 5-hydroxytryptamine (5-HT) derivative with potent, selective, and unique high intrinsic activity at 5-$HT_{1B/1D}$ receptors in models relevant to migraine. J Pharmacol Exp Ther 1999;290:83-95.
25. John GW, Perez M, Pauwels PJ, Le Grand B, Verscheure Y, Colpaert FC. Donitriptan, a unique high efficacy 5-$HT_{1B/1D}$ agonist. Key features and acute antimigraine potential. CNS Drug Reviews 2000;6:278-289.
26. Dukat M. Donitriptan (Pierre Fabre). Curr Opin Investig Drugs 2001;2:415-418.
27. Tom B, De Vries P, Heiligers JP, Willems EW, Kapoor K, John GW, Saxena PR. Effects of donitriptan on carotid haemodynamics and cardiac output distribution in anaesthetized pigs. Cephalalgia 2002;22:37-47.
28. Villalón CM, De Vries P, Rabelo G, Centurión D, Sánchez-López A, Saxena PR. Canine external carotid vasoconstriction to methysergide, ergotamine and dihydroergotamine: a role of 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors. Br J Pharmacol 1999;126:385-394.
29. Martignoni E, Facchinetti F, Rossi F, Sances G, Genazzani AR, Nappi G. Neuroendocrine evidence of deranged noradrenergic activity in chronic migraine. Psychoneuroendocrinology 1989;14:357-363.
30. Facchinetti F, Martignoni E, Nappi G, Fioroni L, Sances G, Genazzani AR. Premenstrual failure of alpha-adrenergic stimulation on hypothalamus-pituitary responses in menstrual migraine. Psychosom Med 1989;51:550-558.
31. Fozard JR. Comparative effects of four migraine prophylactic drugs on an isolated extracranial artery. Eur J Pharmacol 1976;36:127-139.
32. Reichl R, Walland A. Inhibition of neurosympathetic cerebroarterial constriction by clonidine in cats. Eur J Pharmacol 1980;68:349-357.

33. Willems EW, Trion M, De Vries P, Heiligers JP, Villalon CM, Saxena PR. Pharmacological evidence that α_1- and α_2-adrenoceptors mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetized pigs. Br J Pharmacol 1999;127:1263-1271.
34. Willems EW, Heiligers JPC, De Vries P, Kapoor K, Tom B, Villalón CM, Saxena PR. α_1-adrenoceptor subtypes mediating vasoconstriction in the carotid circulation of anaesthetised pigs: possible avenues for antimigraine drug development. Cephalalgia 2001;21:110-119.
35. Piascik MT, Hrometz SL, Edelmann SE, Guarino RD, Hadley RW, Brown RD. Immunocytochemical localization of the α_{1B} adrenergic receptor and the contribution of this and the other subtypes to vascular smooth muscle contraction: analysis with selective ligands and antisense oligonucleotides. J Pharmacol Exp Ther 1997;283:854-868.
36. Knepper SM, Buckner SA, Brune ME, DeBernardis JF, Meyer MD, Hancock AA. A-61603, a potent α_1-adrenergic receptor agonist, selective for the α_{1A} receptor subtype. J Pharmacol Exp Ther 1995;274:97-103.
37. Docherty JR. Subtypes of functional α_1- and α_2-adrenoceptors. Eur J Pharmacol 1998;361:1-15.
38. Villalón CM, Ramirez-San Juan E, Castillo C, Castillo E, Lopez-Munoz FJ, Terrón JA. Pharmacological profile of the receptors that mediate external carotid vasoconstriction by 5-HT in vagosympathectomized dogs. Br J Pharmacol 1995;116:2778-2784.
39. Saxena PR. Cranial arteriovenous shunting, an *in vivo* animal model for migraine. In: Olesen J, Moskowitz MA, eds. Experimental headache models. Vol. 27. Philadelphia, USA: Lippincott-Raven Publishers, 1995:189-198.
40. Heyck H. Pathogenesis of migraine. Res Clin Stud Headache 1969;2:1-28.
41. Descombes J-J, Menant Y, Barou A, Cordi A, Verbeuren TJ. S19014 is a partial agonist at alpha-adrenoceptors that selectively contracts the veins. Pharmacol Toxicol 1998;83 (Suppl. 1):92.
42. De Vries P, Willems EW, Heiligers JPC, Villalón CM, Saxena PR. Constriction of porcine carotid arteriovenous anastomoses as indicator of antimigraine activity: the role of 5-$HT_{1B/1D}$, as well as unidentified receptors. In: Edvinsson L, ed. Migraine & headache pathophysiology. London: Martin Dunitz Ltd., 1999
43. MaassenVanDenBrink A, Reekers M, Bax WA, Ferrari MD, Saxena PR. Coronary side-effect potential of current and prospective antimigraine drugs. Circulation 1998;98:25-30.
44. Saxena PR, Tfelt-Hansen P. Triptan, 5-$HT_{1B/1D}$ receptor agonists in the acute treatment of migraine. In: Olesen J, Tfelt-Hansen P, Welch KMA, eds. The headaches. Philadelphia: Lippincott Williams & Wilkins, 2000:411-438.
45. Goadsby PJ, Edvinsson L. The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats. Ann Neurol 1993;33:48-56.
46. Durham P, Russo A. New insights into the molecular actions of serotonergic antimigraine drugs. Pharmacol Ther 2002;94:77.
47. Buzzi MG, Carter WB, Shimizu T, Heath H, 3rd, Moskowitz MA. Dihydroergotamine and sumatriptan attenuate levels of CGRP in plasma in rat superior sagittal sinus during electrical stimulation of the trigeminal ganglion. Neuropharmacology 1991;30:1193-1200.
48. Zagami AS, Lambert GA. Stimulation of cranial vessels excites nociceptive neurons in several thalamic nuclei of the cat. Exp Brain Res 1990;81:552-566.

49. Ebersberger A, Averbeck B, Messlinger K, Reeh PW. Release of substance P, calcitonin gene-related peptide and prostaglandin E2 from rat dura mater encephali following electrical and chemical stimulation in vitro. Neuroscience 1999;89:901–907.
50. Burstein R, Yarnitsky D, Goor-Aryeh I, Ransil BJ, Bajwa ZH. An association between migraine and cutaneous allodynia. Ann Neurol 2000;47:614-624.
51. Goadsby PJ, Edvinsson L, Ekman R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. Ann Neurol 1990;28:183-187.
52. Moskowitz MA, Cutrer FM. SUMATRIPTAN: a receptor-targeted treatment for migraine. Annu Rev Med 1993;44:145-154.
53. May A, Buchel C, Turner R, Goadsby PJ. Magnetic resonance angiography in facial and other pain: neurovascular mechanisms of trigeminal sensation. J Cereb Blood Flow Metab 2001;21:1171-1176.
54. Goadsby PJ, Lipton RB, Ferrari MD. Migraine--current understanding and treatment. N Engl J Med 2002;346:257-270.
55. Doggrell SA. Migraine and beyond: cardiovascular therapeutic potential for CGRP modulators. Expert Opin Investig Drugs 2001;10:1131-1138.
56. Goadsby PJ. New directions in migraine research. J Clin Neurosci 2002;9:368-373.
57. Filla SA, Winter MA, Johnson KW, Bleakman D, Bell MG, Bleisch TJ, Castano AM, Clemens-Smith A, del Prado M, Dieckman DK, Dominguez E, Escribano A, Ho KH, Hudziak KJ, Katofiasc MA, Martinez-Perez JA, Mateo A, Mathes BM, Mattiuz EL, Ogden AM, Phebus LA, Stack DR, Stratford RE, Ornstein PL. Ethyl (3S,4aR,6S,8aR)-6-(4-ethoxycar- bonylimidazol-1-ylmethyl)decahydroiso-quinoline-3-carboxylic ester: a prodrug of a GluR5 kainate receptor antagonist active in two animal models of acute migraine. J Med Chem 2002;45:4383-4386.
58. Sang CN, Ramadan NM, Chappell AS, Freitag FG, Smith TR, Silberstein SD, Tepper SJ. A double-blind, placebo-controlled study to investigate the efficacy and tolerability of 1.2 mg/kg intravenous LY293558 versus 6 mg subcutaneous sumatriptan versus placebo in patients with an acute migraine attack. Neurology 2001;56 (Suppl. 3):A218.
59. MacArthur L, Eiden L. Neuropeptide genes: targets of activity-dependent signal transduction. Peptides 1996;17:721–728.
60. Asakura K, Kanemasa T, Minagawa K, Kagawa K, Yagami T, Nakajima M, Ninomiya M. α-Eudesmol, a P/Q-type Ca^{2+} channel blocker, inhibits neurogenic vasodilation and extravasation following electrical stimulation of triegeminal ganglion. Brain Res 2000;873:94–101.
61. Silberstein SD. Review of botulinum toxin type A and its clinical applications in migraine headache. Expert Opin Pharmacother 2001;2:1649-1654.
62. Poungvarin N. The first world report of botulinum A toxin injection for status migrainosus. J Med Assoc Thai 2001;84:1199-1203.
63. Binder WJ, Brin MF, Blitzer A, Pogoda JM. Botulinum toxin type A (BOTOX) for treatment of migraine. Dis Mon 2002;48:323-335.
64. Honey AC, Bland-Ward PA, Connor HE, Feniuk W, Humphrey PP. Study of an adenosine A1 receptor agonist on trigeminally evoked dural blood vessel dilation in the anaesthetized rat. Cephalalgia 2002;22:260-264.
65. Goadsby PJ, Hoskin KL, Storer RJ, Edvinsson L, Connor HE. Adenosine A1 receptor agonists inhibit trigeminovascular nociceptive transmission. Brain 2002;125:1392-1401.
66. Akerman S, Williamson DJ, Kaube H, Goadsby PJ. Nitric oxide synthase inhibitors can antagonize neurogenic and calcitonin gene-related peptide induced dilation of dural meningeal vessels. Br J Pharmacol 2002;137:62-68.

67. Goldstein DJ, Roon KI, Offen WW, Ramadan NM, Phebus LA, Johnson KW, Schaus JM, Ferrari MD. Selective serotonin 1F (5-HT_{1F}) receptor agonist LY334370 for acute migraine: a randomised controlled trial. Lancet 2001;358:1230-1234.
68. Srikiatkhachorn A, Suwattanasophon C, Ruangpattanatawee U, Phansuwan-Pujito P. 2002 Wolff Award. 5 -HT2A receptor activation and nitric oxide synthesis: a possible mechanism determining migraine attacks. Headache 2002;42:566-574.
69. Brandes JL. Global Trends in Migraine Care: Results from the MAZE Survey. CNS Drugs 2002;16:13-18.

CHAPTER 9

Thesis summary

9. Thesis summary

9.1. Summary in English

Chapter 1 describes in brief the experimental models of migraine and its drug discovery from the age of triptans to the exploration of novel therapeutic targets, including α-adrenoceptors and the calcitonin gene related peptide receptors. Triptans, serotonin 5-$HT_{1B/1D}$ receptor agonists, serve as the mainstay for migraine treatment during the latter part of the twentieth century. Many migraine sufferers were relieved of their migraine in a way that they had not previously seen, clinical trial guidelines were redefined and revised and clinical studies were well organised and uniform. Sumatriptan has now been followed by other triptans: zolmitriptan, naratriptan, rizatriptan, almotriptan eletriptan and frovatriptan; donitriptan has finished preclinical evaluation and is currently in development. Ergotamine, the mainstay of specific acute treatment for most of the twentieth century after its initial description in the nineteenth century, now has few indications in which it is the treatment of choice. Most patients do prefer triptans when asked. However, some patients who would benefit from such treatment may not get them because of diagnostic or financial issues, and, in contrast, some patients overuse the medicines. Furthermore, sumatriptan is has some limitations, for example, low oral bioavailability, short half-life, metabolic breakdown via monoamine oxidase and its inability to cross the blood brain barrier. Therefore, there is utmost need for discovery of new molecules for migraine, which can overcome the limitations of the triptans.

As propounded initially, dilatation of intracranial and extracerebral arteries and arteriovenous shunts is involved in the pathophysiology of migraine. This has been demonstrated in the temporal arteries of migraineurs. Later, it was proposed that neurogenic inflammation, involving vasodilatation and plasma protein extravasation, could also play a role in migraine. A number of neuropeptides, including the calcitonin gene-related peptide (CGRP), are released, of which the latter plays a dominant role. In fact, an increase in CGRP levels in the jugular venous blood has been demonstrated in migraine and cluster headache patients, and the CGRP levels are normalised after treatment with sumatriptan. Thus, it appears that inhibition of CGRP release or its receptors could be beneficial in aborting migraine headaches. Accordingly, specific low molecular CGRP antagonists, like BIBN4096BS and SB-(+)-273779, are being developed. BIBN4096BS is the first CGRP antagonist under clinical trials in migraine patients.

A possible role of α-adrenoceptors as a new avenue for migraine therapy is also discussed. It has been shown that several acutely acting antimigraine agents, including the ergots (ergotamine and dihydroergotamine) and the triptans, produce a potent vasoconstriction in the canine and porcine carotid vasculature mediated by 5-$HT_{1B/1D}$ receptors. Interestingly, the canine carotid vasoconstrictor responses of the ergot alkaloids are mediated by 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors. The above lines of evidence, combined with the high affinity of ergotamine and dihydroergotamine at α-adrenoceptors, suggests that their therapeutic efficacy may partly be explained by an action mediated *via* α-adrenoceptors.

The experiments in the following chapters have been designed to elucidate therapeutic role of novel drugs like A61603, S19014, BIBN4096BS, donitriptan (F11356, F12640) and CGRP receptors in porcine model of migraine.

Chapter 2 is based on the evidence that CGRP may play an important role in the pathogenesis of migraine. Increased concentrations of immunoreactive α-CGRP have been observed in the jugular venous blood of migraineurs during the headache phase. We investigated the effects of the novel CGRP antagonist BIBN4096BS on the regional distribution of cardiac output and on the carotid and systemic haemodynamic changes induced by α-CGRP (10, 30 and 100 $pmol.kg^{-1}.min^{-1}$). BIBN4096BS (100, 300 and 1000 $\mu g.kg^{-1}$, i.v.) caused a small decrease in cardiac output, which was not significantly different from that in vehicle-treated animals but did not affect heart rate, mean arterial blood pressure or systemic vascular conductance. The highest dose of BIBN4096BS moderately decreased vascular conductance in the lungs, kidneys, spleen and adrenals. Vascular conductance in other tissues, including the brain, heart, gastrointestinal system, skin and skeletal muscles, remained unchanged. Consecutive intracarotid infusions of human α-CGRP increased total carotid conductance and blood flow and decreased mean arterial blood pressure as well as arteriovenous oxygen saturation difference ($A\text{-}VSO_2$); the responses to CGRP were dose dependently blocked by BIBN4096BS. These findings suggest that BIBN4096BS might be an effective drug in aborting migraine headaches with minimal side effects, but its therapeutic efficacy in migraine therapy will ultimately depend on its pharmacokinetic properties. The experiments also show that that the endogenous CGRP does not seem to play an important physiological role in regulating basal vascular tone.

Chapter 3. In this chapter we investigated the effects of CGRP receptor antagonist BIBN4096BS on capsaicin-induced porcine haemodynamic changes in a porcine model for migraine. Capsaicin produces trigeminal sensory neuron stimulation, leading to the release of CGRP in the pigs. Intracarotid infusions of capsaicin (0.3, 1, 3 and 10 $\mu g.kg^{-1}.min^{-1}$, i.c.) did not alter heart rate but caused an increase in the mean blood pressure, which was not modified by BIBN4096BS. Capsaicin significantly increased carotid blood flow and conductance in both arteriovenous anastomotic and capillary fractions and decreased $A\text{-}VSO_2$. These effects were dose-dependently antagonised by BIBN4096BS. Vascular conductances to the different tissues of the head, except that of salivary gland and brain, were also significantly increased by capsaicin and the response was antagonised by BIBN4096BS. As expected, capsaicin infusion more than doubled CGRP concentration in the jugular blood. Interestingly, the release of CGRP was potentiated by BIBN4096BS suggesting that blockade of prejunctional inhibitory CGRP autoreceptors by BIBN4096BS may have produced a positive feed back on the release of neuronal CGRP. The above results show that BIBN4096BS behaves as a potent antagonist of capsaicin-induced carotid haemodynamic changes that are mediated via the release of CGRP. Therefore, this compound may prove effective in the treatment of migraine.

Chapter 4. It has recently been shown that both α_1- and α_2-adrenoceptors mediate the constriction of porcine carotid arteriovenous anastomoses, but no attempt was made to identify the specific subtypes (α_{1A}, α_{1B} and α_{1D}) involved. Therefore, using the α_1-adrenoceptor antagonists, 5-methylurapidil (α_{1A}), L-765,314 (α_{1B}) and BMY 7378 (α_{1D}), the present study was designed to elucidate the specific subtype(s) of α_1-adrenoceptors involved in the above response. Intracarotid infusions of phenylephrine induced a dose-dependent decrease in total carotid and arteriovenous anastomotic conductance. These carotid vascular effects were abolished by L-765,314, and were only attenuated by 5-methylurapidil and BMY 7378. Furthermore, intravenous bolus injections of phenylephrine produced a dose-dependent pressor response, which was only affected by 5-methylurapidil, while the other antagonists were ineffective. These results, coupled with

the binding affinities of the above antagonists at the different α_1-adrenoceptor subtypes, suggest that both α_{1A}- and α_{1B}-adrenoceptors mediate constriction of carotid arteriovenous anastomoses in anaesthetised pigs. In view of the less ubiquitous nature of α_{1B}- compared to α_{1A}-adrenoceptors and a minor role played α_{1B}-receptors in the vasoconstriction of the peripheral blood vessels, the development of potent and selective α_{1B}-adrenoceptor agonists may prove to be important for the treatment of migraine.

Chapter 5. As stated in Chapter 4, the pharmacological profile of α_1-adrenoceptors mediating constriction of porcine carotid arteriovenous anastomoses resembles that of α_{1A}- and α_{1B}-adrenoceptor subtypes. In an attempt to verify the involvement of α_{1A}-adrenoceptors, we used a potent α_{1A}-adrenoceptor agonist A61603 and found that intracarotid administration of A61603 (0.3, 1, 3 and 10 $\mu g.kg^{-1}$) dose-dependently decreased porcine carotid blood flow and vascular conductance. This decrease was exclusively due to a constriction of carotid arteriovenous anastomoses; the capillary blood flow and conductance remained unchanged. Surprisingly, the responses to A61603 were little modified by prior treatment with 5-methylurapidil, prazosin or a combination of prazosin and rauwolscine. The 5-$HT_{1B/1D}$ receptor antagonist GR127935 and ketanserin also failed to modify carotid vascular responses to A61603, but, interestingly, methiothepin proved to be a potent antagonist. Taken together, the present results show that A61603 is a relatively poor agonist at the α_{1A}-adrenoceptor in pigs and that the carotid vasoconstriction produced by A61603 is mediated by a novel methiothepin-sensitive receptor/mechanism. A selective agonist at this novel receptor, without causing major systemic haemodynamic changes could well prove its worth in the management of migraine.

Chapter 6 assesses the antimigraine potential of a novel α-adrenoceptor agonist S19014. As stated above, both α_1- and α_2-adrenoceptors can mediate the constriction of porcine carotid arteriovenous anastomoses, which effectively serve as an experimental model predictive of antimigraine activity. S19014 (1, 3, 10 and 30 $\mu g.kg^{-1}$) produced an initial short lasting pressor response, but dose-dependently decreased total carotid and arteriovenous anastomotic blood flows (maximum change: 18±2% and 34±5%, respectively) and conductances (maximum change: 14±2% and 30±4%, respectively). In contrast, S19014 increased capillary blood flow and conductance (maximum change: 40±23% and 49±27%, respectively) and did not much affect the distribution of cardiac output to peripheral organs. Whereas prazosin was ineffective, rauwolscine attenuated the responses to S19014, thus establishing the involvement of one or more α_2-adrenoceptor subtypes. We further evaluated the human coronary side-effect potential of S19014 and compared it with sumatriptan. The maximal contraction to S19014 was smaller than that to sumatriptan, particularly in precontracted coronary arteries. The above results suggest that S19014 could be effective in the treatment of migraine with improved cardiovascular tolerance.

Chapter 7. Donitriptan is a new triptan that possesses a high affinity as well as efficacy at 5-$HT_{1B/1D}$ receptors. We investigated the effects of donitriptan on carotid haemodynamics and complete distribution of cardiac output in anaesthetised pigs. Donitriptan dose-dependently decreased total carotid blood flow and vascular conductance and this effect was entirely due to a selective reduction in the cephalic arteriovenous anastomotic fraction. The dose of donitriptan that decreased arteriovenous anastomotic conductance by 50% was found to be 47±16 $\mu g.kg^{-1}$ (92±31 $nmol.kg^{-1}$). Donitriptan did not decrease vascular conductances in or blood flows to a number of organs, including the heart and kidneys; in fact, vascular conductances in the skin, brain and skeletal muscles increased. Cardiac output

was slightly decreased by donitriptan, but this effect was also confined to the non-nutrient part (peripheral arteriovenous anastomoses). The 5-$HT_{1B/1D}$ receptor antagonist GR127935 substantially reduced the haemodynamic effects of donitriptan. These results show that donitriptan selectively constricts arteriovenous anastomoses via 5-HT_{1B} receptor activation. The drug should be able to abort migraine headaches without affecting blood flows to vital organs.

Chapter 8 discusses the findings of the present investigation in relation with the recent and ongoing efforts being made to further improve drug therapy in migraine. In parallel, the challenge is also to diagnose migraine early and offer patients appropriate and effective therapy. Physicians particularly need to reach patients who do not realise they have migraine and those who have lapsed from care.

9.2. Samenvatting in het Nederlands

Hoofdstuk 1 beschrijft kort de experimentele modellen van migraine en de ontdekking van medicijnen ervoor uit de tijd van triptanen tot de verkenning van nieuwe therapeutische doelen, waaronder α-adrenoceptors en de calcitonin gen-gerelateerde peptide (CGRP) receptoren. Triptanen, serotonine 5-$HT_{1B/1D}$ receptor agonisten, dienen als de voornaamste steunpilaar voor de behandeling van migraine aan het eind van de twintigste eeuw. Veel mensen met migraine werden van hun migraine verlost op een manier die nog nooit eerder waargenomen was, klinische richtlijnen voor proeven werden opnieuw gedefinieerd en aangepast en klinische studies werden goed georganiseerd en uniform gemaakt. Sumatriptan is nu opgevolgd door andere triptanen: zolmitriptan, naratriptan, rizatriptan, almotriptan eletriptan en frovatriptan; donitriptan is door de preklinische evaluatie heen en is momenteel in ontwikkeling. Ergotamine, het voornaamste middel voor de acute behandeling voor het grootste deel van de twintigste eeuw, is na de eerste beschrijvingen in de negentiende eeuw nu weinig in gebruik in de behandeling van migraine. De meeste patiënten hebben een voorkeur voor triptanen. Sommige patiënten die veel baat bij een dergelijke behandeling zouden hebben krijgen deze niet om diagnostieke of financiële redenen en, in contrast, gebruiken sommige patiënten de medicijnen te veel. Bovendien heeft sumatriptan enkele beperkingen, zoals bijvoorbeeld een lage orale biologische beschikbaarheid, een korte halfwaardetijd, afbraak door monoamine oxidase en het feit dat het niet door de bloed-hersenen barrière heen kan. Daarom is het hard nodig dat er nieuwe moleculen worden ontdekt voor migraine, waardoor de beperkingen van triptanen wegvallen.

Zoals in eerste instantie is geopperd, is de verwijding van intracraniale en extracerebrale bloedvaten betrokken bij de pathofysiologie van migraine. Dit is aangetoond in de temporale arteriën van migrainepatiënten. Later werd verondersteld dat neurogene ontsteking met plasma-eiwitlekkage ook een rol kan spelen bij migraine. Een aantal vrijgekomen neuropeptides, waaronder de CGRP, spelen later een dominante rol. Inderdaad, een toename van het CGRP gehalte in het bloed in de halsaderen is aangetoond bij patiënten met migraine en clusterhoofdpijn, welke werd teruggebracht tot een normaal niveau na behandeling met sumatriptan. Zodoende lijkt het erop dat het blokkeren van de afgifte van CGRP of de receptor bij kan dragen aan de verbetering van hoofdpijn bij migraine. Dienovereenkomstig hiermee worden specifieke lage moleculaire CGRP receptor antagonisten ontwikkeld, zoals BIBN4096BS en SB-(+)-273779. BIBN4096BS is de eerste CGRP antagonist dei klinisch getest wordt op migrainepatiënten.

Over een mogelijke rol van α-adrenoceptoren als een nieuwe manier van behandeling van migraine wordt ook gediscussieert. Het is aangetoond dat een aantal middelen ter bestrijding van een acute migraineaanval, waaronder de ergot alkaloïden (ergotamine en dihydroergotamine) en de triptanen, een krachtige vaatvernauwing veroorzaken in halsslagaders bij honden en varkens door middel van 5-$HT_{1B/1D}$ receptoren. Interessant is dat de respons van de ergot alkaloïden in de halsslagaders van honden door middel van 5-$HT_{1B/1D}$ receptoren en α_2-adrenoceptoren tot stand komt. De hiervoor beschreven bewijzen in combinatie met een hoge affiniteit van ergotamine en dihydroergotamine tot de α-adrenoceptoren, suggereren dat de therapeutische effectiviteit voor een deel verklaard kan worden door een actie *via* α-adrenoceptoren.

De experimenten in volgende hoofdstukken zijn opgezet om de therapeutische rol van nieuwe medicijnen zoals A61603, S19014, BIBN4096BS, donitriptan (F11356, F12640) en CGRP receptoren in het model van migraine bij varkens op te helderen.

Hoofdstuk 2 is gebaseerd op het bewijs dat CGRP een belangrijke rol kan spelen bij het ontstaan van migraine. Verhoogde concentraties van immuunreactieve α-CGRP zijn waargenomen in de halsslagaderen van migrainepatiënten tijdens de hoofdpijnfase. We onderzochten de effecten van de nieuwe CGRP antagonist BIBN4096BS op de regionale distributie van cardiale output en op systematische haemodynamische veranderingen veroorzaakt door α-CGRP bij verdoofde varkens. BIBN4096BS veroorzaakte een kleine afname in hartminutenvolume, wat niet veel verschilde van dat in de controle dieren, maar dit had geen effect op de hartslag, bloeddruk of systemische vasculaire geleiding. De hoogste dosis BIBN4096BS zorgde voor een matige afname van de bloedtoevoer naar de longen, nieren en bijnieren. Infusie van humaan α-CGRP in de halsslagader verhoogde de totale bloeddoorstroming in de halsslagader en verlaagde de gemiddelde bloeddruk evenals arterieveneuze zuurstofverzadigingsverschil (A-VSO_2); de reactie op CGRP werd doseringsafhankelijk geblokkeerd door BIBN4096BS. Deze vondst suggereert dat BIBN4096BS een effectief middel kan zijn om migrainehoofdpijn tegen te gaan met minimale bijwerkingen. De therapeutische effectiviteit in de behandeling van migraine zal echter uiteindelijk afhangen van de farmacokinetische eigenschappen van het middel.

Hoofdstuk 3. In dit hoofdstuk onderzochten we de effecten van de CGRP receptor antagonist BIBN4096BS op de door capsaicin veroorzaakte haemodynamische veranderingen in de halsslagader van een varkensmodel voor migraine. Capsaicin prikkelt sensorische neuronen, hetgeen leidt tot de afgifte van CGRP in de varkens. Infusie van capsaicin in de halsslagader gaf geen verandering in de hartslag maar veroorzaakte een toename van de gemiddelde bloeddruk, hetgeen niet beïnvloed werd door BIBN4096BS. Capsaicin zorgde voor een significante stijging van de bloeddoorstroming in de halsslagader alsmede in de arterieveneuze 'shunt' en capillair fracties; er was een afname in A-VSO_2. Deze effecten werden tegengegaan door BIBN4096BS. Vasculaire geleiding in de verschillende weefsels in het hoofd, behalve in de speekselklieren en de hersenen, nam ook significant toe door capsaicin en deze reactie werd tegengegaan door BIBN4096BS. Zoals verwacht verdubbelde de CGRP concentratie in het bloed in de halsslagaders na infusie van capsaicin. De afgifte van CGRP werd niet geremd maar zelfs versterkt door BIBN4096BS, wat zou betekenen dat blokkering van presynaptische CGRP autoreceptoren door BIBN4096BS een positieve feedback tot gevolg kan hebben op de afgifte van CGRP. Alles bij elkaar genomen onderschrijven deze resultaten het idee dat BIBN4096BS een veelbelovend antimigraine middel zou kunnen zijn.

Hoofdstuk 4. Onlangs is aangetoond dat stimulatie van zowel α_1- als α_2-adrenoceptoren leidt tot vernauwing van arterieveneuze anastomoses in het halsslagadergebied van varkens. Het is echter nog niet bekend welke specifieke receptor subtypen (α_{1A}, α_{1B} en/of α_{1D}) hierbij belangrijk zijn. Daarom was deze studie opgezet om de specifieke subtypen van α_1-adrenoceptoren die betrokken zijn bij de hiervoor beschreven reactie te definiëren, waarbij gebruik gemaakt is van de α_1-adrenoceptor antagonisten 5-methylurapidil (α_{1A}), L-765,314 (α_{1B}) en BMY 7378 (α_{1D}). Intra-arteriële infusie van phenylephrine veroorzaakte een afname van de doorstroming in de carotis arterieveneuze anastomosen. Dit effect werd geblokkeerd door L-765,314, en werd alleen verminderd door 5-methylurapidil en BMY 7378. Bovendien leidde de intraveneuze toediening van fenylephrine tot een dosisafhankelijke toename van de arteriële bloeddruk, welke alleen werd verminderd door 5-methylurapidil, terwijl de andere antagonisten ineffectief waren. Deze resultaten, gekoppeld met de binding affiniteiten van de bovengenoemde antagonisten tot de verschillende α_1-adrenoceptor subtypen, suggereren dat zowel α_{1A}- als α_{1B}-adrenoceptoren verantwoordelijk zijn voor de vernauwing van de arterieveneuze anastomoses in de halsslagadergebied van verdoofde varkens. Gezien de minder overheersende aard van α_{1B}- in vergelijking met α_{1A}-adrenoceptoren en het feit dat α_{1B}-receptors geen grote rol speelt bij de regulatie van de bloeddruk, kan de ontwikkeling van krachtige en selectieve α_{1B}-adrenoceptor agonisten belangrijk blijken te zijn voor de behandeling van migraine.

Hoofdstuk 5. Het onderzoek beschreven in hoofstuk 4 liet zien dat de α_1-adrenoceptoren, die de vernauwing van varken carotis arterieveneuze anastomosen veroorzaken mogelijk van α_{1A} en α_{1B} subtypen zijn. In een poging om de betrokkenheid van α_{1A}-adrenoceptoren te verifiëren, gebruikten we een potente α_{1A}-adrenoceptor agonist A61603 en hebben we vastgesteld dat intracarotide toediening van A61603 dosis-afhankelijk de doorbloeding en vasculaire geleiding in de halsslagader deed afnemen. Deze daling was geheel te wijten aan een selectieve vernauwing van de arterieveneuze anastomosen; de capillary doorbloeding en geleiding bleven onveranderd. Verassend was dat de reactie op A61603 maar weinig werd veranderd door 5-methylurapidil, prazosine of een combinatie van prazosine en rauwolscine. De 5-$HT_{1B/1D}$ receptor antagonist GR127935 en de 5-HT_2 receptor antagonist ketanserine slaagden er ook niet in om de vasculaire reactie op A61603 in de halsslagaders te veranderen, maar interessant genoeg bleek methiothepine een krachtige antagonist te zijn. Alles bij elkaar genomen laten de resultaten zien dat A61603 een relatief slechte agonist is voor de α_{1A}-adrenoceptor in de varkens en dat de vernauwing in de halsslagaderen dat door A61603 veroorzaakt werd, mogelijk verloopt via een nieuw, niet-adrenerg mechanisme.

Hoofdstuk 6 bestudeert de mogelijke toepassing van de nieuwe α-adrenoceptor agonist S19014 bij migraine. Zoals hierboven vermeld, kunnen zowel α_1- als α_2-adrenoceptoren de vernauwing van de arterieveneuze anastomosen in de halsslagadergebied van varkens bewerkstellen, hetgeen kan dienen als een experimenteel model om anti-migraine activiteit te voorspellen. Toediening van S19014 leidde tot een kortdurende verhoging van de bloeddruk, maar verlaagde de totale doorbloeding en vasculaire geleiding in de halsslagaders uitsluitend door een vernauwing van de arterieveneuze anastomosen. S19014 had echter niet veel invloed op het hartminutenvolume en de doorbloeding van de verschillende organen. Terwijl prazosine ineffectief was, verminderde rauwolscine de reactie op S19014. Verder hebben we de mogelijkheid van bijwerkingen van S19014 bij humane kransslagaders geëvalueerd en hebben deze met sumatriptan vergeleken. De maximale samentrekking bij S19014 was minder dan die bij sumatriptan, vooral in de van tevoren gecontraheerde

kransslagaderen. Bovenstaande resultaten suggereren dat S19014 effectief kan zijn bij de behandeling van migraine met mogelijk minder cardiovasculair risico.

Hoofdstuk 7. Donitriptan is een nieuwe triptaan, dat zowel een hoge affiniteit als effectiviteit heeft bij de 5-$HT_{1B/1D}$ receptoren. We onderzochten de effecten van donitriptan op de halsslagaderdoorbloeding en de complete distributie van het hartminutenvolume in verdoofde varkens. Donitriptan liet een dosisafhankelijk afname te zien in de totale doorbloeding en vasculaire geleiding in de halsslagaders en dit effect was volledig te wijten aan een selectieve vernauwing van de arterieveneuze anastomosen. De dosis donitriptan dat de vasculaire geleiding van de arterieveneuze anastomosen deed afnemen met 50% bleek 47±16 $\mu g.kg^{-1}$ (92±31 $nmol.kg^{-1}$) te zijn. Donitriptan-toediening leidde niet tot een vermindering van de vasculaire geleiding en doorbloeding van een groot aantal organen, waaronder het hart en de nieren; de vasculaire geleiding in de huid, hersenen en skeletspieren nam zelfs toe. Het hartminutenvolume nam licht af bij het gebruik van donitriptan, maar dit effect was ook te wijten aan de afname van arterieveneuze anastomotische doorbloeding. De 5-$HT_{1B/1D}$ receptor antagonist GR127935 deed de haemodynamische effecten van donitriptan aanzienlijk afnemen. Deze resultaten tonen aan dat donitriptan een selectieve vernauwing van de arterieveneuze anastomosen veroorzaakt door de activering van de 5-HT_{1B} receptor. Het middel zou hoofdpijn bij migraine moeten kunnen couperen, zonder dat het invloed heeft op de bloedtoevoer naar vitale organen.

CHAPTER 10

Appendix

10. Appendix

10.1. Acknowledgements

I seize this rare opportunity to express my deep sense of gratitude and affection for Prof. Dr. P.R. Saxena, my promotor, for offering me to work for a PhD in the cardiovascular laboratory. He has a dynamic personality with exceptional academic and managerial skills from whom I learnt a lot. Prof. Saxena is an authority in his field and I feel proud to have been under his guidance. I wish to attain the same heights and moral values of life as enshrined in him. I am indebted to Mrs. Mukta Saxena, his gracious wife, who always showered a motherly touch all through my stay in Holland and at times of need. God bless their family.

I sincerely thank Prof. Dr. A.H.J. Danser and Dr. R.G. Schoemaker, who helped me as and when, required during my doctoral period. My sincere thanks to Prof. Carlos Villalón, (México), who during his short visits to Rotterdam gave critical suggestions in my manuscripts and helped in my preparations for Departmental seminars.

Jan Heiligers, a man with lot of commitment and sincerity towards PhD fellows, a perfect technical and surgical hand and above all a talented artist, from whom I learnt all the surgical skills. He is an asset to this laboratory and with his help I could achieve my goal with ease. Thank you Jan, for all the help extended.

Edwin Willems, popularly known amongst us as 'Ed' or 'Eddyman', is a loveable personality. He introduced me to the "porcine model of migraine" and explained in detail all the computer programs associated with cardiovascular haemodynamics in addition to those related to microsphere experiments. I have shared some of the best moments of my stay in Holland with Edwin. I wish him all success for his future career endeavours.

I thank Dr. H.S. Sharma for his untiring help and constructive suggestions in my research work and the small sittings we shared discussing the practical exercises for medical students. He also introduced me to some applications of molecular techniques in pharmacology.

I also thank my colleagues and friends, Andor, Antoinette, Beril, Eric, Ingrid, Jasper, Martin, Pankaj, Peter, Rémon, Roeland, Sue, Uday, Wendy and Wenxia, with whom I shared some lighter moments of life. We remained as a family in the department and cheered each up other every now and then during our experiments.

I sincerely thank Mr.Corné Tak for his computer skill assistance, and Magda Busscher-Lauw and Birgitte Breemerkamp for their excellent secretarial assistance.

I would specially like to acknowledge my family members. My father, mother and my in-laws have been just the perfect parents and have been so loving and encouraging in my academics. My wife Nidhi and daughter Saumya were highly accommodative and patient during my study period. I also thank my sisters who called me quite often to boost my moral.

I thank the following persons for their generous gifts of chemical compounds: Christine Vayssettes-Courchay and Tony J. Verbeuren (S19014), Institut de Recherches Internationales Servier I.R.I.S., France; Dr. Henri Doods (CGRP), Boehringer Ingelheim Pharma KG,

Biberach, Germany; Dr. Gareth W. John (Donitriptan), Centre de Recherche Pierre Fabre, France.

I am indeed indebted to all the animals, which were sacrificed for the cause of mankind. In the end, without the blessings of God my present venture would have been impossible.

10.2. About the author

Kapil Kapoor was born in Lucknow, Uttar Pradesh, India on the 15th of December 1966. He graduated Intermediate in 1984 from Lucknow, India, where after he finished the Bachelor of Medicine and Bachelor of Surgery (M.B;B.S) in 1990; followed by Doctor of Medicine (M.D.) in Pharmacology in 1997 from the King George's Medical College, Lucknow, India. During his medical curriculum the Indian Pharmacological Society awarded him a paper of Honors in Physiology and Biochemistry and the U.K. Sheth Gold Medal for the best paper in Clinical Pharmacology.

Between 1997 to 1998 he worked as a part-time Medical Officer in a Ranbaxy Specialties Limited, New Delhi sponsored project on 'Clinical trials of sustained release diltiazem and ramipril in mild to moderate hypertension'. Thereafter, he was awarded a Research Associate fellowship in 1998 from the Council of Scientific and Industrial Research, New Delhi to work at Central Drug Research Institute, Lucknow. During this time his major research focused on a project entitled 'Neuropharmacological evaluation of Dementia models'. He was bestowed with 'Servier Young Investigator award' instituted by Institut De Recherches Internationales Servier, France for his work on Ginkgo biloba (extracts) and Bacosides as cognitive enhancers.

In the year 2000, he initiated his PhD-project under the supervision of Prof. Dr. P.R. Saxena, Chairman of the Department of Pharmacology of Erasmus Medical Center, Rotterdam, The Netherlands, on exploring new avenues in the management of migraine.

Presently, he has been appointed as a Scientist in the Pharmacology Division of Central Drug Research Institute, Lucknow, India.

10.3. Publications

10.3.1. M.D. (Pharmacology) Thesis, Lucknow University, Lucknow, India

Pharmacokinetic correlation of metronidazole toxicity with special reference to its neurotoxicity as measured electrophysiologically.

10.3.2. Full papers

1. Kapoor K, Willems E W, MaassenVanDenBrink A, Heiligers JPC, Vayssettes-Courchay C, Verbeuren TJ, Cordi A, Villalón CM, Saxena PR. Assessment of antimigraine potential of a novel α-adrenoceptor agonist S19014: effects on porcine carotid and regional haemodynamics and human coronary artery. Cephalalgia 2003;In press.
2. Kapoor K, Arulmani U, Heiligers JPC, Garrelds IM, Willems EW, Doods H, Villalón CM, Saxena PR. Effects of the CGRP receptor antagonist BIBN4096BS on capsaicin-induced carotid haemodynamic changes in anaesthetised pigs. Br J Pharmacol 2003;140:329-338.

3. Kapoor K, Arulmani U, Heiligers JPC, Willems EW, Doods H, Villalón CM, Saxena PR. Effects of BIBN4096BS on regional cardiac output distribution and on CGRP-induced carotid haemodynamic responses in the pig. Eur J Pharmacol 2003;475:69-77.

4. Tom B, De Vries P, Heiligers JP, Willems EW, Kapoor K, John GW, Saxena PR. Effects of donitriptan on carotid haemodynamics and cardiac output distribution in anaesthetized pigs. Cephalalgia 2002;22:37-47.

5. Willems EW, Heiligers JPC, De Vries P, Kapoor K, Tom B, Villalón CM, Saxena PR. α_1-adrenoceptor subtypes mediating vasoconstriction in the carotid circulation of anaesthetized pigs: possible avenues for anti-migraine drug development. Cephalalgia 2001;21:110-119.

6. Willems EW, Heiligers JP, De Vries P, Tom B, Kapoor K, Villalon CM, Saxena PR. A61603-induced vasoconstriction in porcine carotid vasculature: involvement of a non-adrenergic mechanism. Eur J Pharmacol 2001;417:195-201.

7. Das A, Kapoor K, Sayeepriadarshini AT, Dikshit M., Palit G, Nath C. Immobilisation stress induced changes in brain acetylcholinesterase activity and cognitive functions in mice. Pharmacol Res 2000;42:213-217.

8. Kapoor K, Chandra M, Nag D, Gupta RC, Paliwal JK, Saxena RC. An evaluation of metronidazole toxicity-A prospective study. Int J Clin Pharm Res 1999;19:83-88.

10.3.3. Book Chapters

1. Kapoor K, Nath C, Saxena RC. Pharmacological evaluation of anti-diabetic drugs. In: National Symposium on current trends in the management of Diabetes mellitus. Saxena RC (Editor), 1999, Lucknow, India.

2. Kapoor K, Saxena RC. Experimental evaluation of drugs with cardiovascular effects. In: Current trends in the management of coronary artery disease. Pant KK, Saxena RC (editors) 1997, Lucknow, India.

10.3.4. Abstracts

1. Kapoor K, Willems EW, Heiligers JPC, Saxena PR. Effects of BIBN4096BS, a novel CGRP antagonist, on capsaicin induced haemodynamic changes in anaesthetized pigs Pharmacologist 2002; 44 (2 suppl 1):A151.

2. Kapoor K, Nath C, Singh HK. A comparative pharmacological evaluation of Ginkgo biloba (extract) and bacosides as cognitive enhancers. Proceedings of International congress on frontiers in Pharmacology and Therapeutics in 21st century, New Delhi, 1999.

3. Kapoor K, Chandra A, Bagati A, Sood OP, Pant N, Saxena RC. A clinical study on efficacy and tolerability of sustained release (SR) diltiazem and ramipril in mild to moderate hypertension. Naunyn-Schmiedeberg's Arch Pharmacol 1998;358 (Suppl 1):1268.

4. Kapoor K, Chandra M, Nag D, Gupta RC and Saxena RC. Metronidazole neurotoxicity- a type 'C' drug reaction. Ind J Physiol Pharmacol 1997;41 (Supplement); Proceedings of 43rd Annual APPICON held at Lucknow, India, Dec. 27-29.

5. Kapoor K, Nag D, Saxena RC. Clinical, electrophysiological and pharmacokinetic evaluation of metronidazole toxicities. Ind J Pharmacol 1997;29.

6. Kapoor K, Nag D, Saxena RC. Neurophysiological changes due to metronidazole therapy - a cross over study. Proceedings of the First FAONS and First IBRO Regional Congress at Pattaya, Thailand, October 20-23, 1996.

7. Kapoor K, Saxena RC, Gupta RC, Paliwal JK, Chandra M. Pharmacokinetics of metronidazole in the healthy and diseased Indian population. Proceedings of the VI World Conference on Clinical Pharmacology and Therapeutics and V Congress of ISCPT at Buenos Aires, Argentina, August 4-9, 1996.

8. Kapoor K, Sharma B; Saxena RC. Study of serum phenobarbital levels in childhood seizures. Proceedings of XIV Indian Academy of Neurosciences Conference at Bombay, February 9-11, 1996.

9. Kapoor K, Pant KK, Nath C. Pro-depressant effect of verapamil. Proceedings of the fourth IBRO World Congress of Neurosciences, 9-14 July, 1995, Kyoto, Japan.

10. Kapoor K, Nag D, Saxena RC. Effect of sodium valproate on cognitive functions in epileptics 4th UP Neurosciences meet, 25 March 1995 at the Department of Neurology, King George's Medical College, Lucknow.

11. Kapoor K, Saxena RC, Agarwal GN, Bansal KM. Role of hormones and adaptogens in the management of cancer breast. Proceedings of the World Congress on Biotechnological developments in medicinal substances of plant and marine origin, 19-22 February, 1995, KGMC, Lucknow.

10.4. List of abbreviations

^{o}C	: Degrees Celsius
γ	: Gamma (radiation)
^{3}H	: Tritium-radiolabelled hydrogen
μg	: Microgram (10^{-6} g)
5-HT	: 5-Hydroxytryptamine (serotonin)
5-methylurapidil	: 5-Methyl-6[[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]amino]-1,3-dimethyluracil
A_1	: Adenosine receptor
ADM	: Adrenomedullin
A61603	: N-[5-(4,5-dihydro-1H-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydro-naphthalen-1-yl]methanesulphonamide
ANOVA	: Analysis of variance
AVA	: Arteriovenous anastomotic (arteriovenous anastomoses)
A-VSO_2	: Difference between arterial and jugular-venous oxygen saturation
BIBN4096BS	: (1-piperidinecarboxamide,N-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-piperazinyl]carbonyl]pentyl]amino]-1-[(3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2H)-quinazolinyl)-, [R-(R*,S*)]-)
BMY7378	: 8-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]-ethyl]-8-azaspiro[4,5 decane-7,9- dione dihydrochloride
BRL15572	: (1-(3-chlorophenyl)-4-[3,3-diphenyl(2-(*S,R*)hydroxypropanyl) piperazine
BTX-A	: Botulinum toxin-A

Ca^{2+} : Calcium ion
cAMP : Cyclic adenosine monophosphate
cGMP : Cyclic guanosine 5´-monophosphate
Ce : Cerium
CGRP : Calcitonin gene-related peptide
CINVESTAV (IPN): Center of Research and Advanced Studies (Instituto Politecnico Nacional)
CNS : Central nervous system
CP122288 : (5-methylaminosulphonylmethyl-3-(*N*-methoxy-pyrrolidin-2*R*-yl-methyl)-1*H*-indole)
CP99,994 : (2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenyl-piperidine
CRLR : Calcitonin receptor like receptor
CRW : Centraal Research Werkplaats
CSD : Cortical spreading depression
CYP3A4 : Cytochrome P450 isoenzyme
D_2 : Dopamine$_2$ receptor subtype
ECG : Electrocardiography
$ED_{50\%}$: The dose of a drug that, on average, changed baseline values of a variable by 50%
ERK : Extracellular signal regulated kinases
e.g. : For example
E_{max} : Maximal response
eNOS : Endothelial nitric oxide synthase
et al. : and colleagues
F12640 : 4-(4-(2-[3-(2-aminoethyl)-1H-indol-5-yloxyl]-acethyl)-piperazin-1-yl)-benzonitril mesylate
FP : Flow pulse
G : G-protein
GPCR : G-protein coupled receptor
GR127935 : N-[methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide hydrochloride
h : Hour(s)
HCl : Hydrochloric acid
HEK 293 : Human embryonic kidney cells
HR : Heart rate
5-HT : 5-Hydroxytryptamine; Serotonin
i.c. : Intracarotid
i.e. : Namely
IHS : International Headache Society
i.m. : Intramuscular
in : Intranasal
IP_3 : Inositol triphosphate
IUPHAR : International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification
i.v. : Intravenous
JNK : Jun amino terminal kinases
K^+ : Potassium ion
KeV : Kilo electro-volt (radioactive γ-radiation)
kg : Kilogram (10^3 g)

L-765,314 : 4-Amino-2-[4-[1-(benzyloxycarbonyl)-2(S)-[[(1,1-dimethylethyl) amino] carbonyl]-piperazinyl]-6,7-dimethoxyquinazoline
LY334370 : 4-fluoro-*N*-[3-(1-methyl-4-piperidinyl)-1*H*-indol-5-yl]-benzamide
M : Molar concentration (mol.litre^{-1})
MA : Massachusetts (USA)
MAP : Mean arterial blood pressure
MAPK : Mitogen activated protein kinase
mg : Milligram (10^{-3} g)
MIDAS : Migraine Disability Assessment scale
min : Minute(s)
ml : Milliliter(s)
mmHg : Millimeter mercury (pressure)
*m*RNA : Messenger RNA
n : Number of animals used
Na^+ : Sodium ions
NaOH : Sodium hydroxide
Nb : Niobium
ng : Nanogram (10^{-9} g)
NK : Neurokinin
NO : Nitric Oxide
nNOS : Neuronal nitric oxide synthase
NPY : Neuropeptide Y
P : Probability
pA_2 : Negative logarithm to base 10 of the molar concentration of the antagonist that makes it necessary to double the concentration of the agonist needed to elicit the original submaximal response
pCO_2 : Negative logarithm to base 10 of the carbon-dioxide (CO_2) concentration
PD_2 : Also referred as pEC_{50}
pEC_{50} : Negative logarithm to base 10 of an agonist concentration eliciting half the maximum effect
pH : Negative logarithm of base 10 of the hydrogen (H) concentration
PI : Phosphoinositol
pK_i : Negative logarithm of a concentration of a competing ligand in a competition assay that would occupy 50% of the receptors if no radioligand would be present
pO_2 : Negative logarithm of oxygen (O_2) concentration
RAMPs : Receptor associated membrane proteins
RBI : Research Biochemicals International (SIGMA-Aldrich)
RCPs : Receptor component proteins
RPR100893 : (3a*S*,4*S*,7a*S*)-7,7-diphenyl-4-(2-methoxyphenyl)-2-[(*S*)-2-(2-methoxyphenyl)proprionyl]perhydroisoindol-4-ol
Ru : Ruthenium
S19014 : spiro[(1,3-diazacyclopent-1-ene)-5:2'-(4',5'-dimethylindane)
SAP : Systolic blood pressure
SB224289 : 2,3,6,7-tetrahydro-1´-methyl-5-[2´-methyl-4´(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carbonyl]furo[2,3-*f*]indole-3-spiro-4´-piperidine hydrochloride
SB-273779 : N-methyl-N-(2-methylphenyl)-3-nitro-4-(2-thiazolyl sulfinyl)nitrobenzanilimide

Sc	: Scandium
SC	: Subcutaneous
s.d.	: Standard deviation
SEM	: Standard error of the mean
SK-N-MC	: Human neuroblastoma cell
Sn	: Tin
SP	: Substance P
TCBF	: Total carotid blood flow
SVC	: Systemic vascular conductance
TGN	: Trigeminal nerve
T_{max}	: Time to reach peak plasma concentration
$T_{1/2\beta}$	: Terminal elimination half life
TNC	: Trigeminal nucleus caudalis
UK	: United Kingdom
USA	: United Stated of America
V_d	: Volume of distribution
VIP	: Vasoactive intestinal polypeptide
vs.	: Versus