VASCULAR EFFECTS OF ANTIMIGRAINE DRUGS

PHARMACOLOGY OF HUMAN IN VITRO MODELS IN MIGRAINE

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VASCULAR EFFECTS OF ANTIMIGRAINE DRUGS

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VASCULAIRE ASPECTEN VAN ANTIMIGRAINE GENEESMIDDELEN

FARMACOLOGIE VAN HUMANE *IN VITRO* MODELLEN IN MIGRAINE

PROEFSCHRIFT

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Nederlandse Hoofdpijn Vereniging

to Loulou

to the memory of my grandparents

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Part I

Introduction

Here are the young men,
a weight on their shoulders
Here are the young men,
well where have they been?
We knocked on the doors
of hell's darker chambers
Pushed to the limits
we dragged ourselves in
Watched from the wings as
the scenes were replaying
We saw ourselves now as
we never have seen
Portrayal of the traumas and degeneration
The sorrows we suffered
and never were freed
*Where have they been**?***

Weary inside, now our hearts
lost forever
Can't replace the fear
or the thrill of the chase
These rituals showed up the door
for our wanderings
Opened and shut, then slammed
in our face
*Where have they been**?***

Joy Division – Decades

Chapter 1

Migraine

1.1 Epidemiology and diagnostic criteria

Migraine is defined as an idiopathic, paroxysmal neurological disorder with moderate to severe attacks of unilateral, throbbing headache exacerbated by physical activity. The migraine attack is accompanied by associated features such as nausea, vomiting, photophobia and phonophobia (Headache Classification Committee of the International Headache Society, 1988). Since migraine is a common illness, it imposes a tremendous health burden on both patient and society (Solomon & Price, 1997). Prevalence rates of migraine vary geographically and its occurrence is dependent on age (most common from age 25-55 years), gender (three times more common in women than in man) and income (affecting lower socio-economic groups more, see Lipton & Stewart, 1997; Silberstein & Lipton, 1996). In about one third of patients (Rasmussen & Olesen, 1992), an aura may precede the migraine headache within one hour (*migraine with aura*), consisting of focal neurological (scintillating scotoma), sensory (pins or needle feeling or numbness) and/or motor (weakness or paralysis) symptoms. The majority of patients, however, do not present such symptoms (*migraine without aura*) (Ferrari, 1998).

Migraine attacks *per se* are not necessarily an abnormal feature, considering that anyone may experience one or two migraine attacks in life. Migraine patients are therefore defined as individuals who have had at least two attacks with aura or at least five attacks without aura. To study migraine scientifically, the International Headache Society (IHS) provided some strict and uniform criteria to determine whether a patient is suffering from migraine (Headache Classification Committee of the International Headache Society, 1988) (see Table 1.1 for migraine with and without aura).

1.2 Pathophysiology

Migraine attacks seem to involve physiological mechanisms, which can be divided in three distinct phases: an initiating trigger, an aura and finally the headache (Ferrari, 1998). Attacks occur when the threshold is reduced or when the triggers are strong and frequent. Genetic factors, such as a P/Q-type calcium channel dysfunction (Ophoff *et al*., 1996), seem to act as predisposing factors. Internal and environmental

Table 1.1 IHS classification and diagnostic criteria for migraine without aura and migraine with aura (Headache Classification Committee of the International Headache Society, 1988).

Migraine without aura

A. At least five 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 location
    2. Pulsating quality
    3. Moderate or severe intensity
    4. Aggravation by walking upstairs 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 associated head trauma, vascular or non-vascular intracranial disorders, exposure to or withdrawal from (toxic) substances, non-cephalic infection, metabolic disorders or cranial or facial disorders
    2. History and/or physical and neurological examinations do suggest such disorder, but 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 to the disorder

Migraine with aura

A. At least two attacks fulfilling B
B. At least three of the following four 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 over more than 4 minutes, or two 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:
    1. History, physical and neurological examinations do not suggest associated head trauma, vascular or non-vascular intracranial disorders, exposure to or withdrawal from (toxic) substances, non-cephalic infection, metabolic disorders or cranial or facial disorders
    2. History and/or physical and neurological examinations do suggest such disorder, but 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 to the disorder

factors, such as hormonal fluctuations, fatigue, relaxation after stress, meteorological changes and substance abuse may modulate this threshold (Rasmussen, 1993).

Migraine generator

Although there is limited information available about the trigger phase of migraine, there are indications that the initial trigger involves the brainstem as 'migraine generator'. Positron emission tomography studies demonstrated that during the headache phase cerebral blood flow increased in cortical sensory association areas and unilaterally in the brainstem and that this activation persisted after complete relief from headache (Diener & May, 1996; Weiller *et al*., 1995). In healthy volunteers, capsaicin-provoked headache failed to demonstrate this brainstem activation (May *et al*., 1998).

Migraine aura

As mentioned above, in about one third of migraine patients an aura is experienced before the headache phase. The aura is associated with a reduction in cerebral blood flow (Olesen *et al*., 1981) that falls below a critical value. This decrease in cerebral blood flow may induce a 'cortical spreading depression', a short-lasting depolarisation wave starting at the occipital cortex and moving across the cortex at a speed of 3-5 mm/min, followed by a depression of neuronal activity (Lauritzen, 1994). Although cortical spreading depression has been demonstrated in animal models (Moskowitz *et al*., 1993; Read *et al*., 1997), definite evidence that this mechanism also applies in humans is lacking. However, the aura symptoms and the cortical spreading depression share characteristics, such as the velocity of its spread and the patterns of changes in blood flow, suggesting that both phenomena have a similar neurobiological origin.

Headache phase

The reduction in cerebral blood flow is followed by the headache phase, which is characterised by a vasodilatation of extracerebral large arteries and arteriovenous anastomoses (e.g. in the dura mater, base of the skull and scalp). This vasodilatation is probably due to changes in activity of the neurones innervating these blood vessels, with subsequent release of vasodilator peptides and transmitters. Immunohistochemical studies have demonstrated the presence of several vasodilators in perivascular nerve fibres supplying intracranial blood vessels, including vasoactive intestinal peptide (VIP), nitric oxide (NO), substance P, neurokinin A and calcitonin gene related peptide (CGRP) (for

review see Gulbenkian *et al*., 1999). Dilated cranial blood vessels lead to enhanced blood volume following each cardiac stroke and rapid diastolic runoff, with a consequent augmentation in pulsations within the affected blood vessel. These pulsations may well be responsible for the throbbing nature of the headache during a migraine attack. The augmented pulsations can then be sensed by 'stretch' receptors in the vessel wall activating perivascular afferent terminals of the trigeminal sensory nerve, which in turn, may also release neuropeptides. This release can consequently reinforce vasodilatation and perivascular sensory nerve activity, intensifying the migraine attack (see Saxena, 1994). Axonal conduction transmits nociceptive information towards the trigeminal nucleus caudalis and higher brain centres such as thalamus and hypothalamus for the registration of pain, photo- and phonophobia and nausea (Figure 1.1).

1.3 Current antimigraine drugs

Mild to moderate migraine attacks may be treated with specific and non-specific drugs including rapidly absorbable NSAIDs (aspirin, naproxen, diclofenac and tolfanemic acid), analgesics (paracetamol), narcotics (codeine, pethidine and morphine) or combination preparations (Diener *et al*., 1998; Ferrari, 1998; Limmroth & Diener, 1998). Antiemetic compounds such as metoclopramide and domperidone are able to speed up the gastric emptying and may thus, when taken early in the migraine attack, improve the absorption of other drugs (Diener *et al*., 1998; Ferrari, 1998). The choice of drug, dose and route of administration depends on the characteristics and frequency of the attack and on the specific preferences and contraindications of the patient (Ferrari, 1998).

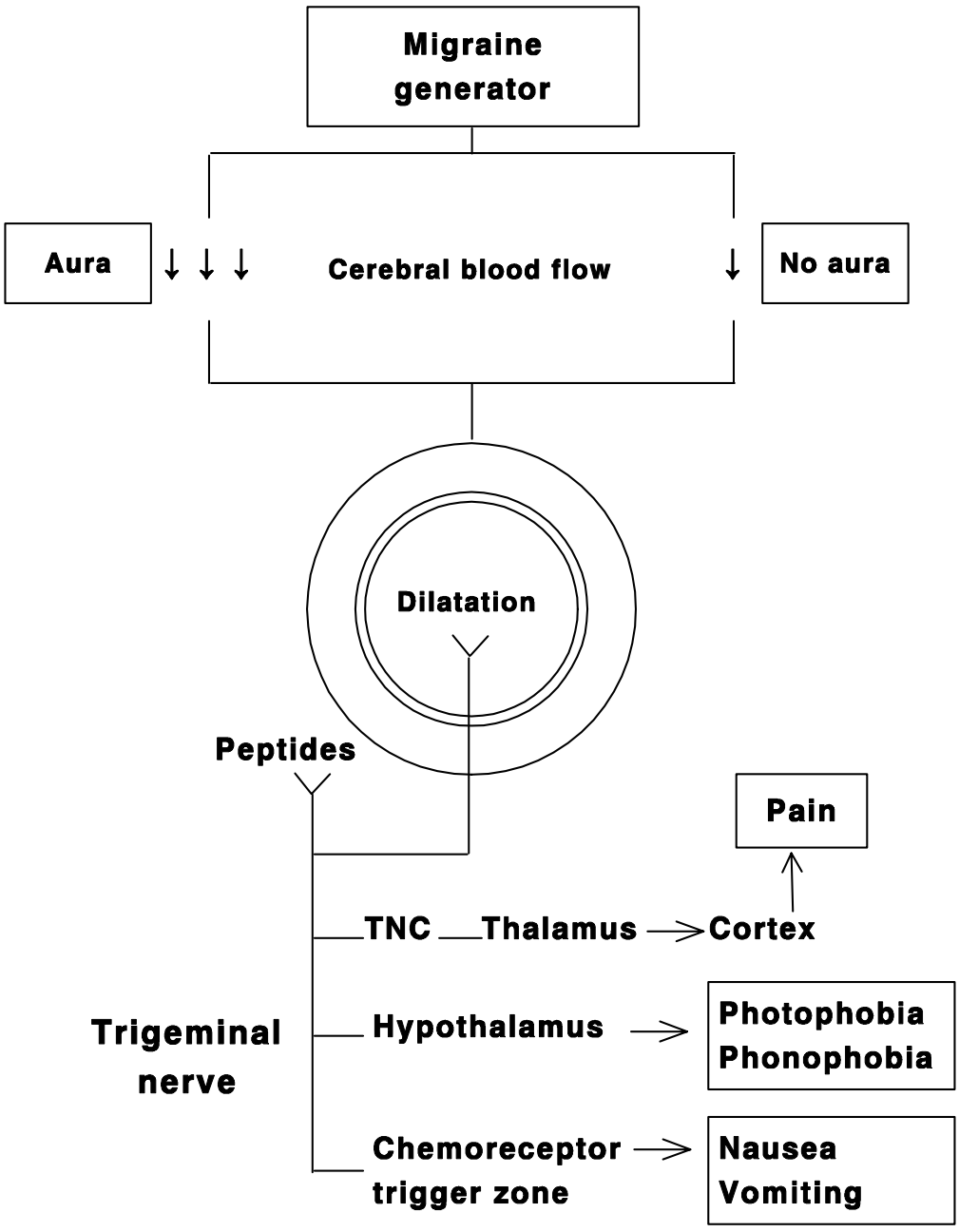

Figure 1.1 Diagram showing putative changes in migraine. For details see text. Based on De Vries *et al*., 1999 and Saxena, 1994.

Prophylactic drugs

The most important indications to start preventive treatment are headache frequency of more than two attacks per month, no or inappropriate response to, contraindication to, or overuse of acute therapy, or when optimal abortive therapies have produced intolerable side effects. The goals of migraine preventive therapy are to: (1) reduce attack frequency, severity and duration; (2) improve responsiveness to treatment of acute attacks: and (3) improve function and reduce disability. The mode of action of prophylactics is mostly unknown (Goadsby, 1997), and animal models to test the efficacy of the drugs are not available. Furthermore, while most modern clinical trials of acute migraine treatment rely on uniform endpoints with minor variations, endpoints in migraine prevention trials are more diverse (for review see Ramadan *et al*., 2000). In general, prophylactic treatment has a limited efficacy (Ramadan *et al*., 1997) and includes the α_2-adrenoceptor agonist clonidine (Louis *et al*., 1985), the β-blockers propranolol and metoprolol, the 5-HT_2 receptor antagonists methysergide, pizotifen and lisuride and the calcium channel blocker flunarizine (Diener *et al*., 1998).

Ergot alkaloids

For decades, ergot alkaloids have been the only specific drugs for the acute treatment of migraine. Although these drugs are widely used, their efficacy has been poorly demonstrated (Dahlöf, 1993; Meyler, 1996) mostly due to methodological flaws in the clinical trials, which, since 1991 have been improved and are now standardised by the IHS (International Headache Society Committee on Clinical Trials in Migraine, 1991). Ergotamine and dihydroergotamine are vasoconstrictors, but they also inhibit the neurogenic plasma extravasation in the rat (Buzzi *et al*., 1992). Ergotamine and dihydroergotamine display affinity for a high number of receptors, which include α-adrenoceptors, dopamine receptors and 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1F} and 5-HT_2 receptors (Pauwels, personal communication). This lack of selectivity probably explains the side-effect potential of the drugs such as nausea, vomiting, vertigo, gastric symptoms, dry mouth, restlessness and chest symptoms (Meyler, 1996). In addition, incidental overdose or chronic overuse may induce ergotism leading to cyanosis, necrosis and infarctions of the heart and brain (Tfelt-Hansen *et al*., 1995). Both ergot alkaloids have a low oral and rectal bioavailability, resulting in a large interpatient variability in the amount of drug reaching the circulation (Humbert *et al*., 1996; Little *et al*., 1982).

Furthermore, the clinical response is not related to the plasma concentration of the drug (Martin *et al.*, 1995; Tfelt-Hansen & Paalzow, 1985), which is probably due to the formation of active metabolites and the slow washout of the compounds from their receptor biosphere (MaassenVanDenBrink *et al.*, 1998; Martin *et al.*, 1995). Despite the number of potential side effects the ergot alkaloids are still widely used (for review and consensus see Tfelt-Hansen *et al.*, 2000).

Sumatriptan

In the last decade of the twentieth century, tremendous progress was made in the acute therapy of migraine. Sumatriptan (for chemical structure see Figure 1.2), a member of a new class of drugs known as 5-$HT_{1B/1D}$ (previously 5-HT_1-like) receptor agonists, has provided the lead (Humphrey *et al.*, 1990; Humphrey *et al.*, 1988). The introduction of sumatriptan by Humphrey and colleagues was based on the findings that: (1) urinary excretion of 5-hydroxyindole acetic acid (5-HIAA) increases (Sicuteri *et al.*, 1961), while platelet 5-HT decreases during migraine attacks (Hinterberger *et al.*, 1968); (2) migraine-like symptoms can be precipitated by reserpine and alleviated by 5-HT: and (3) ergotamine and methysergide elicit a selective carotid vasoconstriction (at least partly via 5-HT_1-like receptors), which is confined to cephalic arteriovenous anastomoses that seem to be involved in migraine pathophysiology (De Vries *et al.*, 1999; Ferrari & Saxena, 1993; Saxena & Tfelt-Hansen, 2000). Based on the above, tryptamine derivatives were synthesised to achieve selectivity at craniovascular 5-HT_1-like receptors and this culminated in the design of sumatriptan (Humphrey & Feniuk, 1991).

The discovery of the relatively selective 5-HT_1 (i.e. 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1F} and to a minor extent 5-HT_{1A}, see Table 1.2) receptor agonist sumatriptan was a major improvement in the acute treatment of migraine. The drug is highly effective and is generally well tolerated (Brown *et al.*, 1991; The Subcutaneous Sumatriptan International Study Group, 1991). Despite its great utility in migraine treatment, sumatriptan has certain limitations such as low oral bioavailability (14%), high frequency of headache recurrence (20-40%, Fowler *et al.*, 1991; Lacey *et al.*, 1995), possibly due to a short half-life, and adverse events of which heaviness, pressure or tightness in the chest are reported in up to 15% of patients (Brown *et al.*, 1991). Furthermore, the drug is contraindicated in patients with coronary artery disease because of its potential to constrict coronary arteries (see Chapter 3). These shortcomings of sumatriptan, in addition to the excellent sales potentials in migraine, have prompted several pharmaceutical companies to

develop 5-$HT_{1B/1D}$ receptor agonists (the so-called 'second generation' triptans), which would be at least as effective as sumatriptan, but devoid of its shortcomings.

Second generation triptans

As mentioned above, several companies developed 5-$HT_{1B/1D}$ receptor agonists (for chemical structures see Figure 1.2) with a particular aim for a higher oral bioavailability, a longer duration of action with lower headache recurrence, greater selectivity for the carotid over the coronary vascular bed and with fewer if any chest related symptoms. Since the introduction of sumatriptan, five second-generation triptans have been marketed (zolmitriptan, naratriptan, rizatriptan, eletriptan and almotriptan), while frovatriptan is still awaiting approval by the Food and Drug Administration and European Medicines Evaluation Agency (Palmer & Dalton, 1999). Donitriptan (F11356, John *et al.*, 1999) and IS-159 (Dingemanse *et al.*, 2000) are still in the earlier phases of investigation. The clinical development of a few others has been discontinued because of lack of advantage compared to sumatriptan (alniditan and BMS181885) or because of severe alteration in hepatic function (avitriptan). In general, the second-generation triptans display a higher affinity at the 5-HT_{1B} and 5-HT_{1D} receptors than sumatriptan, whereas for other 5-HT receptors the binding affinity profile varies (see Table 1.2 and Chapter 2 for details on the pharmacology of the triptans). Pharmacokinetically, the second-generation triptans display improved oral properties compared to sumatriptan (see Table 1.3), oral bioavailability is much higher and more consistent and they proclaim a substantial increased lipophilicity and thus can cross the blood brain barrier for a possible central action (see Chapter 2). Clinical efficacy data obtained in randomised clinical trials with and between the triptans are currently under evaluation (for reviews on clinical data see Deleu & Hanssens, 2000; Goadsby, 1998; Roon, 2000; Saxena & Tfelt-Hansen, 2000) and show that the second-generation triptans display similar pharmacotherapeutic properties as compared to sumatriptan. Furthermore, all triptans caused typical adverse events, or so-called triptan symptoms (Mathew, 1997), which vary for the different triptans but all include heaviness, and pressure or tightness in the chest (Saxena & Tfelt-Hansen, 2000). As was observed with sumatriptan, all second-generation triptans have the propensity to contract the human isolated coronary artery and are, therefore, contraindicated in patients with coronary artery disease (Saxena & Tfelt-Hansen, 2000).

Overall, more head to head clinical trials are needed to determine the place of each of these 5-$HT_{1B/1D}$ receptor agonists. Although differences among the triptans appear to

be small, the pharmacokinetics vary and, therefore, the availability of effective therapeutic options may improve the management of individual patients.

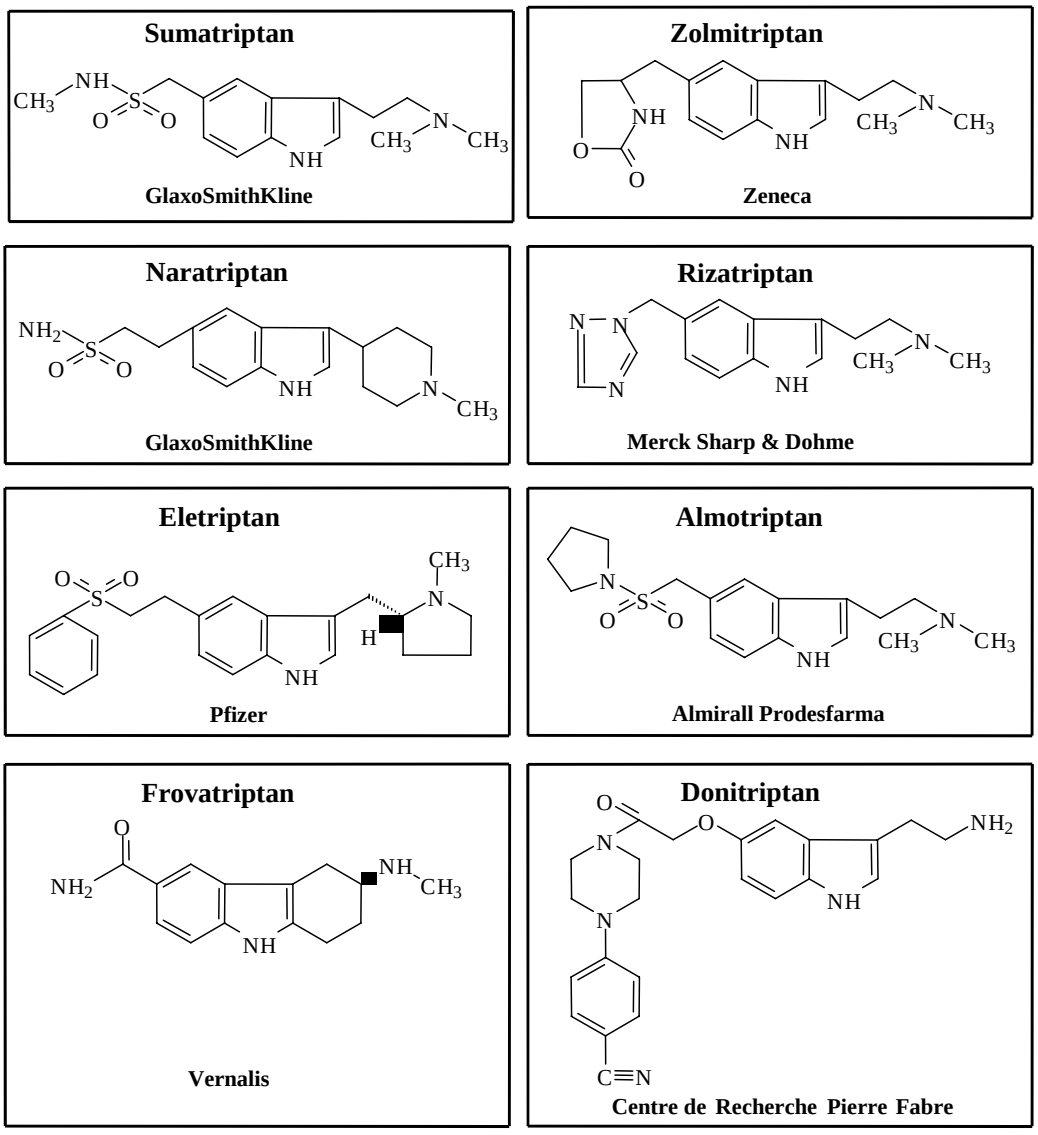

Figure 1.2 Chemical structure of triptans

Chapter 2

Mechanisms of action of antimigraine drugs

2.1 5-HT$_1$ receptor agonists

The introduction of sumatriptan resulted in extensive research investigating the therapeutic targets of acutely acting antimigraine drugs. The vascular theory launched by Wolff suggested that the migraine aura is caused by intracranial vasoconstriction, which is countered by a reactive vasodilatation of the large extracranial arteries causing the headache (Wolff, 1963). Furthermore, the observations as mentioned in Chapter 1.3 (increase of 5-HIAA urinary excretion, precipitation of migraine symptoms by reserpine and alleviation by 5-HT and a selective carotid vasoconstriction by ergotamine) suggest a pivotal role for 5-HT in arterial vasoconstriction (see Saxena, 1990) and was the rationale behind the introduction of sumatriptan. Although originally developed to induce contraction of putatively distended cranial blood vessels via postsynaptic 5-HT$_1$ receptors (Humphrey *et al*., 1990; Humphrey & Feniuk, 1991), sumatriptan was also found to interact with presynaptic receptors on trigeminovascular afferents, whose activation is important in the development of meningeal neurogenic inflammation (Humphrey & Goadsby, 1994, for details see below). Since then, sumatriptan-sensitive sites of action in the pain-transmitting pathways have been identified centrally, suggesting an additional mechanism of action within the central nervous system (Goadsby, 1997, for details see below).

Receptor-binding profile

Sumatriptan, a closely related 5-HT derivative, was developed as the first cranioselective vasoconstrictor, designated as a 5-HT$_1$-like receptor agonist (Humphrey *et al*., 1990; Humphrey *et al*., 1988) devoid of the peripheral vasoconstrictor properties mediated via the 5-HT$_2$ receptor (Bradley *et al*., 1986). Since the introduction of sumatriptan the 5-HT$_1$-like receptors have been characterised and subdivided in 5-HT$_{1A}$, 5-HT$_{1B}$ (previously known as 5-HT$_{1D\beta}$), 5-HT$_{1D}$ (5-HT$_{1D\alpha}$), 5-HT$_{1E}$, 5-HT$_{1F}$ and 5-HT$_7$ receptors (Hoyer *et al*., 1994; Hoyer & Martin, 1997; Martin & Humphrey, 1994). Sumatriptan and the second-generation triptans display a high affinity at the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Table 1.2), with sumatriptan and rizatriptan being the weakest at the 5-HT$_{1B}$ receptor. All triptans have a moderate affinity at the 5-HT$_{1A}$ receptor. Similarly, the compounds have a

moderate to high affinity for the 5-HT_{1F} receptor, except donitriptan, IS-159 and the non-triptan alniditan (Leysen *et al*., 1996). A high degree of selectivity is observed over the other 5-HT receptors, although some triptans display affinity at the 5-HT_7 receptor, which mediates smooth muscle relaxation (Eglen *et al*., 1997; Saxena *et al*., 1998). Finally, it is worth mentioning that donitriptan displays a moderate affinity at the 5-HT_{2A} receptor.

Craniovascular 5-HT$_1$ receptors

As shown in Table 1.4 and further described in Chapter 3, a number of human isolated blood vessels contract in response to second-generation triptans with a potency that is similar to that of sumatriptan (Bou *et al*., 2000; Kaumann *et al*., 1993; Longmore *et al*., 1998; MaassenVanDenBrink *et al*., 2000; Nilsson *et al*., 1999; Parsons *et al*., 1998; Razzaque *et al*., 1999; van den Broek *et al*., 2000). Comparative pharmacological studies revealed a positive correlation between the vascular 5-HT_1 receptor, mediating cerebral artery contraction, and the cloned human 5-HT_{1B}, but not the 5-HT_{1D}, receptor. This latter observation indicates that the 5-HT_{1B} receptor is the target for the triptans in cranial blood vessels (Hamel & Bouchard, 1991; Hamel *et al*., 1993; Nilsson *et al*., 1999; Razzaque *et al*., 1999). Other studies demonstrated expression of the 5-HT_{1B}, but not 5-HT_{1D}, receptor mRNA (Bouchelet *et al*., 2000; Hamel *et al*., 1993; Razzaque *et al*., 1999; Verheggen *et al*., 1998) and protein (Longmore *et al*., 1998; Nilsson *et al*., 1999) in human cranial vessels. In addition to 5-HT_{1B} receptors, expression of 5-HT_{1F} receptor mRNA has been detected (Bouchelet *et al*., 2000). But, as was observed for the cloned 5-HT_{1D} receptor, no correlation between the vascular 5-HT_1 and the 5-HT_{1F} receptor was observed (Hamel *et al*., 1993; Kaumann *et al*., 1993). Furthermore, the lack of contractile effects of the selective 5-HT_{1F} receptor agonists LY334370 and LY344864 in human isolated cerebral arteries (Bouchelet *et al*., 2000; Elhusseiny & Hamel, 2001) imply that this receptor does not mediate vasoconstriction. Interestingly, LY334370 seems to be effective in the treatment of migraine (Gossen *et al*., 2000; Granier *et al*., 2000), suggesting that 5-HT_{1B} receptor-induced vasoconstrictor activity may not be required for antimigraine activity. Unfortunately, it has not been demonstrated that the plasma concentrations reached after administration of LY334370 are devoid of vasoconstrictor activity, based on its moderate affinity for the 5-HT_{1B} receptor (pK_i: 6.87; Johnson *et al*., 1997). In addition, no data are available on vasoactive metabolites as is the case for zolmitriptan (Peck *et al*., 1998) and rizatriptan (Goldberg *et al*., 2000). Be that as it may, the effectivity of LY334370 in

migraine has no bearing on the importance of the 5-HT_{1B} receptor in mediating the therapeutic action of the triptans, as illustrated by the clinical efficacy the non-triptan, alniditan (Roon *et al*., 1999), which has little affinity for the 5-HT_{1F} receptor (Leysen *et al*., 1996).

Peripheral 5-HT_1 receptors on trigeminovascular afferents

Moskowitz and colleagues extensively studied the implication of the trigeminovascular system in the manifestation of migraine pain, suggesting a possible neuronal locus of action of sumatriptan that involves blockade of nociceptive information (Moskowitz *et al*., 1979). The trigeminal fibres innervating human cerebral vessels contain vasodilator neuropeptides substance P, neurokinin A and CGRP (Edvinsson, 1991), of which the latter is released after depolarisation of the trigeminovascular ganglion or its perivascular nerve terminals (Goadsby *et al*., 1988). The release of vasodilator neuropeptides subsequently leads to the development of a neurogenic inflammation. Furthermore, CGRP is released into the extracerebral circulation during a migraine attack (Goadsby *et al*., 1990) and these levels are normalised after administration of sumatriptan in parallel with headache relief (Goadsby & Edvinsson, 1993). Sumatriptan may block the release of neuropeptides and the subsequent dural plasma protein extravasation through inhibitory presynaptic 5-HT_1 receptors, located on trigeminovascular afferents (Buzzi *et al*., 1992; Buzzi & Moskowitz, 1991). Interestingly, mRNAs encoding for the 5-HT_{1B} and 5-HT_{1D} receptor (Bouchelet *et al*., 1996), as well as the receptor proteins (Longmore *et al*., 1997) are present on human trigeminal ganglia. Peripherally located nerve terminals, however, only contain the 5-HT_{1D} receptor (Longmore *et al*., 1997), indicating that this presynaptic receptor is most likely the target for sumatriptan in controlling neurogenic inflammation. Indeed, the triptans (although yet to be established for frovatriptan and IS-159) inhibit plasma protein plasma extravasation following stimulation of the trigeminal nerve (Limmroth *et al*., 2001; Gras *et al*., 2000; Gupta *et al*., 2000; Hargreaves & Shepheard, 1999; Limmroth *et al*., 1999) in animal models. However, angiography techniques failed to demonstrate plasma protein extravasation in patients during a migraine attack (May *et al*., 1998). Interestingly, the selective 5-HT_{1D} receptor agonist PNU-142633 (McCall, 1999), as well as other potent plasma protein extravasation inhibitors, such as the endothelin receptor antagonist bosentan (May *et al*., 1996) and the Neurokinin-1 receptor antagonist lanepitant (Goldstein *et al*., 1997), proved to be ineffective in the treatment of migraine. Furthermore, CP122288, a restricted sumatriptan derivative that has superior efficacy in

inhibiting plasma protein extravasation compared to sumatriptan (over a thousand fold, see Shepheard *et al*., 1997), was ineffective in the treatment of migraine at doses devoid of vasoconstrictor action (Roon *et al*., 2000). Another receptor active in plasma protein extravasation is the 5-HT_{1F} receptor. The selective 5-HT_{1F} receptor agonists LY334370 and LY344864 inhibit plasma protein extravasation in rat and guinea pig (Johnson *et al*., 1997; Phebus *et al*., 1997). These observations, together with the antimigraine activity of LY334370 (see above), may prove that the 5-HT_{1F} receptor is an interesting new presynaptic target for the acute treatment of migraine. As mentioned in the previous paragraph, the effective antimigraine drug alniditan lacks affinity at the 5-HT_{1F} receptor, indicating that antimigraine efficacy does not require activation of this receptor.

Central 5-HT_1 receptors in the trigeminal nucleus caudalis

An additional level of interest in the acute treatment of migraine has been the cells located centrally in the trigeminal nucleus caudalis that transduce intracranial noiciceptive information to higher integrative centres. These cells represent an attractive neuronal therapeutic target that could be dissociated from vascular effects. Interestingly, binding sites that recognise 5-HT_1 receptor ligands, such as sumatriptan, have been localised at this level in humans (Pascual *et al*., 1996). Goadsby and colleagues have demonstrated that intravenous administration of zolmitriptan (Goadsby & Edvinsson, 1994), naratriptan (Goadsby & Knight, 1997) or eletriptan (Goadsby & Hoskin, 1999) inhibited action potentials generated in the trigeminal nucleus caudalis after superior sagittal sinus stimulation, as well as *c-fos* mRNA expression, indicating neuronal activity (Hoskin *et al*., 1999). Similarly in rats, rizatriptan (Cumberbatch *et al*., 1997) and eletriptan (Knyihar-Csillik *et al*., 2000) inhibit such potentials evoked by dural stimulation. However, due to its poor brain penetration, intravenous sumatriptan did not alter trigeminal evoked activity (Kaube *et al*., 1993), nor did it affect *c-fos* expression in the trigeminal nucleus caudalis following trigeminal ganglion stimulation (Shepheard *et al*., 1995). This raises the question of whether central trigeminal inhibition is a key factor in the action of 5-$HT_{1B/1D}$ receptor agonists. On the other hand, it remains to be clarified whether during migraine headaches the blood-brain barrier is partly disrupted. Indeed, after infusion of hyperosmolar mannitol, which disrupts the blood-brain barrier, both trigeminal evoked activity (Kaube *et al*., 1993) and *c-fos* expression (Shepheard *et al*., 1995) were inhibited by sumatriptan. Additional 5-HT_1 receptors in the nucleus tractus solitarus and area prostrema (Pascual *et al*., 1996), regions that are devoid of blood-brain barrier, could be

accessible even to non-brain-penetrant compounds such as sumatriptan and potentially explain some of their antiemetic effects. The exact nature of the 5-HT_1 receptor subtype involved in the central inhibition in human remains to be clarified. On the basis of receptor mRNA and protein localisation in the trigeminal nucleus caudalis (see above) one can argue for the 5-HT_{1D} receptor (Longmore *et al*., 1997) or even the 5-HT_{1F} receptor. 5-HT_{1F} receptor involvement is supported by observations in rat, where LY334370 inhibited activation of second-order neurons in the trigeminal nucleus caudalis produced by electrical stimulation of the dura mater (Shepheard *et al*., 1999). The recent and forthcoming development of 5-HT_1 receptor subtype-specific compounds will help to further identify which receptor subtype is involved in the central action of the triptans.

2.2 5-HT_{2B} receptor antagonists

Although the exact role of 5-HT in the pathogenesis of migraine headache remains unknown, it has been hypothesised that migraine is a low serotonergic syndrome, primarily based on physiological studies on the evaluation of auditory evoked potentials. The amplitude of these evoked potentials is inversely related to central serotonergic transmission and in migraine patients a marked increase in amplitude was observed between attacks (Wang *et al*., 1996). This latter finding supports a low 5-HT transmission and abnormal cortical processing of sensory information (Hegerl & Juckel, 1993). It has been suggested that the optimal goal in migraine management may be to stabilise 5-HT neurotransmission (Raskin, 1993) since both neuronal and platelet stores of 5-HT are mobilised during a migraine attack (Humphrey, 1991). There is considerable circumstantial evidence that the 5-HT_{2B} receptor on human cranial blood vessels is activated by release of 5-HT and causes vasodilatation followed by a sequence of events leading to the migraine headache (Fozard & Kalkman, 1994; Schmuck *et al*., 1996). Clinical evidence to support the role of the 5-HT_{2B} receptors in the development of migraine comes from the characteristics of some of the most common used migraine prophylactics, such as pizotifen, methysergide, cyproheptadine, amitriptyline, mianserin, chlorpromazine and propranolol which are non-selective antagonists at the 5-HT_2 receptor (Goadsby, 1997). On the other hand, the effectiveness of these drugs in migraine has been debated (Tfelt-Hansen & Saxena, 2000). However, administration of the 5-HT_{2B} receptor agonist meta-chlorophenylpiperazine (mCPP), yielding plasma concentrations compatible with activation of this receptor, produces a throbbing migraine-like headache accompanied

by nausea, vomiting and photophobia (Brewerton *et al*., 1988; Leone *et al*., 2000). Endothelial NO formation, occurring as a consequence of 5-HT_{2B} receptor activation, appears to play a pivotal role with regard to both vasodilatation and plasma protein extravasation (Fozard & Kalkman, 1994; Olesen *et al*., 1995; Schmuck *et al*., 1996). Furthermore, migraine sufferers appear to be supersensitive to NO (in the form of nitro-glycerine or glyceryl trinitrate) exposure (Olesen *et al*., 1993; Olesen *et al*., 1994; Thomsen *et al*., 1993). NO, once released from the endothelial cells, can diffuse into the smooth muscle layer to induce dilatation of pain-sensitive intracranial arteries and also has the potential to activate trigeminovascular sensory nerve terminals that contain substance P and CGRP (Wei *et al*., 1992). The success of a non-selective NO synthetase inhibitor L-NG-methylarginine hydrochloride in the treatment of migraine headache (Lassen *et al*., 1998) supports this hypothesis. It should be mentioned, however, that the mCPP- or nitro-glycerine-induced headache is at its peak 8-12 hours after administration (Brewerton *et al*., 1988; Olesen *et al*., 1993), suggesting a still unknown mechanism after the initial NO formation, following 5-HT_{2B} receptor activation. Based on the premise that the release of 5-HT and subsequent activation of endothelial 5-HT_{2B} receptors are early and important steps in the pathogenesis of migraine headache, clinical trials with specific 5-HT_{2B} receptor antagonists could clarify the role for this link in migraine pathogenesis.

2.3 5-HT_7 receptor antagonists

A putative role of the 5-HT_7 receptor in the regulation of cerebrovascular tone and migraine has been postulated on the basis of the ability of 5-HT to produce smooth muscle relaxation, through an endothelium-independent mechanism, in dog cerebral arteries (Terron, 1998; Terron, 1998; Terron & Falcon-Neri, 1999) via a receptor with a pharmacological profile that resembles the 5-HT_7 receptor. Furthermore, there is a high expression of 5-HT_7 transcripts in animal and human brain vessels (Schmuck *et al*., 1996; Schoeffter *et al*., 1996; Ullmer *et al*., 1995). The 5-HT_7 receptor is positively coupled to adenylate cyclase (Tsou *et al*., 1994), increasing cyclic adenosine monophosphate (cAMP) in cultured smooth muscle cells from human brain vessels (Cohen *et al*., 1996). It is thus possible that a similar relaxant mechanism operates in human brain vessels (via serotonergic nerve terminals located at the adventitial-medial border) by eliciting a direct dilatation of meningeal blood vessels without an interaction with the endothelial cells. Interestingly, most antimigraine prophylactics display a relatively high affinity at the

cloned 5-HT_7 receptor (Terron, 1998) and are able to block 5-HT_7 receptor-induced relaxation (Terron & Falcon-Neri, 1999). The recent development of selective 5-HT_7 receptor antagonists (Forbes *et al*., 1998; Lovell *et al*., 2000) may fully elucidate the role of the 5-HT_7 receptor in human cranial vessels and its function in the development of migraine headache.

2.4 CGRP receptor antagonists

Calcitonin gene-related peptide (CGRP) is a 37-amino acid peptide produced by alternative processing of calcitonin gene transcripts (Amara *et al*., 1982). CGRP containing nerves are closely associated with blood vessels and upon release of CGRP, a pronounced vasodilator response is observed in cerebral arteries (Edvinsson *et al*., 1987; Jansen *et al*., 1992). Furthermore, CGRP released from sensory fibres originating in the trigeminal ganglia, dilates cerebral arteries (Goadsby & Edvinsson, 1993) and during a migraine attack, levels of CGRP are increased (Goadsby *et al*., 1990). It has been postulated that inhibition of CGRP release (presynaptic) or prevention of CGRP-induced dilatation of cranial blood vessels (postsynaptic) leads to attenuation of a migraine attack. Furthermore, elevated levels of CGRP are normalised on administration of sumatriptan, concomitant with relief of headache pain (Goadsby & Edvinsson, 1993). This action of sumatriptan may be related to prejunctional 5-$HT_{1B/1D}$ receptors located on trigeminal terminals, preventing CGRP release and subsequent inhibition of neurogenic vasodilatation (Humphrey & Feniuk, 1991). The mode of action of CGRP and the clinical application of CGRP antagonists are of great interest in migraine pathophysiology and therapy, but the lack of non-peptidergic or stable CGRP antagonists has hampered both fundamental and clinical investigations. CGRP exerts its effects through G-protein-coupled receptors, which have been classified into $CGRP_1$ and $CGRP_2$ receptors (van Rossum *et al*., 1997). Two forms of CGRP, termed α- and β-CGRP, are expressed in humans (Amara *et al*., 1985) and display very similar biological activities but can be pharmacologically distinguished by their agonist potency at $CGRP_1$ and $CGRP_2$ receptors, respectively (Jansen *et al*., 1992; Nilsson *et al*., 1992). *In vitro* pharmacological determination of CGRP receptors involved in cranial artery dilatation has been hampered due to the lack of a potent CGRP antagonist. The weak $CGRP_1$ peptidergic antagonist h-α-$CGRP_{(8-37)}$ only displays moderate affinity for this receptor (Chiba *et al*., 1989; Rist *et al*., 1998) with a possible residual partial agonist activity (Longmore *et al*., 1994; Poyner,

1995). Furthermore, the clinical implication of this antagonist in migraine is poor due to its peptidergic structure. In this light, the introduction of the novel and chemically stable CGRP antagonist BIBN4096BS (Doods *et al*., 2000), may further clarify the importance of CGRP and its receptors in the pathogenesis of migraine. Currently, this compound is under clinical investigation for the acute treatment of migraine headache and the results are awaited with great interest.

2.5 Cortical spreading depression inhibitors

As described in Chapter 1, cortical spreading depression is characterised by a transient, reversible depression of electrocephalographic activity originally linked to the aura phase. The role of cortical spreading depression in humans is controversial because of difficulties in demonstrating this phenomenon clinically. In animal models however, cortical spreading depression leads to cranial vasodilatation, partly caused by the release of CGRP and NO (Wahl *et al*., 1994). Interestingly, sumatriptan appeared to decrease the amplitude of NO release, after locally applied depolarisation in both cats and rats (Read & Parsons, 2000). Inhibition of this vasodilator response seems to be a novel avenue for the development of antimigraine drugs devoid of vasoconstrictor action. Several compounds with distinct pharmacological properties have been described to block cortical spreading depression, such as the diuretic furosemide (Read *et al*., 1997), melatonin (Ebert *et al*., 1999), the inhibitor of organic anion transport probenecid (Taylor *et al*., 1997) and the calcium channel antagonist lomerizine (Hara *et al*., 1998). The antiepileptic drug lamotrigine, a glutamate receptor antagonist blocking voltage-sensitive sodium channels, inhibits cortical spreading depression and is effective in preventing migraine aura symptoms together with affecting headache frequency (Lampl *et al*., 1999). Another study indicated that lamotrigine was ineffective in migraine prophylaxis (Steiner *et al*., 1997). Particular interest lays in the development of the anticonvulsant SB220453 (Chan *et al*., 1999), currently under investigation in clinical trials in migraine prophylaxis. SB220453 potently inhibits cortical spreading depression-evoked NO release via a yet unknown mechanism (Read *et al*., 2000; Smith *et al*., 2000) but is devoid of vasoconstrictor properties (this thesis and MaassenVanDenBrink *et al*., 2000).

Chapter 3

Human blood vessels as *in vitro* models in migraine

3.1 General

As described in Chapters 1 and 2, migraine attacks are known to involve alterations in the regulation of tone of intra- and/or extra-cranial arteries. The subsequent dilatation of these cranial blood vessels then induces the headache (Humphrey *et al*., 1990; Humphrey & Feniuk, 1991; Saxena, 1990; Saxena, 1994). There are several models, both in human and animal, to study the effects and mechanisms of antimigraine drugs on cranial blood vessels, both *in vivo* and *in vitro.* Experimentally induced headaches, in healthy volunteers or migraine patients, provide possibilities to study headache mechanisms in various ways. Arterial reactions can be studied using transcranial Doppler and high-frequency ultrasound imaging to measure regional cerebral blood flow and volume. Blood samples taken before and after a migraine attack can be compared to evaluate biochemical alterations in amines and neuropeptides (Goadsby *et al*., 1988; Goadsby *et al*., 1990). Furthermore, experimental headache induction may be useful in the early investigation of novel pharmacologic compounds for migraine and pharmacologic intervention can be a useful tool in the study of headache mechanisms (Olesen, 1995). It should be mentioned, however, that setting up *in vivo* measurements in man is limited in view of ethical and practical reasons. Several experimental models representing features of migraine pathophysiology have been clarified in laboratory animals (for review see De Vries *et al*., 1999). However, animals do not have migraine or at least cannot express whether they have a migraine or not, nor are there any biological markers that indicate whether an animal has migraine. Furthermore, there are marked differences between the various species and humans, regarding neuronal transmission and receptor pharmacology and localisation in tissues (Hoyer & Middlemiss, 1989; Parsons, 1991). In the light of the above, it is meaningful to apply *in vitro* techniques to blood vessels from man to obtain information about the vascular pathophysiology and evaluation of potential drugs in migraine treatment. The study of contraction and relaxation in ring segments of blood vessels important in migraine therapy is a straight forward method used to examine the vascular effects of drugs and together with molecular biology techniques provides an excellent tool in understanding migraine pharmacology. This chapter will mainly focus on

the effects of acute antimigraine drugs (i.e. triptans) in human isolated blood vessels important in migraine therapy.

3.2 In vitro organ bath pharmacology

Pre-experimental considerations

Many methods have been developed for studying the mechanical responses of blood vessels *in vitro* involving vascular segments. The experimental conditions and methods depend on the size of the vessel, the desired response and equipment available. The first consideration is the choice of the buffer solution used in the experiments with the Krebs bicarbonate solution as the most widely used. In particular the concentration of Ca^{2+} ions (Godfraind & Kaba, 1972; Janssens & Verhaeghe, 1984) and the pH (Verbeuren *et al*., 1978) can influence the reactivity of the isolated blood vessels. The solution is often bubbled with a gas mixture of 5% CO_2 (which will maintain a proper pH) and 95% O_2 to prevent hypoxia and unwanted release of substances (Rubanyi & Paul, 1985; Rubanyi & Vanhoutte, 1985). The temperature is another factor that must be controlled, as it affects vascular reactions and the apparent affinity of certain receptors for agonists and antagonists (Arner & Hogestatt, 1990; Flavahan & Vanhoutte, 1986; Padilla *et al*., 1998; Padilla *et al*., 1997).

Another consideration is whether or not the endothelium should remain intact, since it can profoundly influence both the resting tone and pharmacological reactivity of a drug. A large number of studies have shown that activation of the endothelium leads to release of NO, prostacyclins, prostanoids and endothelins, which can obscure the true behaviour of the drugs acting at vascular smooth muscle receptors. Similarly, receptors present in the smooth muscle can also be present in endothelial cells, which could alter the response to drugs. Leaving the endothelium intact, however, may resemble the physiologic reality closer but requires criteria that define its functional integrity (such as observing relaxation to substance P). Once suspended in organ baths, each individual blood vessel segment should be stretched to a level of maximum responsiveness, the degree of stretch necessary for this condition can be determined by measuring the maximal response with several degrees of pre-stretch. In practise this can be time consuming and an average degree of stretch is determined in pilot experiments and used in subsequent experiments using the same type of blood vessel. After initial resting tension is obtained, a reference contraction should be introduced, which can be used as an internal standard. In general,

potassium-induced depolarisation is applied, but this reference contraction can also be obtained by applying by a well described receptor agonist, preferably without influence on the mechanisms investigated to avoid unwanted receptor activation or desensitisation. Once all pre-experimental conditions have been determined, concentration response curves can be constructed by either single drug concentration exposure or cumulative drug additions of which the latter can be designed with either a single curve or a multiple curve protocol in each segment preparation (for review on pre-experimental considerations see Janssens, 1995; Lew & McPherson, 1996; Martin & Giles, 1996).

Pharmacological parameters

Studying the effect of drugs in human isolated blood vessels can produce valuable parameters. Concentration response curves can be described by fitting the constructed cumulative concentration response curve of a drug, using a non-linear regression analysis, into a concentration effect curve by using the following equation:

$$\mathbf{E = E_{max}/(1 + (EC_{50}/[agonist])^{nH})}$$

Or, when logarithmically transformed:

$$\mathbf{E = E_{max}/(1 + 10^{((pEC_{50}-[agonist])*nH))}}$$

Where E is the effect of an agonist, which is expressed either as an absolute (i.e. mN or grams) or as a relative value (i.e. % of contraction evoked by the agonist or compared to an internal standard). From this non-linear regression analysis three distinct parameters can be extracted. Firstly, information can be obtained from the E_{max} value or maximal-induced contraction by an agonist, which is described as the efficacy or activity of an agonist. In general, an agonist whose maximal response corresponds to the response of a known and well described full agonist in a certain blood vessel is known as a full agonist (intrinsic activity = 1), those whose maximal response falls short of this full response are known as partial agonists (intrinsic activity < 1, Ariëns, 1954). Secondly, the EC_{50} value can be determined, which is described as that concentration of agonist needed to obtain a half-maximal effect of an agonist, also known as the affinity or potency of an agonist; the negative logarithm of EC_{50} is depicted as pEC_{50}. Thirdly, the slope factor or

Hill slope (nH) can be determined, this Hill slope describes the steepness of a concentration response curve, which, when the agonist-receptor system follows the law of mass-action of a single binding site, equals unity (Clark, 1937). A Hill Slope different from unity may be indicative of agonist action at two distinct receptors. Interpretation of the calculated parameters depends on the definition of the parameter and the goal of the experiments. When experiments in isolated blood vessels are performed to predict the effects of an agonist in man, the potency of a drug is based on the half maximal effect this compound, regardless the mechanisms and receptors involved in mediating this contraction. In other words, the (p)EC_{50} for *the overall effect*. Functional pharmacology in isolated blood vessels can also be used to determine which receptors and mechanisms are involved in mediating the effects of a drug. In this latter case, potency is described by the combined effect of the agonist affinity for a receptor (i.e. tendency to bind to the receptor) and efficacy (i.e. ability, once bound, to initiate an effect). In this case, potency is described as the (p)EC_{50} for *the receptor mediating the effect*.

Agonist rank orders of potency and efficacy have long been used in receptor classification but show various limitations. It should be recognised that efficacy of a given agonist is tissue-dependent, since its value depends on the receptor density and the efficiency of the receptor-effector coupling, i.e. a certain agonist can behave as a full agonist in one tissue and manifests partial agonism in another tissue. On the other hand, the potency of an agonist greatly depends on the amount of spare receptors or receptor reserve of a certain tissue, i.e. the higher the receptor reserve the lower concentrations of agonist are required to elicit 50 % of the maximal response. When comparing agonists to provide reliable information on receptor classification, all agonists need to achieve the same maximal effect and Hill slopes should be similar. Only when these conditions are met, true agonist potency-ratios can be determined via partial receptor inactivation by, for example, alkylation (Furchgott & Bursztyn, 1967).

Another way to characterise and classify the receptors and mechanisms involved in agonist-induced effects is via the use of specific receptor antagonists. A receptor antagonist binds to a receptor without inducing an effect (i.e. intrinsic activity = 0) and can be classified by its kinetics of interaction with receptors. The most commonly used and mathematically described interaction is called competitive reversible antagonism, which is the condition when an antagonist binds reversibly to the same recognition sites on the receptor as does the agonist and thus competes for such sites. To describe the effects of a competitive antagonist on the responses to a range of agonist concentrations, the concept

of dose (or better concentration) ratios is useful. Dose ratios, which can be determined experimentally, are defined as the ratio of the concentration of agonist required to elicit an equal effect in the presence and absence of the antagonist. An empirical scale for antagonist potencies based on dose ratios, termed pA scale, was introduced by Schild (1968), from which the most widely used parameter has been pA_2 (the negative logarithm of the concentration of antagonist required to produce a two-fold shift to the right of an agonist concentration response curve). However, pA_2 is an empirical measure of antagonist potency that provides no information on the nature of antagonism. By contrast, an estimate of K_B, the antagonist-receptor dissociation equilibrium constant, can be calculated for antagonists that compete with agonists for unoccupied receptors in a simple, reversible manner. This K_B is a chemical term derived from the rates of onset and offset of antagonist binding to the receptor and is independent of receptor function and location. Arunlakshana and Schild mathematically described the relation between the magnitude of agonist curve rightward shift (i.e. dose ratio) and antagonist concentration (Arunlakshana & Schild, 1959) using the following Schild regression equation:

$$\mathbf{Log\ (\mathit{r}\text{-}1) = \mathit{n} * log\ [B] + pK_B}$$

Where *r* represents the dose ratio, *n* the slope of the Schild plot, log [B] the logarithm of molar concentration of antagonist and pK_B the negative logarithm of the K_B. When the Schild plot yields a straight line with slope of unity (i.e. n=1) the intercept of the abscissa reflects the pK_B, and since the zero value on the ordinate is obtained when r=2, the pK_B corresponds to the pA_2. It bears emphasis that when the Schild plot slope is not unity, the slope of the Schild regression should be constrained to unity in order to provide an estimate of pK_B. When this constraint is not applied, the value of the intercept still provides an estimate of the pA_2, but is no longer valid to estimate pK_B. To estimate a valid antagonist potency against a receptor, three well defined criteria should be regarded. Firstly, increasing concentrations of antagonist should produce successive, parallel rightward displacements of the agonist concentration effect curve with no depression of maximal effect. Secondly, the antagonist affinity should be independent of the concentration used so that the data in the form of a Schild plot yield a slope of unity. In case only one or two concentrations of antagonist are used to construct a Schild plot the respective pK_B or pA_2 is depicted as an apparent value. Thirdly, the antagonist affinity

should be independent of the agonist used, because the agonist simply titrates receptor sites unoccupied by the antagonist. When all these criteria are met and the Schild regression analysis reveals a slope of unity one can provide a clear and solid pharmacologic receptor classification (for review on analysis of concentration response curves see Kenakin, 1993; Martin & Giles, 1996).

3.3 Human cranial arteries as models for therapeutic site of action

As described in Chapter 2, the therapeutic activity of acutely acting antimigraine drugs is most probably mediated by constriction of dilated cranial arteries. Therefore, measurements of contraction in human cranial arteries are an excellent tool for the evaluation of potential drugs for the treatment of migraine as well as investigating the mechanisms involved in craniovascular contraction and thus, unravel cerebrovascular pathophysiological changes during a migraine attack. As described below, three distinct arteries are commonly used as therapeutic models, the middle meningeal (an extracerebral intracranial artery), the intracerebral (basilar and cerebral artery) and extracranial artery (temporal artery).

Middle meningeal artery

The human middle meningeal artery (Figure 3.1) is the largest of the arteries that supply the dura mater, a pain-sensitive, richly vascularised and innervated tissue that is implicated in various cephalalgias, including vascular headache. Sumatriptan has been shown to constrict the human middle meningeal artery *in vivo*, with subsequent relief of migraine headache (Henkes *et al*., 1996). The middle meningeal artery is innervated by afferent sensory fibres containing substance P, neurokinin A and CGRP, most of which originate in the trigeminal ganglion, and sympathetic fibres containing noradrenaline and neuropeptide Y (NPY), emanating predominantly from the superior cervical ganglion (Gulbenkian *et al*., 1999). The presence of mast cells over the entire dural connective tissue and dural blood vessels, with their vasoactive constituents, including 5-HT, makes these blood vessels intriguing with regard to their potential role in regulation of vascular tone, inflammation and migraine. The middle meningeal artery can be obtained in conjunction with neurosurgical operations where a part of the skull needs to be removed to obtain access to disorders such as aneurysms or meningeomes. By opening the skull a redundant part of the middle meningeal artery may be damaged and subsequently removed

from the dural sheath after which the obtained piece of artery can be used for *in vitro* organ bath experiments.

As mentioned above, construction of cumulative concentration response curves to antimigraine drugs can be used to determine its potency and efficacy in migraine. Indeed, sumatriptan and the second-generation triptans tested in this preparation all induced concentration-dependent contractions, indicating therapeutic activity, with marginal differences in both efficacy and potency (Table 1.4 for references), except for donitriptan, which appears to be 50-fold more potent than sumatriptan (Chapter 5). The triptans have a high binding affinity at the human 5-HT_{1B} and 5-HT_{1D} receptors (Table 1.2) and moderate to low affinity for various other receptor types. The vasoconstrictor potency of 5-HT receptor agonists in the middle meningeal artery is positively correlated with measurements of affinity obtained in cell lines expressing the human 5-HT_{1B} receptor, but not the 5-HT_{1D} or 5-HT_{1F} receptor (Razzaque *et al.*, 1999). Due to the lack of specific subtype-selective receptor antagonists, the functional identification of the receptor involved in the triptan-induced contraction has long been hampered. Studies using the non-selective 5-$HT_{1B/1D}$ receptor antagonists GR125743 (reversible) and GR127935 (irreversible) revealed that sumatriptan-induced contraction was potently blocked (Razzaque *et al.*, 1999), but failed to discriminate between the 5-HT_{1B} and 5-HT_{1D} receptors. Eletriptan-induced contraction was similarly blocked by GR125743 as compared to sumatriptan (Chapter 7), indicating that eletriptan and sumatriptan contract the middle meningeal artery via the same mechanisms. Indications that sumatriptan elicits its action predominantly via the 5-HT_{1B} receptor came from the finding that the 5-HT_2 receptor antagonist ketanserin, which also has a high affinity for the 5-HT_{1D} receptor, failed to block sumatriptan-induced contractions (Jansen *et al.*, 1992). Indeed, as described in Chapter 8, the first selective 5-HT_{1B} receptor antagonist SB224289 blocked sumatriptan-induced contractions, whereas the selective 5-HT_{1D} receptor antagonist BRL15572 failed to block this contraction.

In parallel with functional *in vitro* organ bath experiments, molecular biological studies revealed dense and predominant 5-HT_{1B} (and only trace amounts of 5-HT_{1D}) receptor protein and mRNA in the smooth muscle and endothelium of the middle meningeal artery suggesting a role for the 5-HT_{1B} receptor in antimigraine activity (Longmore *et al.*, 1998; Longmore *et al.*, 1997; Schmuck *et al.*, 1996). Apart from 5-HT_{1B} receptor mRNA, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_4 and 5-HT_7 receptor mRNA has been detected in homogenates of the human middle meningeal artery (Schmuck *et al.*,

1996). While the 5-HT_{1F} receptor does not seem to be involved in regulation of blood vessel tone both 5-HT_{2B} and 5-HT_{7} receptors regulate blood vessel dilatation (see Chapter 2). The 5-HT_{2A} receptor mediates contractile responses to 5-HT in this preparation, since the latter is competitively blocked by ketanserin (Jansen *et al.*, 1993). Due to the fact that the 5-$HT_{1B/1D}$ receptor agonists reveal a similar intrinsic activity as compared to 5-HT, both the 5-HT_{1B} as well as the 5-HT_{2A} receptor mediate serotonergic vasoconstriction in this preparation.

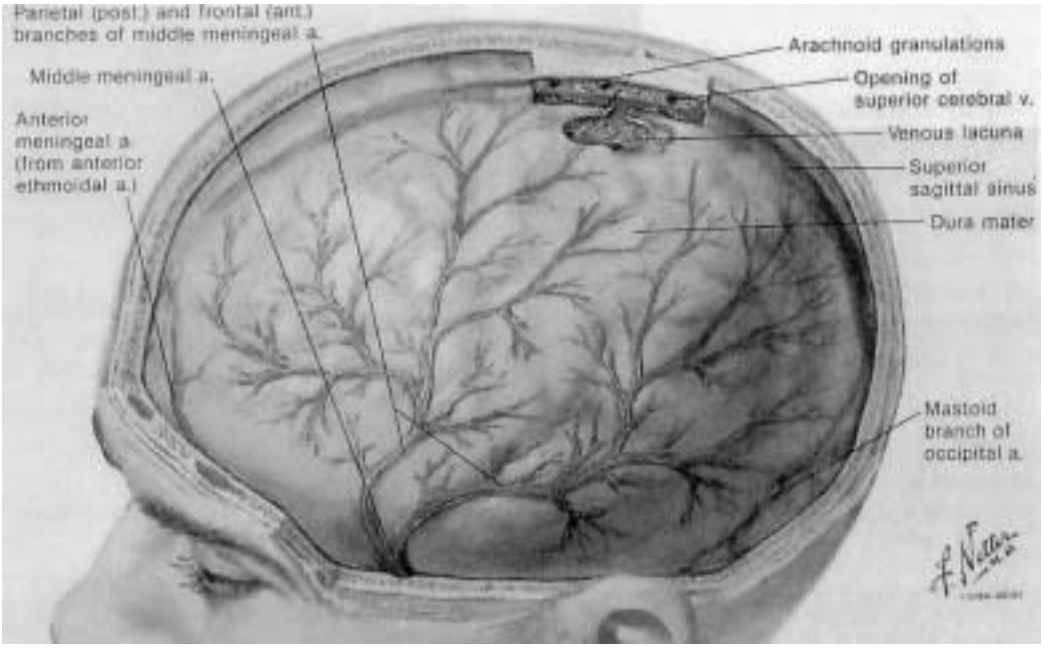

Figure 3.1 Middle meningeal artery and its projection on the dura mater (Edvinsson & Dahl, 2000).

Cerebral arteries

The major cerebral arteries supplying the brain consist of the internal carotid system and the vertebrobasilar system that communicate with one another to form an anastomotic arterial system called the circle of Willis. Branches from the major cerebral (i.e. anterior, middle and posterior) arteries extend along the surface of the brain and then penetrate the pia mater to become the intraparenchymal (pial) vessels that supply the neural tissue of the brain. Immunocytochemical studies have revealed that the large cerebral arteries are innervated with nerve fibres containing NPY, vasoactive intestinal peptide (VIP), substance P, neurokinin A and CGRP, originating from the superior cervical ganglion and small local ganglia at the base of the skull. Sensory fibres seem to derive from the

trigeminal ganglion and from dorsal root ganglia (Gulbenkian *et al*., 1999; Edvinsson *et al*., 1987; Edvinsson *et al*., 1994; Uddman & Edvinsson, 1989). Using transcranial Doppler sonography (for review see, May & Goadsby, 1999) is has been demonstrated that blood flow velocity in middle cerebral artery is significantly higher on the headache side, which returns to normal values after administration of sumatriptan (Humphrey *et al*., 1991). On the other hand, although administration of sumatriptan increased regional cerebral blood flow velocity in middle cerebral arteries and basilar artery, no differences were found between the headache and non-headache side as well as between migraine and headache free periods (Limmroth *et al*., 1996). Despite the differences in observations regarding cerebral blood flow, this intracranial artery can be used as a model to predict antimigraine activity. It has been shown that sumatriptan and other triptans constrict human isolated cerebral arteries (Bouchelet *et al*., 2000; Jansen *et al*., 1993; Nilsson *et al*., 1999) with similar potencies as compared to the isolated middle meningeal arteries (see Table 1.4). Similarly, sumatriptan is blocked by 5-$HT_{1B/1D}$ receptor antagonists, whereas the 5-HT_2 receptor antagonist ketanserin was without effect (Bouchelet *et al*., 2000; Jansen *et al*., 1993; Nilsson *et al*., 1999). In contrast to middle meningeal artery, contraction to 5-HT is not blocked by ketanserin, suggesting that the 5-HT_{1B} receptor exclusive mediates serotonergic vasoconstriction in this preparation. Furthermore, selective 5-HT_{1D} and 5-HT_{1F} receptor agonists failed to constrict the middle cerebral artery (Bouchelet *et al*., 2000). Immunocytochemical studies revealed the presence of 5-HT_{1B} receptor mRNA and protein whereas that of 5-HT_{1D} receptor was absent (Bouchelet *et al*., 1996; Hamel *et al*., 1993; Nilsson *et al*., 1999). Some middle cerebral arteries also expressed the 5-HT_{1F} receptor mRNA (Bouchelet *et al*., 1996).

Temporal artery

The superficial temporal artery (a. temporalis superficialis), the smaller of the two terminal branches of the external carotid artery, appears, from its direction, to be the continuation of that vessel. It begins in the substance of the parotid gland, behind the neck of the mandible, and crosses over the posterior root of the zygomatic process of the temporal bone and divides into two branches, a frontal and a parietal temporal artery. Immunocytochemical studies have revealed that the majority of the nerve fibres displayed immunoreactivity for tyrosine hydroxylase and NPY. A moderate supply of perivascular nerve fibres displayed either acetylcholinesterase activity or immunoreactivity for VIP and CGRP. Only a few nerve fibres displayed substance P and neurokinin A

immunoreactivity (Jansen Olesen *et al*., 1995). In their pioneer work, Graham and Wolff (1938) observed an increase in the amplitude of the superficial temporal artery pulsations during the headache phase of migraine, explaining the throbbing nature of the migraine headache. Furthermore, after administration of ergotamine, the amplitude of the pulsations was reduced and pain was concomitantly relieved. From these data they reasoned that migraine headache is caused by stretching of extracranial arteries and that it is alleviated by vasoconstriction. As was observed in measuring regional cerebral blood flow, more recent studies revealed conflicting results. Using a high-resolution ultrasound equipment it has been demonstrated that, during an attack, the temporal artery diameter on the headache side is larger (Iversen *et al*., 1990). On the other hand, it was observed that only in a minority of patients, dilatation of the superficial temporal artery contributed to the migraine headache (Drummond & Lance, 1983). Despite these conflicting results, this extracranial artery constricts to various antimigraine drugs and therefore, can be used as a model to predict antimigraine activity. Sumatriptan constricted the isolated temporal artery with a similar potency and efficacy as compared to both human middle meningeal and cerebral arteries (Jansen *et al*., 1992; Jansen *et al*., 1993). Furthermore, sumatriptan is potently blocked by the 5-HT_{1B} receptor antagonist SB224289 (Verheggen *et al*., 1998) and not by the 5-HT_{1D} receptor antagonist BRL15572, whereas a 5-HT_2 receptor mediated response at high concentrations is blocked by the 5-HT_2 receptor antagonist mesulergine (Chapter 8). The 5-HT_2 receptor appears to be the predominant contractile serotonergic receptor in this preparation since sumatriptan revealed a markedly lower intrinsic activity as compared to 5-HT (Jansen *et al*., 1993; Verheggen *et al*., 1996), which is not observed in middle meningeal and cerebral artery. Indeed, 5-HT is potently blocked by the 5-HT_2 receptor antagonist ketanserin, whereas non-selective 5-HT_1-like receptor antagonists show little (Verheggen *et al*., 1996) or no effect (Jansen *et al*., 1993). Despite the functional role for the 5-HT_{2A} receptor in this preparation, mRNA could not be detected in homogenised tissue, possibly due to an artery specific splice variant (Verheggen *et al*., 1998). The same study revealed mRNA expression for the 5-HT_{1B}, 5-HT_{1D}, 5-HT_4 and 5-HT_7 receptors, whereas no 5-HT_{1F} receptor mRNA was detected.

3.4 Human Peripheral blood vessels as a model for side-effect potential

Ever since the introduction of ergotamine and dihydroergotamine, various studies reported the occurrence of chest symptoms, such as substernal chest pain, discomfort and even

myocardial infarction (Meyler, 1996; Roithinger *et al*., 1993; Slob *et al.*, 1988; Wayne, 1986), in a proportion of patients using antimigraine drugs. In order to avoid side-effects related to peripheral vasoconstriction, sumatriptan was introduced as a potent cranioselective indole derivative, but as was the case with the ergots, sumatriptan administration revealed similar chest symptoms in up to 15% of patients (Ottervanger *et al*., 1997; Plosker & McTavish, 1994). In addition, there are some reports on myocardial infarction after the use of sumatriptan (Ottervanger *et al*., 1997). These findings suggest that coronary artery constriction is responsible for the reported side-effects. In order to investigate the potential side-effects, the human isolated coronary artery can be used as a model to predict coronary side-effect potential of sumatriptan and the newly developed antimigraine drugs. Similarly, the human isolated saphenous vein can also be used as model for the coronary artery as well as a predictive preparation for possible peripheral effects of these antimigraine drugs.

Coronary artery

To investigate the side-effect potential of antimigraine drugs *in vitro*, the right epicardial coronary artery is most commonly used. In our laboratory, this artery is obtained from 'heart-beating' organ donors, who died of non-cardiac disorders, within 24 hours after explantation. The right coronary artery arises from the right anterior aortic sinus. It passes at first between the conus arteriosus and the right auricula and then runs in the right portion of the coronary sulcus. Immunohistochemistry revealed that the proximal part of epicardial arteries possesses a relatively sparse supply of nerve fibres forming a loose network in the adventitia, whereas the perivascular network increases in density as the vessels are followed distally. In both proximal and distal regions, the majority of nerve fibres display neuropeptide Y and tyrosine hydroxylase immunoreactivity. CGRP- and substance P-immunoreactive nerve fibres are very sparse in the proximal region of the arteries and increase in number distally. Only a few scattered VIP-immunoreactive nerve fibres are found in both arterial regions (Gulbenkian *et al*., 1993).

As was observed in the cranial vasculature, ergotamine reduced myocardial blood flow and increased coronary resistance in migraine patients (Gnecchi-Ruscone *et al*., 1998), which is caused by vasoconstriction of the coronary arteries. Similarly, it has been demonstrated, via angiography, that subcutaneously administered sumatriptan caused a significant rise in systemic pressure with a reduction of coronary artery diameter (MacIntyre *et al*., 1993). *In vitro* studies revealed that sumatriptan constricted the

coronary artery with a lower potency than its respective potency in cranial arteries (see Table 1.4), consistent with the cranioselective nature of this compound (MaassenVanDenBrink *et al*., 2000; Parsons *et al*., 1998; van den Broek *et al*., 2000). This propensity of sumatriptan to constrict the coronary artery was one of the reasons behind developing the second-generation triptans but all, except eletriptan, are equally or slightly more potent in human isolated coronary artery as compared to sumatriptan (Ferro *et al*., 1995; Longmore *et al*., 1996; Longmore *et al*., 1997; MaassenVanDenBrink *et al*., 1998; MaassenVanDenBrink *et al*., 2000; Saxena *et al*., 1997; van den Broek *et al*., 2000). All triptans, except donitriptan (see Chapter 5), constrict the coronary artery with a similar efficacy, whereas they evince a lower efficacy than 5-HT (Bax *et al*., 1993; Ferro *et al*., 1995; MaassenVanDenBrink *et al*., 2000) and their respective efficacies in human cranial arteries (MaassenVanDenBrink *et al*., 2000; Parsons *et al*., 1998; van den Broek *et al*., 2000). The relatively high intrinsic activity of 5-HT indicates that the 5-HT_2 receptor is the most important receptor in this preparation. Indeed, contraction to 5-HT is predominantly blocked by 5-HT_2 receptor antagonists whereas only a small proportion is blocked by 5-HT_1 receptor antagonists (Bax *et al*., 1993; Connor *et al*., 1989; Kaumann *et al*., 1994). Contraction to sumatriptan is blocked by non-selective 5-$HT_{1B/1D}$ receptor antagonists (MaassenVanDenBrink *et al*., 2000; van den Broek *et al*., 2000), whereas the 5-HT_{1B} receptor antagonist SB224289 partially blocked this contraction (Chapter 8). The 5-HT_{1D} receptor antagonist BRL15572 failed to block sumatriptan-induced contraction. These data indicate that the 5-HT_{1B} receptor is responsible for the contraction to the triptans in this preparation although other unknown receptors cannot be excluded (see Chapters 7 and 8). Thus, as long as the 5-HT_{1B} receptor is required for the therapeutic efficacy of antimigraine drugs, they will also have the propensity to constrict the coronary artery. Immunocytochemical RT-PCR techniques have revealed predominant mRNA expression coding for the 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2B} receptor, while little or no 5-HT_{1A}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2C} and 5-HT_7 receptor mRNA was detected in human epicardial coronary arteries (Ishida *et al*., 1999; Nilsson *et al*., 1999). Conflicting results have been obtained for 5-HT_{1F} receptor mRNA, where one study demonstrated strong expression (Nilsson *et al*., 1999) and two studies showed little or no expression (Bouchelet *et al*., 2000; Ishida *et al*., 1999). Using selective antibodies against 5-HT_{1B} and 5-HT_{1D} receptors, the 5-HT_{1B} receptor protein (Nilsson *et al*., 1999) has been localised in smooth muscle and endothelium, whereas little or no 5-HT_{1D} receptor protein could be detected.

Similar results were obtained with immunohistochemical techniques used to localise the receptor mRNAs (Chapter 8).

Saphenous vein

The human saphenous vein is frequently used as a graft in coronary bypass surgery. Leftover pieces can be obtained intra-operatively and may be used as a tool in investigating peripheral vasoconstriction. Various experiments have been performed in animal (i.e. canine and rabbit) isolated saphenous veins to screen for antimigraine efficacy (Bou *et al*., 2000; Cohen & Schenck, 1999; Humphrey *et al*., 1989; John *et al*., 1999; Martin *et al*., 1997; Slassi *et al*., 2000), whereas these preparations are also predictive of human coronary artery constriction (Cohen *et al*., 1997) and thus can be used to screen for side-effect potential. The great saphenous vein (v. saphena magna), the longest vein in the body, begins in the medial marginal vein of the dorsum of the foot and ends in the femoral vein about 3 cm below the inguinal ligament. Immunohistochemical methods revealed a peptidergic innervation mainly localised along the vasa vasorum and associated with immunoreactivity of substance P and CGRP (Herbst *et al*., 1992).

Studies in human isolated saphenous vein have demonstrated that sumatriptan, as well as eletriptan, constricted this preparation with a potency that is more closely related to the respective values in coronary artery as opposed to middle meningeal artery (MaassenVanDenBrink *et al*., 2000; van den Broek *et al*., 2000, see Table 1.4). The efficacy profile of these antimigraine drugs, however, appears to be more closely related to the latter preparation, but both compounds have a lower intrinsic activity than 5-HT (Bax *et al*., 1992; MaassenVanDenBrink *et al*., 2000; van den Broek *et al*., 2000). Non-selective 5-HT_1 receptor antagonists blocked sumatriptan-induced contractions (Bax *et al*., 1992; van den Broek *et al*., 2000) but only weakly antagonised eletriptan- (van den Broek *et al*., 2000) and 5-HT- (unpublished results) induced contractions. The selective 5-HT_{1B} receptor antagonist SB224289 potently blocked sumatriptan-induced contractions, whereas the 5-HT_{1D} receptor antagonist BRL15572 was without effect (Chapter 8). The 5-HT_2 receptor antagonist ketanserin potently blocked 5-HT-induced contraction but it was ineffective against sumatriptan (Bax *et al*., 1992). Based on these results the human saphenous vein is likely to contain both contractile 5-HT_{1B} and 5-HT_2 receptors. No immunocytochemical studies have been reported for 5-HT receptor proteins, but 5-HT_{1B}, in contrast to 5-HT_{1D}, receptor mRNA, has been localised in smooth muscle and endothelium of this preparation (Chapter 8).

3.5 Summary

Based on pharmacological *in vitro* and immunohistochemical studies it is apparent that the 5-HT_{1B} receptor is responsible for mediating contraction by the triptans (see Tables 1.4 and 1.5). One of the key characteristics of this constriction to antimigraine drugs is the fact that they are cranioselective, but they also constrict peripheral blood vessels. It is, therefore, important to examine the effects of antimigraine drugs in models mimicking the therapeutic site of action (i.e. cranial arteries) and potential coronary side-effects (i.e. coronary artery) to provide an index of efficacy and safety, respectively.

Aims of the thesis

Based on the questions that were addressed in the previous chapters, the aims of the thesis were:

1. To determine the effects of several current and prospective antimigraine drugs on human isolated blood vessels of relevance to therapeutic activity and side-effect potential (see part II of the thesis).
2. To investigate and characterise which receptors/mechanisms are involved in vascular responses elicited by antimigraine drugs (see part III of the thesis).

Tables

Table 1.2 Receptor binding properties (pK_i values) of the triptans at human 5-HT receptors.

Receptor	Sumatriptan	Zolmitriptan	Naratriptan	Rizatriptan	Eletriptan	Almotriptan	Frovatriptan	Donitriptan	IS-159
5-HT_{1A}	6.0 – 6.9[a,b,c]	6.5 – 6.6[c,d]	7.1 – 7.6[b,c]	6.4[c]	7.4[c]	6.3[f]	7.3[g]	7.6[h]	
5-HT_{1B}	7.4 – 8.5[a,c,i]	7.7 – 8.1[c,d,i]	8.1 – 9.3[c,e,i]	6.9 – 8.1[c,i,]	7.8 – 8.0[c,i]	8.0[f]	8.2 – 8.6[g,i]	9.4[h]	8.5[k]
5-HT_{1D}	8.0 – 8.7[a,c,i]	8.9 – 9.7[c,d,i]	8.3 – 9.2[c,e,i]	7.9 – 8.6[c,i,]	8.8 – 8.9[c,i]	8.0[f]	8.2 – 8.6[g,i]	9.3[h]	8.8[k]
5-HT_{1E}	5.6 – 4.8[a,c,j]	7.7 – 8.0[c,i]	7.7[c]	6.8[c]	7.3[c]		<6.0[g]	5.9[h]	
5-HT_{1F}	7.6 – 7.9[a,c,j]	7.2 – 7.5[c,d,i]	8.2 – 8.4[c,i]	6.8 – 6.9[c,i]	7.7 – 8.0[c,i]		7.0 – 7.2[g,i]	5.5[h]	<5.0[k]
5-HT_{2A}	<5.5[c]	<5.5[c]	<5.5[c]	<5.5[c]	<5.5[c]		<5.3[g]	6.7[h]	
5-HT_{2B}	6.9*	7.2*		6.6*					
5-HT_{7}	5.9[c]	7.0[c]	<5.5[c]	5.7[c]	6.7[c]	<6.5[f]	6.7[g]	6.4[h]	

Data taken from: [a], Leysen *et al*., 1996; [b], Newman-Tancredi *et al*., 1997; [c], Napier *et al*., 1999; [d], Martin *et al*., 1997; [e], Connor *et al*., 1997; [f], Bou *et al*., 2000; [g], Xu *et al*., 1999; [h], John *et al*., 1999; [i], Pauwels & John, 1999; [j], Adham *et al*., 1993; [k], Hamel, 2000; *, P. Gupta, personal communication.

Table 1.3 Pharmacokinetic parameters for triptans

Drug	Dose (mg)	T_{max} (h)	$T_{1/2}$ (h)	Bio availability (%)	C_{max} (nM)	Plasma protein binding (%)	Free C_{max} (nM)	$LogD_{pH\ 7.4}$
Sumatriptan	6 (s.c.)	0.2	2.0	96	244 – 261	14 – 21	193 – 224	-1.5
	100 (p.o.)	1.5	2.0	14	142 – 183		112 – 157	
Zolmitriptan	2.5 (p.o.)	1.5	2.5	39	9	25*	7	-1.0
	5 (p.o.)	1.5	3.0	46	17		13	
Naratriptan	2.5 (p.o.)	2.0	5.5	74	38	20*	30	-0.2
Rizatriptan	10 (p.o.)	1.0	2.0	40	74 – 93	14*	64 – 80	-0.7
Eletriptan	40 (p.o.)	1.8		50	213	85*	32	+0.5
	80 (p.o.)	1.4	6.3	50	643		96	
Frovatriptan	2.5 (p.o.)	3.0	25.7	30	29	15[#]	25	-1.0
Almotriptan	12.5 (p.o.)	2.5	3.1	80	148	30[†]	104	

Data taken from: Saxena & Tfelt-Hansen, 2000. *, A. McHarg, personal communication; [#], P. Buchan, personal communication; [†], J. Gras, personal communication. $LogD_{pH\ 7.4}$: measure of lipophilicity with increasing numbers indicating greater lipid solubility, s.c.: subcutaneous, p.o.: oral.

Table 1.4 pEC_{50} values of contraction to triptans in human isolated blood vessels.

	Sumatriptan	**Zolmitriptan**	**Naratriptan**	**Rizatriptan**	**Eletriptan**	**Almotriptan**	**Frovatriptan**	**Donitriptan**	**IS-159**
Middle meningeal artery	6.9 – 7.6 [a,b,c,d,e]	7.4[a]		7.0[b]	7.3[c,d]	7.5[f]		9.1[e]	
Cerebral/ Basilar Artery	6.4 – 6.8[g,i]	7.2[g]	7.0[g]			*5.4*[f]	*7.9*[h]		6.8[i]
Coronary artery	5.7 – 6.7 [c,d,e,j,k,l,m]	6.3 – 7.3[j,k]	6.8[j,l]	6.0 – 6.4[j,k,m]	5.5 – 5.7[c,d]	5.3[f]	7.4[h]	7.6[e]	
Saphenous vein	6.1 – 6.2[c,d]				5.9 – 6.1[c,d]				

Data taken from: [a], Razzaque *et al*., 1999; [b], Longmore *et al*., 1998; [c], MaassenVanDenBrink *et al*., 2000; [d], van den Broek *et al*., 2000; [e], Chapter 5; [f], Bou *et al*., 2001; [g], Nilsson *et al*., 1999; [h], Parsons *et al*., 1998; [i], Bouchelet *et al*., 2000; [j], MaassenVanDenBrink *et al*., 1998; [k], Martin *et al*., 1997; [l], Connor *et al*., 1997; [m], Longmore *et al*., 1997. pEC_{50} values for basilar artery in italics.

Table 1.5 Presence of mRNAs of various 5-HT receptor subtypes in blood vessels important in migraine.

	5-HT$_{1A}$	**5-HT$_{1B}$**	**5-HT$_{1D}$**	**5-HT$_{1F}$**	**5-HT$_{2A}$**	**5-HT$_{2B}$**	**5-HT$_{4}$**	**5-HT$_{7}$**
Middle meningeal artery		++	±	+	+	+	+	+
Middle cerebral artery		++	—	±				
Temporal artery		++	+	—			+	+
Coronary artery	—	+	—	— / ± / +	++	+		—

For references see Chapter 3. ++, dense; +, normal; ±, little; —, no expression of receptor mRNA.

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Part II

Therapeutic activity and side-effect potential of antimigraine drugs

Chapter 4

Craniovascular selectivity of eletriptan and sumatriptan in human isolated blood vessels

Summary - Eletriptan is a 5-$HT_{1B/1D}$ receptor agonist with proven efficacy in the acute treatment of migraine. The objective of this study was to assess the craniovascular selectivity of eletriptan and sumatriptan in blood vessels predictive of therapeutic efficacy (human middle meningeal artery) and adverse coronary side effects (human coronary artery and human saphenous vein).

We obtained coronary artery from organ donors (*n*=9), middle meningeal artery from patients (*n*=11) undergoing craniotomy, and saphenous vein from patients (*n*=9) undergoing coronary bypass surgery. Concentration-response curves to eletriptan and sumatriptan were constructed to obtain measurements of efficacy (maximum contraction, E_{max}) and potency (concentration eliciting 50% of E_{max}, EC_{50}). The contraction that is likely to be induced at the maximal free plasma concentration (C_{max}) was determined by calculating C_{max}/EC_{50} ratios and by interpolation of the concentration-response curves.

Eletriptan and sumatriptan induced concentration-dependent contractions of meningeal artery, coronary artery, and saphenous vein. Eletriptan was less potent than sumatriptan in coronary artery, whereas both compounds had similar potency in meningeal artery and saphenous vein. However, the potency of eletriptan and sumatriptan was higher in meningeal artery than in coronary artery (86-fold for eletriptan and 30-fold for sumatriptan) or saphenous vein (66- and 25-fold). The efficacy of eletriptan and sumatriptan was similar within tissues. The predicted contraction by eletriptan (40 mg and 80 mg) and sumatriptan (100 mg) at free C_{max} observed in clinical trials was similar in meningeal artery, whereas in coronary artery and saphenous vein it was lower for 40 mg eletriptan than for sumatriptan. At therapeutic concentrations both eletriptan and sumatriptan contract middle meningeal artery more than coronary artery. This suggests that in patients with healthy coronary arteries, they have a limited propensity to cause adverse coronary side effects. However, both drugs remain contraindicated in patients with coronary artery disease.

Based on: MaassenVanDenBrink, A., van den Broek, R.W.M., de Vries, R., Bogers, A.J.J.C., Avezaat, C.J. & Saxena, P.R. (2000). Craniovascular selectivity of eletriptan and sumatriptan in human isolated blood vessels. *Neurology*, **55,** 1524-30.

4.1 Introduction

The 5-$HT_{1B/1D}$ receptor agonist sumatriptan is effective in the treatment of migraine attacks (Ferrari, 1991; Ferrari & Saxena, 1993; Saxena & Tfelt-Hansen, 2000). However, from a therapeutic perspective, sumatriptan has several limitations, including low oral bioavailability, variable absorption, and a short half-life. Moreover, up to 15% of patients consistently report chest symptoms, including chest pressure, tightness and pain, often mimicking pectoral angina (Brown *et al*., 1991; Plosker & McTavish, 1994; Visser *et al*., 1996). Although extracardiac mechanisms have been invoked, chest symptoms may well be caused by coronary vasoconstriction, which has been observed after sumatriptan both *in vivo* (MacIntyre *et al*., 1992) and *in vitro* (Bax & Saxena, 1993; Chester *et al*., 1993; MaassenVanDenBrink *et al*., 1998). Indeed, the use of sumatriptan, like that of ergotamine, (Galer *et al*., 1991; Yasue *et al*., 1981) has even been associated with myocardial infarction (O'Connor & Gladstone, 1995; Ottervanger *et al*., 1993) and cardiac arrest (Kelly, 1995). These limitations have prompted the development of several new 5-$HT_{1B/1D}$ receptor agonists with improved clinical and pharmacological profiles.

The 5-$HT_{1B/1D}$ receptor agonist eletriptan appears to have several advantages over sumatriptan with regard to its pharmacological and pharmacokinetic characteristics. Eletriptan has a higher affinity for the human recombinant 5-HT_{1B} and 5-HT_{1D} receptor (pK_i: 8.0 for 5-HT_{1B} and 8.9 for 5-HT_{1D} receptor) than sumatriptan (pK_i: 7.4 for 5-HT_{1B} and 8.0 for 5-HT_{1D} receptor) (Napier *et al*., 1999). Eletriptan has also been shown to be more rapidly and consistently absorbed from the gastrointestinal tract than sumatriptan (Morgan *et al*., 1997; Rance *et al*., 1997) and has a longer plasma half-life (Morgan *et al*., 1997). These features of eletriptan may contribute to its superior efficacy versus sumatriptan in comparative clinical trials (Goadsby *et al*., 2000; Pryse-Phillips & on behalf of the Eletriptan Steering Committee, 1999).

Eletriptan is effective in preclinical models that are believed to be predictive of clinical effect. For example, eletriptan effectively reduces carotid arteriovenous anastomotic blood flow in anesthetized pigs (Willems *et al*., 1998) and reverses established plasma protein extravasation in anesthetized rats with a potency and efficacy equivalent to sumatriptan (Gupta *et al*., 1996). Eletriptan has also been shown to exhibit an improved selectivity compared to sumatriptan in reducing carotid artery blood flow when compared with coronary artery diameter and femoral arterial blood flow in the anesthetized dog (Gupta *et al*., 1996). *In vitro* studies show that eletriptan elicits a potent

and concentration-dependent contraction of the dog isolated saphenous vein and basilar artery, where it acts as a partial agonist (Gupta *et al*., 1999).

Vasoconstriction of the large cranial and extracranial blood vessels is considered to be a putative mechanism of the antimigraine action of sumatriptan (Ferrari & Saxena, 1993; Saxena & Tfelt-Hansen, 2000). Indeed, sumatriptan has been shown to potently contract the human isolated middle meningeal artery (Hamel *et al*., 1993; Jansen *et al*., 1992). Thus, contraction of the human isolated middle meningeal artery by eletriptan may be indicative of its therapeutic efficacy in migraine patients. Similarly, contraction of the human isolated coronary artery (MaassenVanDenBrink *et al*., 1998) and, possibly, also of the saphenous vein (Cohen *et al*., 1998) to antimigraine drugs may predict the cardiovascular safety profile of the triptans in migraine patients without cardiovascular artery disease.

In the present study, we determined the potency (EC_{50}) and efficacy (E_{max}) of eletriptan and sumatriptan in contracting the human isolated middle meningeal artery, coronary artery and saphenous vein. Contractions to serotonin (5-hydroxytryptamine; 5-HT) were also studied as controls. As described previously (MaassenVanDenBrink *et al*., 1998), we related our findings to the therapeutic free plasma concentrations of eletriptan and sumatriptan in migraine patients.

4.2 Patients and methods

Tissue preparations

Human isolated middle meningeal artery

Middle meningeal arteries were obtained from 11 patients (six men, five women; age 26-73 years; mean age±s.e.mean: 50±5 years) undergoing craniotomy at the neurosurgical unit of the University Hospital 'Dijkzigt', Erasmus University Medical Centre, Rotterdam. During the surgical procedure, a part of the skull is temporarily removed and the dura mater, together with a small piece of the middle meningeal artery, is intentionally cut to obtain access to the brain. This piece of the artery was placed in a plastic tube filled with ice-cold (0-4°C) physiological saline and immediately transported to the laboratory.

After arrival at the laboratory, the middle meningeal artery was placed in a cold, oxygenated Krebs buffer solution of the following composition: 119 mM NaCl, 4.7 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$ and 11.1 mM glucose; pH 7.4. To avoid spontaneous contractions during the experiment, the

cyclo-oxygenase inhibitor indomethacin (0.1 μM) was added to the Krebs solution. In the experiments where relaxation to eletriptan and sumatriptan was studied after a precontraction with 5-HT, the Krebs solution was enriched with a cocktail of antagonists and re-uptake inhibitors (atropine, mepyramine, mesulergine, prazosin and imipramine: all 0.1 μM; corticosterone: 10 μM). The artery was cleaned from connective tissue, and no attempt was made to remove the endothelium.

Human isolated coronary artery

The right epicardial coronary artery was obtained from nine heart beating organ donors who died of non-cardiac disorders less than 24 h before the tissue was taken to the laboratory (six cerebrovascular accident, three trauma of the head; six men, three women; age 5-57 years; mean age±s.e.mean: 37±6 years). The hearts were provided by the Rotterdam Heart Valve Bank (Rotterdam, The Netherlands) 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 0-4°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. Vessel segments containing macroscopically visible atherosclerotic lesions were excluded.

Human isolated saphenous vein

Leftover human saphenous vein was obtained postoperatively from nine patients (seven men, two women; age 60 - 78 years; mean age±s.e.mean: 69±2 years) undergoing coronary bypass surgery at the cardiothoracic surgical unit of the University Hospital 'Dijkzigt', Erasmus University Medical Centre, Rotterdam. The tissue was immediately placed in cold (0-4°C) physiological saline and transported to the laboratory within 15 min. After arrival at the laboratory, the vein was cleaned of connective tissue and placed in a cold, oxygenated Krebs buffer solution of the same composition as used for coronary artery.

Isometric tension measurements

Vessels were cut into ring segments of approximately 3-4 mm length and suspended on stainless steel hooks in organ baths (10 ml for middle meningeal artery, 15 ml for coronary artery and saphenous vein), containing Krebs buffer solution as described above for each tissue. The buffer was aerated with 95% O_2 and 5% CO_2 and was maintained at 37°C. The segments were allowed to equilibrate for at least 30 min and were washed every 15 min. Changes in tension were measured with an isometric force transducer (EMKA Technology, Paris, France: middle meningeal artery; Harvard, South Nattick, Massachusetts, U.S.A: coronary artery and saphenous vein) and were recorded with IOX 1.103 software (EMKA Technology, Paris, France: middle meningeal artery) or on a flatbed recorder (Servogor 124, Goerz, Neudorf, Austria: coronary artery and saphenous vein). Preparations were stretched to a stable pretension of about 4 mN for middle meningeal artery, 15 mN for coronary artery or 10 mN for saphenous vein.

Experimental protocols

Human isolated middle meningeal artery

Vessel segments were exposed two to three times to 0.1 μM prostaglandin $F_{2\alpha}$ (K^+ was avoided as it frequently increased basal tone; unpublished observations) to 'prime' the tissue stable contractions. Subsequently, the segments were contracted with 1 μM prostaglandin $F_{2\alpha}$ and endothelial functional integrity was assessed by observing relaxation to substance P (10 nM). Contractions were expressed as a percentage of contraction to 1 μM prostaglandin $F_{2\alpha}$. After a 30-min incubation period, concentration response curves were constructed with eletriptan, sumatriptan and 5-HT (in all cases 0.1 nM-100 μM). Where enough segments could be obtained from one artery, experiments were performed in a paired parallel manner (i.e. all compounds were tested in different segments obtained from the same artery; n=4). Otherwise, experiments were performed in a non-parallel manner (three arteries, resulting in n=1 for eletriptan, sumatriptan and 5-HT).

Since high concentrations (>1 μM) of eletriptan and sumatriptan relaxed the middle meningeal artery (see Results), these relaxant responses were further investigated in segments precontracted with 100 μM 5-HT (n=4).

Human isolated coronary artery and saphenous vein

Segments were exposed to K^+ (30 mM) twice. After precontraction with prostaglandin $F_{2\alpha}$ (1 μM), the functional integrity of the endothelium was verified by observing relaxation to substance P (1 nM) in coronary artery or to bradykinin (1 μM) in saphenous vein (substance P is nearly inactive in saphenous vein). Following washout, 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 eletriptan, sumatriptan and 5-HT (all 1 nM-100 μM) were constructed in a paired, parallel set-up. The contractions were expressed as a percentage of contraction to 100 mM K^+.

Relation with clinical plasma concentrations

The E_{max} represents the efficacy, and the EC_{50} the potency, of a drug in eliciting a response. Thus, the lower the EC_{50} of a drug, the more likely it is to cause vasoconstriction at lower plasma concentrations; the E_{max} is only of importance when a drug is present in high enough concentrations, as dictated by its potency (see MaassenVanDenBrink *et al.* (1998). To assess the ability of eletriptan and sumatriptan to contract the blood vessels during clinical use, we calculated the ratio between the maximal free (corrected for protein binding) plasma concentration (free C_{max}) of these drugs after the clinically used oral dose (eletriptan: 40 mg and 80 mg, sumatriptan: 100 mg) (Ferrari, 1998; Goadsby, 1998; Hettiarachchi, 1999; Saxena & Tfelt-Hansen, 2000) and the EC_{50} value of the compounds. In addition, we determined the contraction that would occur at free C_{max}. Free C_{max} values were used because the fraction of a drug that is bound to plasma proteins only serves as a reservoir and is pharmacologically inactive (Du Souich *et al*., 1993; Proost *et al*., 1996; Wright *et al*., 1996).

Data analysis and presentation

The concentration response curves obtained with eletriptan, sumatriptan and 5-HT were analyzed using GraphPad software (GraphPad software Inc., San Diego, California, U.S.A.) to determine EC_{50} values. In case a concentration response curve did not reach a plateau, the contraction in response to the highest concentration was considered as E_{max}. Contraction occurring at free C_{max} was calculated by interpolation of the individual concentration response curves, using a sigmoidal function (SlideWrite Plus, Advanced Software Inc., Encinitas, California, U.S.A.).

EC_{50} values were transformed into pEC_{50} values ($-^{10}\log EC_{50}$) before statistical analyses to obtain a normal distribution. The EC_{50} values of the different compounds obtained in the three blood vessels were compared using analysis of variance, followed by Tukey's post hoc test (*between* tissue unpaired comparison). Analysis of variance, followed by Tukey's post hoc test was also used to compare the EC_{50} and E_{max} values of the compounds (*within* tissue comparison). Analysis of variance was performed in a paired manner for coronary artery and saphenous vein and in an unpaired manner for middle meningeal artery. The relaxation of middle meningeal artery observed with eletriptan and sumatriptan was compared using an unpaired t-test.

For analysis on the predicted contraction to eletriptan and sumatriptan at free C_{max}, the mean of the reported C_{max} (Lacey *et al*., 1995; Milton *et al*., 1998; Plosker & McTavish, 1994) and plasma protein binding (Scott, 1994) values of eletriptan and sumatriptan were used. Since predicted contractions to eletriptan (40 or 80 mg) and sumatriptan (100 mg) at free C_{max} were not normally distributed, as assessed with the Kolmogorov-Smirnov test, they were compared with non-parametric statistics. In middle meningeal artery, Kruskal-Wallis test (*within* tissue unpaired comparison) was used, while in coronary artery and saphenous vein the predicted contractions were compared using Friedman test (*within* tissue paired comparison). Both Kruskal-Wallis and Friedman tests were followed by Dunn multiple post hoc comparisons. No comparison was made between predicted contractions induced by 40 and 80 mg eletriptan because these values were obtained from the same concentration-response curve and thus were not independent observations. Data are presented as mean±s.e.mean in figures. In all cases significance was assumed when $P<0.05$.

Compounds

Eletriptan hydrogen bromide and sumatriptan succinate (extracted from tablets) were a gift from Pfizer Limited (Sandwich, Kent, UK). Prostaglandin $F_{2\alpha}$ (tris salt), substance P acetate, bradykinin acetate and 5-hydroxytryptamine creatinine sulfate (serotonin; 5-HT) were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). Indomethacin hydrochloride was obtained from the pharmacy of University Hospital 'Dijkzigt', Erasmus University Medical Centre, Rotterdam. Indomethacin was dissolved in DMSO and further diluted in distilled water. All other compounds were dissolved in distilled water.

Ethical approval

The Ethical Committee of the Erasmus University Medical Centre Rotterdam approved this study.

4.3 Results

Basic contractile properties

In vessel segments of middle meningeal artery, relaxation to substance P (10 nM) amounted to 40±9% of precontraction with 1 μM prostaglandin $F_{2\alpha}$ (10±2 mN, *n*=11). Coronary artery segments relaxed in response to substance P (1 nM), the response amounting to 48±16% of precontraction (30±4 mN) induced by prostaglandin $F_{2\alpha}$ (1 μM, *n*=9). Coronary artery contraction to 100 mM K^+ was 43±12 mN. In segments of saphenous vein, relaxation to bradykinin (1 μM) was 42±7% of precontraction (10±3 mN) induced by 1 μM prostaglandin $F_{2\alpha}$ (*n*=9). Contraction to 100 mM K^+ was 17±4 mN.

Contractile responses to eletriptan, sumatriptan and 5-HT

Human isolated middle meningeal artery

As shown in Figure 4.1 (*left panel*), eletriptan, sumatriptan and 5-HT contracted the human isolated middle meningeal artery in a concentration-dependent manner up to a concentration of 1 μM. Higher concentrations of the agonists relaxed the artery; at 100 μM the relaxant response was 11±7% (5-HT), 31±7% (sumatriptan) or 87±9% (eletriptan) of their individual maximal contraction. The E_{max} and EC_{50} of the contractile response to 5-HT, sumatriptan and eletriptan did not differ significantly (Table 4.1).

To further investigate the relaxant responses to sumatriptan and eletriptan, the compounds were studied after precontraction with 5-HT (100 μM). As shown in Figure 4.1 (*right panel*), the relaxation by eletriptan (134±29% of precontraction with 100 μM 5-HT) was significantly higher than that by sumatriptan (20±5%).

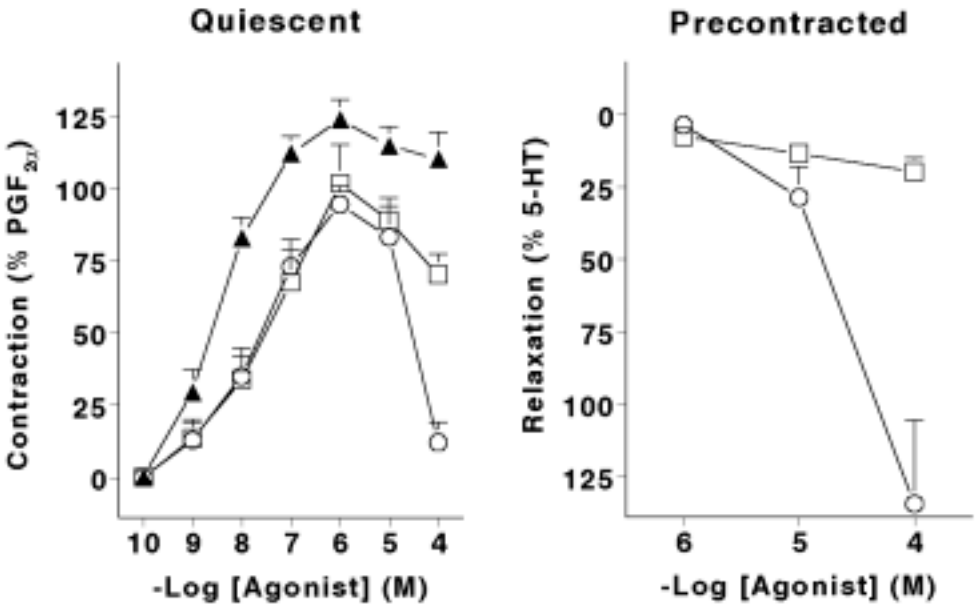

Figure 4.1 Human middle meningeal artery. *Left panel*: Contractile responses (*n*=5) to eletriptan (○), sumatriptan (□) and 5-HT (▲). *Right panel*: Relaxant responses (*n*=4) to eletriptan (○) and sumatriptan (□) in vessel precontracted with 100 μM 5-HT.

Human isolated coronary artery

Eletriptan, sumatriptan and 5-HT all caused concentration-dependent contractions of the coronary artery (Figure 4.2). The EC_{50} of eletriptan and sumatriptan was significantly higher (i.e. the potency was significantly lower) than that of 5-HT, whereas the E_{max} of these compounds was significantly smaller than that of 5-HT. Furthermore, eletriptan was significantly less potent (i.e., the EC_{50} value was significantly smaller) compared to sumatriptan (Table 4.1).

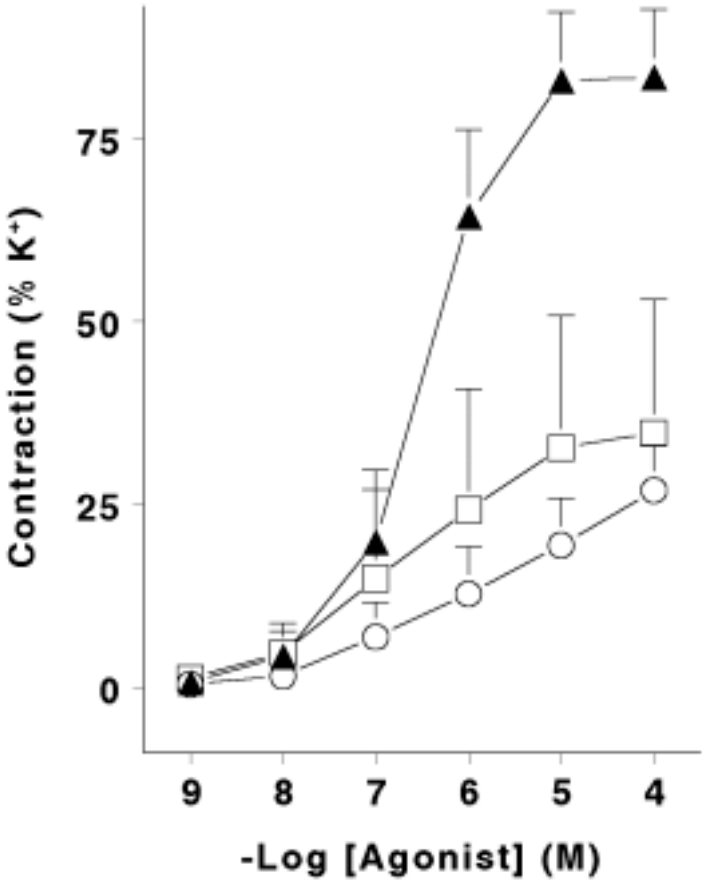

Figure 4.2 Contractile responses to eletriptan (○), sumatriptan (□) and 5-HT (▲) in the human coronary artery (*n*=9).

Human isolated saphenous vein

Eletriptan, sumatriptan and 5-HT all caused concentration-dependent contractions of the saphenous vein (Figure 4.3). The EC_{50} of both eletriptan and sumatriptan was significantly higher than that of 5-HT, whereas the E_{max} of these compounds was significantly lower than that of 5-HT (Table 4.1).

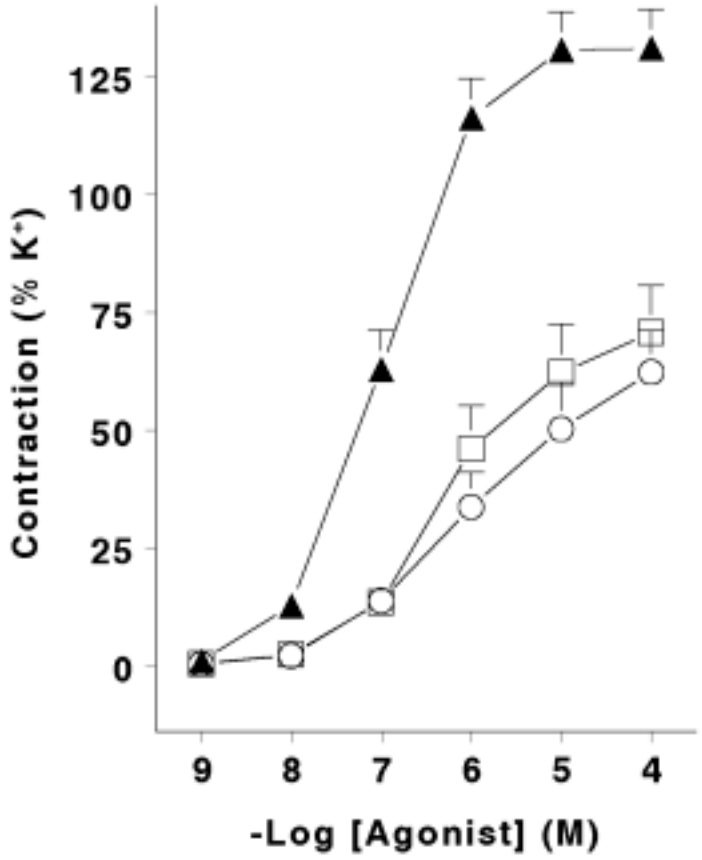

Figure 4.3 Contractile responses to eletriptan (○), sumatriptan (□) and 5-HT (▲) in the human saphenous vein (*n*=9).

Comparison of contractile responses between blood vessels

Both eletriptan and sumatriptan were significantly more potent (i.e. lower EC_{50}) in the middle meningeal artery than in coronary artery or saphenous vein (Table 4.1). This selectivity (i.e. EC_{50} middle meningeal artery divided by EC_{50} coronary artery or saphenous vein) for eletriptan was 86-fold for coronary artery and 66-fold for saphenous vein; for sumatriptan the cranioselectivity was 30-fold for coronary artery and 25-fold for saphenous vein for sumatriptan. 5-HT was also significantly more potent in the middle meningeal artery than in coronary artery (25-fold) or saphenous vein and (7-fold). In addition, 5-HT was more potent in the saphenous vein than in coronary artery (Table 4.1). Because E_{max} values between different tissues may differ depending on the internal

standard used (prostaglandin $F_{2\alpha}$ or K^+), the E_{max} values were not compared *between* tissues.

Predicted contraction at therapeutic plasma concentrations

The maximal plasma concentrations (C_{max}) attained at therapeutic doses of eletriptan and sumatriptan were corrected for plasma protein binding to obtain free C_{max} values. As described in detail earlier,(MaassenVanDenBrink *et al*., 1998) we calculated the ratio between the reported free plasma C_{max} after administration of a clinically effective dose (see Table 4.2) and the EC_{50} value of the compounds in contracting the human isolated middle meningeal and coronary artery and saphenous vein (Figure 4.4). The data show that the C_{max}/EC_{50} ratios in middle meningeal artery are considerably higher than in coronary artery or saphenous vein. Whereas in the middle meningeal artery the C_{max}/EC_{50} ratio of eletriptan (40 and 80 mg) and sumatriptan (100 mg) are similar, this ratio seems to be smaller for eletriptan in the coronary artery and saphenous vein.

In addition to the analysis described above, we determined the contraction occurring at free C_{max} by interpolation of the concentration response curves (see 'data analysis'). At free C_{max}, the predicted contractions of middle meningeal artery to eletriptan (40 mg and 80 mg) and sumatriptan (100 mg) were similar. In contrast, the predicted contraction of coronary artery and saphenous vein for the 40-mg eletriptan dose was significantly smaller than that for sumatriptan (Table 4.2).

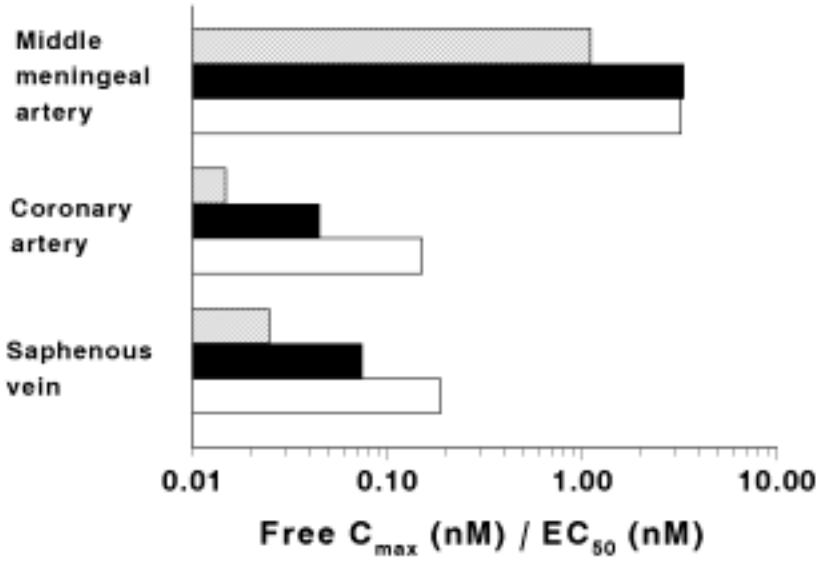

Figure 4.4 Relationship between the reported C_{max} concentration in patients (see Table 4.2) and EC_{50} values of eletriptan (40 mg, hatched columns; 80 mg, filled columns) and sumatriptan (100 mg, open columns) in contracting human isolated middle meningeal artery, coronary artery and saphenous vein. Note that a free C_{max}/EC_{50} ratio of one indicates that, if same conditions were applicable in patients as in present *in vitro* experiments, the drug would elicit 50% of its maximum contraction. In view of use of C_{max} values from literature, data were not subjected to statistical analysis.

Table 4.1 EC_{50} and E_{max} values of eletriptan, sumatriptan and 5-HT for the contraction of human blood vessels.

	Middle meningeal artery (*n*=5)		**Coronary artery (*n*=9)**		**Saphenous vein (*n*=9)**	
	EC_{50} (nM)	**E_{max} (% $PGF_{2\alpha}$)**	**EC_{50} (nM)**	**E_{max} (% K^+)**	**EC_{50} (nM)**	**E_{max} (% K^+)**
Eletriptan	50±28	98±6	4299±1624[b,c]	27±6	3299±1635[c]	62±9
Sumatriptan	53±21	103±13	1597±465[a,c]	35±18	1327±577[c]	71±11
5-HT	21±11	123±7	529±166[a,b,c]	83±9[a,b]	138±30[a,b,c,d]	130±9[a,b]

[a]Significantly different from the EC_{50} or E_{max} of eletriptan in the respective tissue (i.e. *within* tissue comparison).
[b]Significantly different from the EC_{50} or E_{max} of sumatriptan in the respective tissue (i.e. *within* tissue comparison).
[c]Significantly different from the EC_{50} in middle meningeal artery for the respective compound (i.e. *between* tissue comparison).
[d]Significantly different from the EC_{50} in coronary artery for the respective compound (i.e. *between* tissue comparison).

Table 4.2 C_{max} of eletriptan and sumatriptan following oral dose, plasma protein binding values and free C_{max} for eletriptan and sumatriptan, as well as the predicted contraction occurring at free C_{max} in middle meningeal artery, coronary artery and saphenous vein.

	Oral dose	**C_{max} (nM)**	**Plasma protein binding (%)**	**Free C_{max} (nM)**	**Predicted contraction at free C_{max} (range)**		
					Middle meningeal artery (% $PGF_{2\alpha}$)	**Coronary artery (% K^+)**	**Saphenous vein (% K^+)**
Eletriptan	40 mg	213*	83-88*	30	50 (29-74)	4 (0-25)[d]	7 (0-11)[d]
	80 mg	643[a]		90	72 (43-92)	7 (2-44)	13 (1-23)
Sumatriptan	100 mg	142-183[b]	14-21[c]	112-157	74 (46-113)	16 (0-125)	17 (2-44)

*A.D. McHarg, personal communication. [a], Milton *et al*., 1998; [b], Lacey *et al*., 1995; Plosker & McTavish, 1994; [c], Scott, 1994.
[d] Significantly different from sumatriptan in the respective blood vessel (i.e. *within* tissue comparison). No significant differences were observed between 80 mg eletriptan and sumatriptan in the respective blood vessel.

4.4 Discussion

Contraction to eletriptan and sumatriptan

In the present study, we investigated contraction elicited by eletriptan and sumatriptan in human isolated blood vessels predictive of therapeutic efficacy (middle meningeal artery) and coronary side-effect potential (coronary artery, saphenous vein). Both eletriptan and sumatriptan contracted these blood vessels in a concentration-dependent manner and exhibited selectivity for contracting the human isolated middle meningeal artery relative to coronary artery (86-fold for eletriptan and 30-fold for sumatriptan) as well as saphenous vein (66-fold for eletriptan and 25-fold for sumatriptan). Whereas there was no difference in potency between eletriptan and sumatriptan in the middle meningeal artery or saphenous vein, eletriptan was less potent than sumatriptan in coronary artery. This is in accordance with another study at our laboratory, where eletriptan was significantly less potent than sumatriptan in contracting the human isolated coronary artery (Van den Broek *et al*., 1999).

It is to be noted that, compared to sumatriptan, high concentrations of eletriptan elicited a more marked relaxation of middle meningeal artery (Figure 4.1). This relaxant response may be mediated by the 5-HT_7 receptor (Eglen *et al*., 1997; Hoyer *et al*., 1994; Saxena *et al*., 1998), since eletriptan has a higher affinity for the 5-HT_7 receptor than sumatriptan (pK_i: 6.7 for eletriptan and 5.9 for sumatriptan (Napier *et al*., 1999)). On the other hand, eletriptan (and sumatriptan) did not relax coronary artery, where, at least, the 5-HT_7 receptor mRNA has been located (Nilsson *et al*., 1999; Ullmer *et al*., 1995). We do not know whether the 5-HT_7 receptor is expressed in saphenous vein, but, in any case, neither eletriptan nor sumatriptan induced a relaxation. Irrespective of the mechanism involved, the relaxant response to eletriptan in middle meningeal artery is not clinically relevant because it was observed at a concentration of 100 μM, which is over a 1000-fold higher than its therapeutic plasma concentration (see Table 4.2). In the middle meningeal artery, contraction to eletriptan and sumatriptan reached the same E_{max} as contraction to 5-HT, whereas in the coronary artery and saphenous vein the maximum contraction to 5-HT was 2- to 3-fold higher than that of eletriptan and sumatriptan. This confirms that in the middle meningeal artery no or few 5-HT_2 receptors are present (Hamel & Bouchard, 1991; Hamel *et al*., 1993; Longmore *et al*., 1998), in contrast to coronary artery and saphenous vein where contraction to 5-HT is mainly mediated by 5-HT_2 receptors (Bax *et al*., 1993; Bax *et al*., 1992; Connor *et al*., 1989). The potency rank order of the

compounds obtained in the coronary artery and saphenous vein was similar, suggesting that the human isolated saphenous vein may serve as a model for human coronary artery contraction to antimigraine drugs.

Predicted contraction at therapeutic plasma concentrations

Both eletriptan and sumatriptan had a significantly lower EC_{50} (higher potency) in the middle meningeal artery than in the coronary artery or saphenous vein. This higher potency in the middle meningeal artery is favorable for antimigraine drugs, conferring selectivity for cerebral over coronary blood vessels. Indeed, the predicted contraction by eletriptan and sumatriptan at free C_{max} was high in the middle meningeal artery, while contraction of the coronary artery and saphenous vein was generally low for both compounds (Table 4.2). The comparison of the magnitude of contraction in middle meningeal artery versus coronary artery and saphenous vein is hampered by the fact that contractions in middle meningeal artery were expressed as percentage of contraction to 1 μM prostaglandin $F_{2\alpha}$ (K^+ was avoided as it frequently increased basal tone), while in coronary artery and saphenous vein they were expressed as percentage of contraction to K^+ (100 mM). However, the contraction to 1 μM prostaglandin $F_{2\alpha}$ in the coronary artery and saphenous vein was about 60-70% of the contraction obtained with 100 mM K^+ (see Results, basic contractile properties). Thus, the use of prostaglandin $F_{2\alpha}$- or K^+-induced contraction for reference most probably does not account for the high efficacy of eletriptan and sumatriptan in contracting middle meningeal artery.

The predicted coronary artery contraction at free C_{max} was lower for 40 mg eletriptan than for sumatriptan (100 mg), whereas there was no difference in predicted middle meningeal artery contraction (see Table 4.2). This data is supported by a recent study in patients without significant obstructive coronary artery disease who underwent diagnostic cardiac catheterisation. Eletriptan was infused at 3.33 μg/kg/min (that resulted in a mean free C_{max} equivalent to that observed after an oral dose of 40 mg) and it produced no significant changes in the mean proximal, middle or distal coronary artery diameters (Muir *et al*., 1999). However, one of the ten patients investigated exhibited a segmental constriction of the proximal right coronary artery that may have been catheter-induced (Muir *et al*., 1999) or perhaps caused by the drug. In contrast, intravenous infusion of sumatriptan (total dose of 48 μg/kg) reduced the coronary artery diameter significantly with 13% (MacIntyre *et al*., 1992). It should, however, be noted

that the plasma concentration obtained with this infusion yielded 3-4 times the C_{max} obtained with 100-mg oral dose of sumatriptan. In summary, it appears that the 40-mg dose of eletriptan may have a low therapeutic burden, including coronary side effects, whilst retaining therapeutic efficacy.

The interpretation of the above data may be influenced by certain factors, including the presence of circulating active metabolites of the parent drug (MaassenVanDenBrink *et al*., 1998). In contrast to sumatriptan (Duquesnoy *et al*., 1998; Fowler *et al*., 1991), a pharmacologically active N-desmethyl metabolite has been described for eletriptan (Milton *et al*., 1996). This metabolite is similar to the parent compound with respect the affinity at 5-HT_{1B} and 5-HT_{1D} receptors, but its plasma levels are 7-9 fold lower than eletriptan concentrations in humans (A.D. McHarg, personal communication). Therefore, N-desmethyl eletriptan is unlikely to contribute to the pharmacological action of eletriptan during clinical use. Further, the calculations of the predicted contraction to eletriptan and sumatriptan were based on plasma concentrations obtained outside migraine attacks. Whereas it is known that the absorption of 100 mg oral sumatriptan is not significantly affected during migraine attacks (Cutler *et al*., 1991; Plosker & McTavish, 1994), no such data are available about C_{max} values following therapeutic doses (40 or 80 mg) of eletriptan. However, the C_{max} following 30 mg oral eletriptan is reduced by 31% during migraine attacks (Johnson *et al*., 1997). Extrapolation of this finding may mean that plasma concentrations following 40- and 80-mg doses of eletriptan are also decreased during a migraine attack. This may lead to a reduced coronary artery constriction, but then the constriction of middle meningeal artery, probably mediating therapeutic efficacy, may also be reduced.

In any case, the predicted coronary artery contraction to both doses of eletriptan as well as to sumatriptan is such that myocardial ischemia is unlikely to occur in patients with healthy coronary arteries. This is consistent with data obtained in this laboratory for other triptans (MaassenVanDenBrink *et al*., 1998). In contrast, in patients with pre-existing coronary artery lesions who have only a limited coronary reserve (Winbury & Howe, 1979), even a small coronary artery contraction could lead to myocardial ischemia. A similar phenomenon may also be observed in patients with "variant" angina pectoris, who have increased coronary artery sensitivity to 5-HT (McFadden *et al*., 1992). Therefore, both eletriptan and sumatriptan remain contraindicated in coronary artery disease.

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4.6 Post-Publication Peer Review: Craniovascular selectivity of eletriptan and sumatriptan in human isolated blood vessels

Determining safety and appropriate use of medications are key concerns in drug development. We therefore consider it necessary to correct any misconceptions that might arise from this article by VanDenBrink and colleagues. The authors rightly conclude that sumatriptan and eletriptan have limited propensity to cause adverse coronary side effects, but incorrectly imply that eletriptan is relatively more selective for meningeal vis-à-vis coronary arteries than sumatriptan. Their misleading conclusion is unfounded because of their use of inappropriate analyses, such that: **1.** The contractions of human isolated coronary artery to both sumatriptan and eletriptan were both small and variable, with some S.E.M. values being greater than 50% of the mean. **2.** Contractions to eletriptan, but not sumatriptan, continued to elevate with increasing concentration without plateauing. This distorts EC_{50} estimates which should only be used for comparison of agonist potencies when a response plateau has been clearly demonstrated for each agonists and the maxima are shown to be the same (reference 2, below). **3.** It makes no sense to estimate separate EC_{50} values from the concentration- contraction curves for eletriptan and sumatriptan knowing that the two curves are statistically indistinguishable. **4.** Comparison of contractile effects *in vitro* relative to estimated clinical plasma concentrations, provided by the authors, must be interpreted cautiously because of the lack of robustness of the quantitative data for both parameters. Because of extensive plasma protein binding of eletriptan, its free concentration has been calculated on the assumption that the protein binding involved is a linear phenomenon unaffected by other possible therapies, which is not the case in reality where non equilibrium kinetics apply.

With either drug, the plasma concentrations normally achieved are associated with little or no coronary artery constriction. Further suggesting that there might be less contractile effects with eletriptan than sumatriptan are misleading. Thus, there is a general consensus that all the triptans have similar pharmacology and a similar but small degree of ability to constrict human conduit coronary arteries. This should not confound the view that all triptans have a good safety profile when used properly in appropriate patient populations, devoid of ishemic heart disease (reference 1).

P.P.A. Humphrey Dr. W. Feniuk Dr. R. Salonen; Neurology 2001, 57: 162-a

1) Goadsby, PJ, Hargreaves, RJ. Mechanisms of action of serotonin 5- $HT_{1B/1D}$ agonists: insights into migraine pathophysiology using rizatriptan. Neurology, 55 (9 suppl 2):S8-14, 2000).
2) Kenakin, T. pharmacologic analysis of drug-receptor interaction. Raven, New York, second edition (1993).

4.6 Response to: Post-Publication Peer Review: Craniovascular selectivity of eletriptan and sumatriptan in human isolated blood vessels

The overall conclusion and indeed the 'take-home' message in our paper (MaassenVanDenBrink et al.; reference 1) is that "at therapeutic concentrations both eletriptan and sumatriptan contract middle meningeal artery more than coronary artery, suggesting that in patients with healthy coronary arteries they have a limited propensity to cause adverse coronary side effects [, but] both drugs remain contraindicated in patients with coronary artery disease". While agreeing with this conclusion, Humphrey and colleagues term our analyses 'inappropriate' and our finding that eletriptan may be a little more selective for meningeal vis-à-vis coronary arteries than sumatriptan 'misleading' and 'unfounded'. We believe that the choice of these adjectives is rather harsh and not borne by their critique, which we address in the same order. **1**. We know that coronary artery contractions to sumatriptan often show a relatively large variability (references 2,3) and, therefore, our experiments were conducted in parallel, i.e. both eletriptan and sumatriptan were studied in segments obtained from each of the 9 coronary arteries. Despite this variability, which was more conspicuous with sumatriptan (see Fig. 2; reference 1), the difference in the EC_{50} values was statistically discernible. **2**. As pointed out in our paper, concentrations of eletriptan and sumatriptan higher than 10^{-4} M (which are some 500 times the C_{max} values and produced contractions that were statistically not different) could not be used. One may argue whether or not the maximum coronary contraction (E_{max}) was reached with eletriptan, the 'distortion' of EC_{50} estimate is relatively small and, that too, to the 'disadvantage' of eletriptan, whose EC_{50} would then be still higher; this is supported by computer simulation with extrapolated Emax values. Moreover, comparable agonists maxima (which is in all likelihood the case here) may be a requirement when one is using agonists EC_{50} ratios for the purpose of receptor classification4, but are not essential for analyses of the kind used in the present case, in particular, the estimation of predicted contractions following drug C_{max}. **3**. The concentration contraction curves for eletriptan and sumatriptan consist of a family of 9 paired curves and whether or not the mean curves were "statistically indistinguishable", it is strange that Humphrey *et al*. allege that "it makes no sense to estimate separate EC_{50} values from the concentration-contraction

curves for eletriptan and sumatriptan". Surely, the proximity of curves does not preclude estimation of EC_{50} values for the two compounds. **4**. We are quite aware that one must be cautious with interpretation of in vitro experimental data for clinical situations, where laboratory conditions do not always apply (see Conclusion and the last part of Discussion; reference 1). There is nothing wrong in using therapeutic plasma concentration for our analyses. If this concentration is affected by concomitant medications, one may need to adjust the drug doses accordingly.

Lastly, we like to point out that the methods and analyses applied here were very much the same as in our earlier publication (reference 3), dealing with human coronary artery contractions elicited by sumatriptan, ergots as well as some other triptans. One may perhaps also argue that the contraction to sumatriptan (10^{-4} M) had not reached a plateau (see Fig. 1; reference 3), but then we reported that "all [investigated] drugs … were more potent (lower EC_{50} values) than sumatriptan in contracting the human isolated coronary artery". Our sentiments expressed then (reference 3), now (reference 1) or elsewhere (references 5,6) remain the same: all triptans, including sumatriptan, have a good safety profile in otherwise healthy subjects, but they must remain contraindicated in patients with coronary artery disease.

In conclusion, while we refute the critique expressed by Humphrey and colleagues, we feel consoled that this critique is from such reputed scientists as Humphrey and colleagues, who obviously agree with our 'take- home' message.

P.R. Saxena, R.W.M. van den Broek, A. MaassenVanDenBrink; Neurology 2001, 57: 163-a

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Chapter 5

Human middle meningeal and coronary artery contractions by donitriptan and sumatriptan: Prediction of therapeutic plasma concentration of donitriptan.

Summary - Donitriptan is a potent, high efficacy agonist at 5-$HT_{1B/1D}$ receptors. We investigated the contractile effects of donitriptan and sumatriptan on human isolated blood vessels of relevance to therapeutic efficacy in migraine (middle meningeal artery) and coronary adverse events (coronary artery). Furthermore, using the concentration response curves in the middle meningeal artery, we predicted the plasma concentration needed for the therapeutic effect of donitriptan. Both donitriptan and sumatriptan contracted the middle meningeal artery with similar apparent efficacy (E_{max}: 103±8% and 110±12%, respectively), but the potency of donitriptan (pEC_{50}: 9.07±0.14) was significantly higher than that of sumatriptan (pEC_{50}: 7.41±0.08). In the coronary artery, the contraction to donitriptan was biphasic with a significantly higher maximal response (E_{max}: 29±6%) than sumatriptan (E_{max}: 14±2%; pEC_{50}: 5.71±0.16), yielding two distinct pEC_{50} values (8.25±0.16 and 5.60±0.24). Incubation with the 5-HT_2 receptor antagonist ketanserin (10 μM) eliminated the low affinity component of the concentration response curve of donitriptan and the resultant E_{max} and pEC_{50} were 9±2% and 7.33±0.21, respectively. Ketanserin was without effect on the sumatriptan-induced contraction. Based on the middle meningeal artery contraction, concentrations (C_{max}) of donitriptan that may be expected to have a therapeutic efficacy equivalent to that of 50 and 100 mg sumatriptan are predicted to be around 2.5 and 4.3 nM, respectively. Such concentrations are likely to induce only a small coronary artery contraction 2.9±1.5% and 3.8±2.0%, respectively; these are not different from those by C_{max} concentrations of sumatriptan (1.7±0.4% or 2.2±0.4%). The present results suggest that, like sumatriptan, donitriptan exhibits cranioselectivity and would be effective in aborting migraine attacks with a similar coronary side-effect profile as sumatriptan.

Based on: van den Broek, R.W.M., MaassenVanDenBrink, A., Mulder, P.G.M., Bogers, A.J.J.C., Avezaat, C.J. & Saxena, P.R. (2001). Comparison of donitriptan and sumatriptan-induced contractile responses in the human middle meningeal and coronary arteries. *Eur. J. Pharm.*, **submitted**.

5.1 Introduction

The introduction of the 5-$HT_{1B/1D}$ receptor agonist sumatriptan (Humphrey and Feniuk, 1991) has been followed by a number of 'second-generation' triptans (Connor et al., 1997; Martin, 1997; Parsons et al., 1998; Gupta et al., 1999), all of which effectively abort migraine headache (Goadsby, 1998; Diener and Limmroth, 1999; Deleu and Hanssens, 2000; Millson et al., 2000; Tfelt-Hansen et al., 2000; Ferrari et al., 2001). Despite the higher affinity at 5-$HT_{1B/1D}$ receptors, better oral bioavailability, increased brain penetration and longer plasma half-life, somewhat surprisingly, the newer triptans show only subtle differences with sumatriptan in the efficacy and tolerability (Goadsby, 1998; Deleu and Hanssens, 2000; Fox, 2000; Millson et al., 2000; Tfelt-Hansen et al., 2000; Ferrari et al., 2001). In the light of the above, Centre de Recherche Pierre Fabre (Castres, France) have reported another triptan, donitriptan (F11356: hydrochloride salt or F12640: mesylate salt), which displays a uniquely high affinity and, more importantly, intrinsic efficacy at recombinant human 5-$HT_{1B/1D}$ receptors with negligible affinity for the 5-HT_{1F} receptor (Table 5.1). In animal experiments, donitriptan has been shown to have a long duration of action, to gain access to the brain and to be well-tolerated (John et al., 1999; 2000). The therapeutic potential of the drug is currently being evaluated in migraine patients.

It is now generally accepted that the therapeutic action of sumatriptan and other triptans is mainly due to constriction of dilated intra- and extracranial blood vessels, although other mechanisms interfering with the trigeminovascular system may also be involved (De Vries et al., 1999; Tfelt-Hansen et al., 2000; Feniuk and Humphrey, 2001). The constriction of cranial blood vessels by triptans is much more marked than that of the peripheral arteries, such as the coronaries (Longmore et al., 1998; Parsons et al., 1998; MaassenVanDenBrink et al., 2000; Van den Broek et al., 2000). Despite this cranioselectivity, the triptans do have a propensity to cause cardiovascular adverse events, including myocardial ischaemia and infarction in predisposed individuals (Kelly, 1995; Ottervanger et al., 1997; Main et al., 1998).

In the present study, we have investigated the contractile effects of donitriptan and sumatriptan on human isolated blood vessels of relevance to therapeutic efficacy (middle meningeal artery) and coronary adverse events (coronary artery). Furthermore, using the concentration response curves in the middle meningeal artery and the basic assumptions about the mechanism of action of triptans, we have made an attempt to predict the free

(protein-unbound) plasma concentration needed for therapeutic effect of donitriptan, which would shortly undergo clinical trials.

5.2 Material and methods

Tissue collection

Human isolated middle meningeal artery

Human middle meningeal arteries were obtained from 7 patients (3 male, 4 female; age 20-72 years) undergoing craniotomy at the neurosurgical unit of the University Hospital 'Dijkzigt', Erasmus University Medical Centre, Rotterdam. During the surgical procedure, a part of the skull is temporarily removed and the duramater, together with a small piece of the middle meningeal artery, is cut to obtain access to the brain. This piece of the artery was placed in a plastic tube filled with ice-cold (0-4 °C) physiological saline and immediately transported to the laboratory. Upon arrival at the laboratory, the artery was placed in a cold oxygenated modified Krebs bicarbonate solution of the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 1.25, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.1; pH 7.4. The cyclo-oxygenase inhibitor indomethacin (0.1 μM) was added to the Krebs solution to prevent prostaglandin synthesis. Excess tissue surrounding the artery was carefully removed and no attempt was made to remove the endothelium. The middle meningeal artery was stored overnight in cold oxygenated Krebs solution and was used the following day.

Human isolated coronary artery

The human right epicardial coronary artery was obtained from 7 ‘heart-beating’ organ donors (5 male, 2 female; 12-65 years), who died of non-cardiac disorders. The hearts were provided by the Heart Valve Bank, Rotterdam, The Netherlands after donor mediation by Bio Implant Services Foundation/Eurotransplant Foundation, Leiden, The Netherlands; for details, see MaassenVanDenBrink et al. (1998). Upon arrival in the laboratory, the right coronary artery was cleaned from the surrounding tissue and placed in a cold, oxygenated Krebs bicarbonate solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 8.3; pH 7.4. No attempt was made to remove the endothelium. The artery was stored overnight in cold oxygenated Krebs solution and was used the following day.

Experimental protocol

Human isolated middle meningeal artery

The middle meningeal artery was cut into circular 3- to 4-mm long segments, which were mounted on metal prongs in 10-ml organ baths containing Krebs bicarbonate solution, aerated with 95% O_2 and 5% CO_2 and maintained at a temperature of 37 ^oC. Changes in isometric tension were measured by a force displacement transducer and recorded with IOX 1.203h software (both EMKA Technology, Paris, France). Segments were stretched to a passive tension of 4 mN and were allowed to stabilise at this level for 60 min (with replacement of Krebs solution every 15 min). All segments were then exposed 2-3 times to 0.1 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to demonstrate reproducibility of the evoked contractions. Subsequently, the segments were pre-contracted with $PGF_{2\alpha}$ (1 μM) and the relaxation response to substance P (10 nM) was used to evaluate the presence of a functional endothelium. After washing, the segments were allowed to equilibrate for 60 min, with replacement of buffer every 15 min. The segments were then used, where possible, in a paired parallel experimental set-up and cumulative concentration response curves to either donitriptan (0.03 nM-1 μM) or sumatriptan (1 nM-10 μM) (both dissolved in 40% v/v polyethylene glycol) were constructed.

Human isolated coronary artery

Vessels were cut into ring segments of approximately 4-mm length and were suspended on stainless steel hooks in 15-ml organ baths containing Krebs bicarbonate solution aerated with 95% O_2 and 5% CO_2 and maintained at a temperature of 37 ^oC. Vessel segments containing macroscopically visible atherosclerotic lesions were not used. Changes in tension were recorded using a Harvard isometric transducer (Harvard Apparatus, South Natick, MA, USA). The segments were allowed to equilibrate for at least 30 min and were washed every 15 min. Segments were stretched to a passive tension of 15 mN. All segments were then exposed to KCl (30 mM) twice, to prime tissue reproducibility. Subsequently, the functional integrity of the endothelium was verified by observing relaxation to substance P (1 nM) after pre-contraction with $PGF_{2\alpha}$ (1 μM). After washout, the tissue was exposed to KCl (100 mM) to determine the maximal contractile response to KCl. The tissue was washed and then was allowed to equilibrate for another period of 30 min. After this equilibration period the segments were divided in a paired parallel experimental set-up and cumulative concentration response curves to

either donitriptan, sumatriptan (both dissolved in 40% v/v polyethylene glycol) or sumatriptan (dissolved in distilled water) were constructed. In addition, we incubated half of the segments with the 5-HT_2 receptor antagonist ketanserin (10 μM) for 30 min prior to constructing concentration response curves to donitriptan and sumatriptan.

Data analysis

Concentration response curves

Contractile responses were expressed as percentage of the contractile response to 1 μM $PGF_{2\alpha}$ (middle meningeal artery) or 100 mM KCl (coronary artery). Initially, we calculated the mean value of the individual maximum contractile responses (E_{max}) to donitriptan and sumatriptan; in case the contractions did not reach a plateau the contraction induced by the highest agonist concentration was considered as E_{max}. Except for donitriptan in the coronary artery, we analysed all concentration response curves with a non-linear regression fitting technique for sigmoidal functions with variable slope using Prism® 3.0 (GraphPad Software Inc., San Diego, CA, USA) to obtain pEC_{50} values for the agonists. The mean concentration response curve to donitriptan in the coronary artery appeared to be biphasic in nature. Indeed, the curve fitted significantly better to a model of two receptor populations than to a sigmoidal model (Goodness of Fit: R=0.99 and 0.97, respectively, $P<0.05$; Prism® 3.0). Thus, two distinct pEC_{50} values of donitriptan-induced contractions in coronary artery were obtained.

Cranioselectivity ratios were calculated as the inverse logarithmic difference between respective pEC_{50} values of donitriptan and sumatriptan in the middle meningeal and coronary arteries (see MaassenVanDenBrink *et al*., 2000; Van den Broek *et al*., 2000).

Prediction of plasma concentration of donitriptan required for therapeutic activity

The average free (protein-unbound) maximum plasma concentration (C_{max}) in human volunteers following 50 mg and 100 mg oral sumatriptan is 81 nM and 135 nM, respectively (see MaassenVanDenBrink et al., 1998; Fox, 2000; MaassenVanDenBrink et al., 2000). The extent of middle meningeal artery contractions elicited at these concentrations of sumatriptan was derived using individual concentration response curves (see Fig. 5.1). Based on the assumption that constriction of dilated cranial blood vessels is the major mechanism of action of triptans in migraine (De Vries et al., 1999; Tfelt-Hansen et al., 2000; Feniuk and Humphrey, 2001), we have attempted to predict therapeutic C_{max}

of donitriptan, i.e. the concentrations of donitriptan that would be needed to elicit a middle meningeal artery contraction equivalent to that caused by 81 nM or 135 nM of sumatriptan. In two out of seven experiments with donitriptan in the middle meningeal artery, the predicted contraction was higher than the individual E_{max} of donitriptan, thus excluding these experiments from this analysis. Therefore, we chose to analyse the mean concentration response curve of donitriptan to predict the mean therapeutic free C_{max} and 95% CI. Lastly, the individual concentration response curves in the coronary artery were used to calculate the extent of coronary artery contraction that may be expected at the (predicted) therapeutic C_{max} of sumatriptan and donitriptan.

Statistical analysis

Differences between E_{max} and pEC_{50} values of concentration response curves as well as the goodness of fit (sigmoidal or biphasic nature of donitriptan in the coronary artery) were analysed according to paired t-test or repeated measures one-way analysis of variance (ANOVA), followed where appropriate (coronary artery) by Tukey's multiple comparison t-test (Prism® 3.0). The 95% confidence interval (95% CI) of cranioselectivity ratios obtained with donitriptan and sumatriptan was calculated and compared as described by Steel and Torrie (1980). In all cases, statistical significance was assumed when $P<0.05$. Except for cranioselectivity ratios and free C_{max} values, where the geometric means with 95% CI are given, all other data are presented as mean±S.E.M.

Ethical approval

The Medical Ethics Committee of the Erasmus University Medical Centre Rotterdam, dealing with the use of human material for scientific experiments, approved the protocols for this investigation.

Compounds

Indomethacin hydrochloride, ketanserin tartrate, prostaglandin $F_{2\alpha}$ tris salt and substance P acetate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Donitriptan mesylate (F12640) was kindly supplied by Centre de Recherche Pierre Fabre (Castres-Cedex, France). Sumatriptan succinate (batch: C1008/122/1) was a gift from Glaxo Wellcome (Ware, Kent, UK). Indomethacin was dissolved in 100% v/v dimethyl sulphoxide and further diluted in distilled water. Donitriptan mesylate (F12640) was

dissolved in 40% v/v polyethylene glycol and sumatriptan was either dissolved in 40% v/v polyethylene glycol or distilled water. All other compounds were dissolved in distilled water.

5.3 Results

Relaxation responses to substance P

In vessel segments of the middle meningeal and coronary arteries (n=7 each) the relaxation to substance P amounted to 26±8% and 58±10% of precontraction to 1 μM $PGF_{2\alpha}$, respectively.

Human middle meningeal artery

As shown in Fig. 5.1 and Table 5.2, both donitriptan (n=7) and sumatriptan (n=5) contracted the middle meningeal artery in a concentration-dependent manner with a similar efficacy (E_{max}: 103±8% and 110±12% of contraction to 1 μM $PGF_{2\alpha}$, respectively). The potency (pEC_{50}) of donitriptan was significantly higher than that of sumatriptan (9.07±0.14 and 7.41±0.08, respectively; $P<0.0001$). This was also the case when only the 5 experiments with donitriptan performed in parallel with sumatriptan were considered (pEC_{50}: 9.05±0.17). The Hill slopes of the concentration response curves to donitriptan (1.2±0.3) and sumatriptan (1.1±0.3) did not significantly differ from each other, nor were they significantly different from unity.

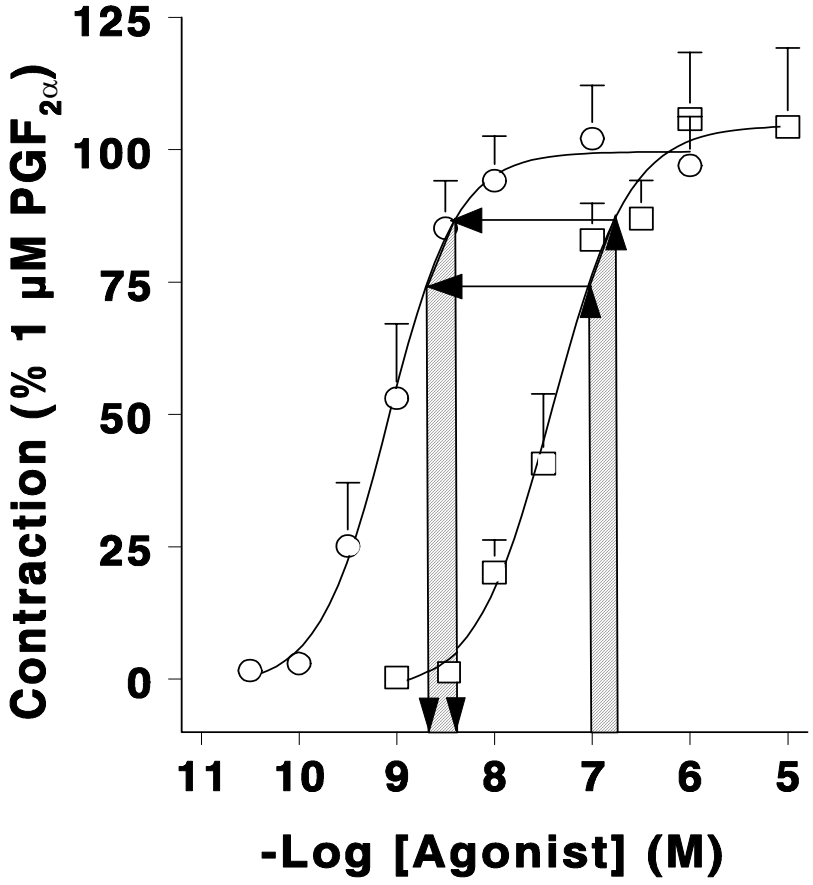


Figure 5.1 Cumulative concentration response curves to donitriptan (○; n=7) and sumatriptan (□; n=5) in the human isolated middle meningeal artery. Symbols and vertical bars represent the means and S.E.M. Arrows represent the prediction of the contraction of the artery at the average free (protein-unbound) C_{max} (81 or 135 nM) after a therapeutic dose of sumatriptan (50 mg or 100 mg oral tablet, respectively) and the predicted therapeutic C_{max} of donitriptan needed to elicit a contraction equivalent to that caused by 81 or 135 nM sumatriptan.

Human coronary artery

The human isolated coronary artery also contracted in response to donitriptan and sumatriptan in a concentration-dependent manner (Fig. 5.2 and Table 5.2). Donitriptan contracted the coronary artery biphasically with an E_{max} of 29±6% of contraction to 100 mM KCl. The concentration response curves to donitriptan were fitted to a model of two receptor populations and revealed two distinct pEC_{50} values (8.25±0.16 and 5.60±0.24). Incubation with the 5-HT_2 receptor antagonist ketanserin (10 µM) revealed a sigmoidal curve with a potency (pEC_{50}) of 7.33±0.21 yielding a significant decrease in E_{max} (9±2%

of contraction to 100 mM KCl) as compared to donitriptan in the absence of ketanserin (P<0.01). The Hill slope of the concentration response curves to donitriptan in the presence of ketanserin was 0.8±0.1, which was not significantly different from unity.

Sumatriptan, dissolved in distilled water, contracted the coronary artery with a potency (pEC_{50}) of 5.71±0.16 and maximal effect (E_{max}) of 14±2% of contraction to 100 mM KCl; these were significantly lower as compared to the pEC_{50} (high affinity component) and E_{max} of donitriptan. The Hill slope of concentration response curves to sumatriptan (0.6±0.1) was significantly different from unity. Incubation with the 5-HT_2 receptor antagonist ketanserin (10 μM) was without effect on either the E_{max} (11±3% of contraction to 100 mM KCl) or the pEC_{50} (5.76±0.14) of sumatriptan, whereas the Hill slope did not differ from unity (0.8±0.3).

Due to the fact that donitriptan was dissolved in 40 v/v polyethylene glycol, we investigated whether the solvent influenced contraction to sumatriptan. Sumatriptan dissolved in 40 v/v polyethylene glycol caused a similar contraction (E_{max}: 10±2% of contraction to 100 mM KCl; pEC_{50}: 6.18±0.29; Hill slope: 0.8±0.3) as compared to sumatriptan dissolved in distilled water.

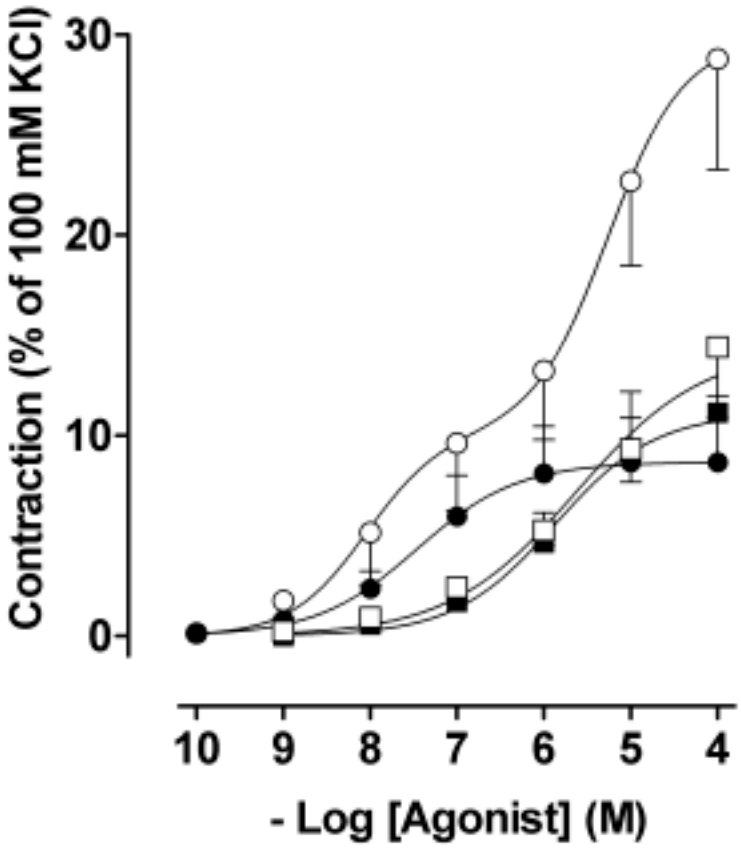


Figure 5.2 Cumulative concentration response curves (n=7 each) to donitriptan in the absence (○) or presence of 10 μM ketanserin (●) and sumatriptan in the absence (□) or presence of 10 μM ketanserin (■). Symbols and vertical bars represent the mean and S.E.M.

Cranioselectivity of donitriptan and sumatriptan

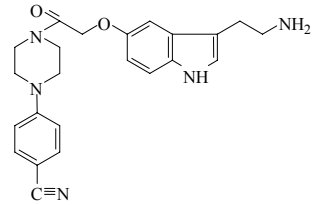
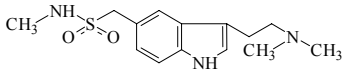
The mean cranioselectivity ratios (inverse logarithm of the difference between the pEC_{50} values in the middle meningeal and coronary arteries) and the respective 95% CI are shown in Table 5.2. Due to the fact that donitriptan showed a biphasic response in the human coronary artery, the pEC_{50} value obtained in the lower (high affinity) part of the curve was used to calculate the cranioselectivity ratio, since the therapeutic concentration is expected to be in this range (see above). Both donitriptan and sumatriptan were significantly more potent in the middle meningeal artery as compared to the coronary artery, whereas the cranioselectivity ratio for donitriptan was slightly lower than that for sumatriptan. After treatment with ketanserin, both donitriptan and sumatriptan had similar cranioselectivity ratios (Table 5.2).

Prediction of plasma concentration of donitriptan required for therapeutic activity

The mean therapeutic free C_{max} following administration of a 50 mg or 100 mg oral tablet of sumatriptan has been reported to be 81 nM and 135 nM, respectively (see MaassenVanDenBrink et al., 1998). As shown in Table 5.3, the contraction to sumatriptan that is predicted to occur at these concentrations was 79±8% and 89±9% of contraction to 1 μM $PGF_{2\alpha}$, respectively, in the middle meningeal artery and 1.7±0.4% and 2.2±0.4% of contraction to 100 mM KCl, respectively, in the coronary artery. Interpolating concentration response curves to donitriptan in the middle meningeal artery, the predicted therapeutic free C_{max} values of donitriptan (i.e. concentration eliciting 79% or 89% of contraction to 1 μM $PGF_{2\alpha}$) were 2.5 nM (95% CI: 1.5-6.5 nM) and 4.3 nM (95% CI: 2.4-12.9 nM), respectively. At these predicted free C_{max} values, the contraction of donitriptan in the coronary artery may amount to 2.9±1.5% and 3.8±2.0% of contraction to 100 mM KCl, respectively; these were not significantly different from those calculated for sumatriptan (Table 5.3).

Table 5.1

Chemical structure and affinity and efficacy of donitriptan and sumatriptan at human 5-$HT_{1B/1D/1F}$ receptors.

	Chemical structure	Inhibition radioligand binding (pK_i)			Inhibition cAMP accumulation (pEC_{50})		Enhancement [^{35}S]GTPγS binding (pEC_{50})	
		5-HT_{1B}	5-HT_{1D}	5-HT_{1F}	5-HT_{1B}	5-HT_{1D}	5-HT_{1B}	5-HT_{1D}
Donitriptan[a]		9.4-10.1	9.3-10.2	5.5	8.91	9.57	8.74	9.08
Sumatriptan		7.8[b]	8.5[b]	7.9[b]	7.16[c]	8.79[c]	6.63[c]	7.75[c]

Data from: [a], John et al. (1999); [b], Leysen et al. (1996); [c], Pauwels et al. (1997). pK_i and pEC_{50} are expressed as –Log M.

Table 5.2 Functional parameters obtained with donitriptan and sumatriptan in the absence or presence of ketanserin (10 μM) in the human isolated middle meningeal and coronary arteries. Data are presented as means±S.E.M. or as mean (95% CI). Except sumatriptan in the middle meningeal artery (n=5), the number of experiments were 7 in each case. The E_{max} values are presented as % of contraction elicited by either 1 μM $PGF_{2\alpha}$ (middle meningeal artery) or 100 mM KCl (coronary artery). NP, Not performed.

	Middle meningeal artery		Coronary artery		Cranioselectivity ratio[a] (95% CI)
	E_{max}	pEC_{50}	E_{max}	pEC_{50}	
Donitriptan	103±8%	9.07±0.14[b]	29±6%[b]	8.25±0.16[b,c] 5.60±0.24[c]	7 (3-16)[d,e]
Donitriptan + ketanserin	NP	NP	9±2%	7.33±0.21[b]	55 (19-162)[d]
Sumatriptan	110±12%	7.41±0.08	14±2%	5.71±0.16	50 (22-113)[d]
Sumatriptan + ketanserin	NP	NP	11±3%	5.76±0.14	45 (22-92)[d]

[a], Cranioselectivity ratio = Inverse logarithm [pEC_{50} (middle meningeal artery) – pEC_{50} (coronary artery)].
[b], Significantly different from respective values obtained with sumatriptan (P<0.05).
[c], Biphasic concentration response curves yielding two distinct pEC_{50} values.
[d], Significantly different from 1 (P<0.05).
[e], Significantly lower than sumatriptan (P<0.05).

Table 5.3 Predicted therapeutic free plasma C_{max} of donitriptan and predicted contraction of middle meningeal and coronary arteries at free therapeutic C_{max} of sumatriptan and donitriptan. Data are presented as means±S.E.M. or as mean (95% CI). Except sumatriptan in the middle meningeal artery (n=5), the number of experiments were 7 in each case.

	Free therapeutic C_{max} (nM)		Predicted contraction at free C_{max}	
	Known[a]	Predicted	Middle meningeal artery (% $PGF_{2\alpha}$)	Coronary artery (% KCl)
Sumatriptan				
50 mg	81		79±8	1.7±0.4
100 mg	135		89±9	2.2±0.4
Donitriptan				
X mg[b]		2.5 (1.5-6.5)	79	2.9±1.5
Y mg[c]		4.3 (2.4-12.9)	89	3.8±2.0

[a], see Fox, 2000; MaassenVanDenBrink *et al*., 1998.
[b], Values based on middle meningeal artery contraction induced by free C_{max} after 50 mg sumatriptan.
[c], Values based on middle meningeal artery contraction induced by free C_{max} after 100 mg sumatriptan.

5.4 Discussion

Efficacy profile in the middle meningeal artery

It is well known that acutely acting antimigraine drugs (ergots and triptans) have a vasoconstrictor effect, particularly on large cranial arteries and arteriovenous anastomoses (De Vries et al., 1999; Tfelt-Hansen et al., 2000). Donitriptan contracted the human isolated middle meningeal artery with a similar maximal response as sumatriptan (see E_{max} values in Table 5.2). The results are in accordance with those obtained in the rabbit isolated saphenous vein (John et al., 2000), an established model to detect agonist activity at 5-HT_{1B} receptors (Valentin et al., 1996; Wurch et al., 1997), but are at variance with the high intrinsic activity of donitriptan at 5-$HT_{1B/1D}$ receptors demonstrated in other experimental models (John et al., 1999; 2000). Indeed, like the rabbit saphenous vein model, the human middle meningeal artery preparation apparently does not distinguish between low and high efficacy 5-HT_{1B} receptor agonists, suggesting the preparation is likely to express 5-HT_{1B} receptors in a high density (Longmore et al., 1997; 1998). It is noteworthy that high efficacy agonist activity is not necessarily observed in every model, and models that distinguish high from low efficacy agonists have a low receptor reserve, due to a relatively low receptor density and/or inefficient second-messenger coupling (Kenakin, 1993; John et al., 2000). We concede that the E_{max} of donitriptan-induced coronary artery contraction in the presence of ketanserin (mediated by the 5-HT_{1B} receptor) was not higher than that of sumatriptan (see Fig. 5.2), despite a low 5-HT_{1B} receptor density in this vessel (Longmore et al., 1998), but we do not have data with regard to receptor coupling.

There is now overwhelming evidence that cranial vasoconstrictor response to triptans is mediated via the 5-HT_{1B} receptor (Bouchelet et al., 1996; Longmore et al., 1997; 1998; Van den Broek et al., 2000; Centurión et al., 2001). Indeed, in accordance with the binding affinities at the human 5-HT_{1B} receptor (see Table 5.1), donitriptan (pEC_{50}: 9.07±0.14) was 48-fold more potent than sumatriptan (pEC_{50}: 7.41±0.08) in contracting the human middle meningeal artery.

Efficacy profile in the coronary artery

Concentration response curves to donitriptan in the human isolated coronary artery revealed a biphasic response with a significantly higher maximum contraction as compared to sumatriptan (E_{max}: 29±6% and 14±2% of the response to 100 mM KCl,

respectively). This biphasic response and higher response amplitude of donitriptan appears to be at variance with findings in the canine isolated coronary artery, where both donitriptan and sumatriptan showed a similar efficacy and sigmoidal shape of the concentration response curve (John et al., 2000). However, in contrast to the predominant role of 5-HT_{2A} over 5-HT_{1B} receptors in the human coronary artery (Connor et al., 1989; Kaumann et al., 1994; Ishida et al., 1999; Nilsson et al., 1999), the 5-HT_2 receptor antagonist ketanserin was unable to block 5-HT-induced contractions in the canine coronary artery, suggesting that the 5-HT_{2A} receptor plays little role in this preparation (Cushing and Cohen, 1992). Therefore, we reasoned that the biphasic response to donitriptan in the present experiments could be due to activation of a 5-HT_2-like (possibly the 5-HT_{2A}) receptor at high concentrations of donitriptan (pK_i: 6.7, John et al., 1999). Indeed, the maximal response (E_{max} as percentage of the response to 100 mM KCl) evoked by donitriptan (29±6%) was significantly reduced in the presence of ketanserin (9±2%) to a similar level as that of sumatriptan (11±3%); the latter was resistant to ketanserin (Table 5.2 and Connor et al., 1989; Kaumann et al., 1994). Furthermore, the concentration response curve to donitriptan in the presence of ketanserin was restored to a sigmoidal function and, as expected from their respective affinities at the 5-HT_{1B} receptor (Table 5.1), yielded a 37-fold higher potency (pEC_{50}: 7.33±0.21) compared to that of sumatriptan (pEC_{50}: 5.76±0.14). It should be mentioned however, that constriction to donitriptan was not blocked by ketanserin (up to 100 μM) in rat isolated aorta (G.W. John, unpublished observations), which is considered as a typical 5-HT_{2A} receptor preparation. Despite this discrepancy it appears that at low, clinically relevant concentrations donitriptan constricts the coronary artery via the 5-HT_{1B} receptor and has similar maximal response amplitude as sumatriptan.

One may argue that in the relatively high concentration employed (10 μM), ketanserin can also act as a 5-HT_{1D} receptor antagonist (Zgombick et al., 1995; Pauwels and Colpaert, 1996). This is probably not relevant because 5-HT_{1D} receptor activation is not associated with vasoconstriction (Ennis et al., 1998; Bouchelet et al., 2000; Centurión et al., 2001).

Cranioselectivity of donitriptan and sumatriptan

It is known that a number of triptans (sumatriptan, rizatriptan, frovatriptan and eletriptan) selectively constrict cranial blood vessels compared to the coronary artery (Longmore et

al., 1998; Parsons et al., 1998; MaassenVanDenBrink et al., 2000; Van den Broek et al., 2000). In the present investigation also, donitriptan as well as sumatriptan exhibited cranioselectivity because they were both more potent in contracting middle meningeal artery than coronary artery (see Table 5.2 for pEC_{50} values). The cranioselectivity ratio, calculated as inverse logarithm of the differences between the respective pEC_{50} values in the middle meningeal and coronary arteries, for donitriptan (7-fold) was smaller than that for sumatriptan (50-fold). However, it must be emphasised that, in view of the predicted therapeutic concentration range (Table 5.3), we have used the pEC_{50} value obtained with the high affinity component of the concentration response curve to donitriptan in the coronary artery. Interestingly, after elimination of the low affinity component of donitriptan by the 5-HT_{2A} receptor antagonist ketanserin, the cranioselectivity ratio was similar to that of sumatriptan (Table 5.2). This latter finding can be explained by the fact that in this preparation, the 5-HT_{2A} receptor is predominant over the 5-HT_{1B} receptor (Connor et al., 1989; Kaumann et al., 1994; Ishida et al., 1999; Nilsson et al., 1999).

Prediction of plasma concentration of donitriptan required for therapeutic activity and predicted contractions

Another way to determine cranioselectivity is to calculate predicted contractions at therapeutic free (protein-unbound) C_{max} of donitriptan and compare them with those for sumatriptan (MaassenVanDenBrink et al., 2000). Since therapeutic free C_{max} of donitriptan is currently not known, we have attempted to predict this (see Fig. 5.1) using two assumptions: (i) cranial vasoconstriction, as reflected by the human isolated middle meningeal artery contraction, is the major mechanism of therapeutic action of triptans (De Vries et al., 1999; Tfelt-Hansen et al., 2000; Feniuk and Humphrey, 2001) and (ii) data obtained in our laboratory setting by and large would apply to the clinical setting. Obviously, we cannot be absolutely sure on either count, but predicted therapeutic C_{max} of donitriptan may also help in determining initial doses to be employed in clinical trials. In any case, a comparison of the predicted and real therapeutic C_{max} values of donitriptan may be useful in the consideration of the mechanism of action of the drug.

On the basis of therapeutic free C_{max} of 50 mg or 100 mg oral sumatriptan (81 and 135 nM, see MaassenVanDenBrink et al., 1998; 2000) and the predicted contractions of the middle meningeal artery obtained at these concentrations of sumatriptan (79% or 89% of the contraction to 1 μM $PGF_{2\alpha}$), donitriptan would need a free C_{max} of ~3-4 nM to be as

effective as sumatriptan (see Table 5.3). The predicted contractions in coronary artery (expressed as percentage of the contraction to 100 mM KCl) to donitriptan were small (3-4%) and similar to those with sumatriptan at therapeutic concentrations. Thus, based on these predictions, the two drugs have an equal propensity to constrict the coronary artery at therapeutic concentrations.

5.5 References

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Chapter 6

The Potential antimigraine compound SB-220453 does not contract human isolated blood vessels or myocardium; a comparison with sumatriptan

Summary - The mechanistically novel benzopyran derivative SB-220453, which is undergoing clinical evaluation in migraine, exhibits a high affinity for a selective, but not yet characterised binding site in the human brain. It inhibits nitric oxide release and cerebral vasodilatation following cortical spreading depression as well as carotid vasodilatation induced by trigeminal nerve stimulation in the cat. The aim of our study was to investigate the contractile properties of SB-220453 on a number of human isolated blood vessels (coronary artery, saphenous vein and middle meningeal artery) as well as atrial and ventricular cardiac trabeculae. While sumatriptan induced marked contractions in all blood vessels investigated, SB-220453 was devoid of any effect. In atrial and ventricular cardiac trabeculae, neither SB-220453, nor sumatriptan displayed a positive or negative inotropic effect. Since SB-220453 did not contract the middle meningeal artery, we conclude that potential antimigraine effects are not mediated via a direct cerebral vasoconstriction. The lack of activity of SB-220453 in coronary artery, saphenous vein and cardiac trabeculae demonstrates the compound is unlikely to display adverse cardiac side effects.

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6.1 Introduction

Sumatriptan, an indole sulphonamide with agonist activity at 5-$HT_{1B/1D}$ receptors, is highly effective in aborting attacks of migraine and cluster headache. The drug is generally well tolerated, but up to 15% of patients consistently report chest symptoms, including chest pressure, tightness and pain, often mimicking angina pectoris (Brown *et al*., 1991; Polvino *et al*., 1994; Visser *et al*., 1996). Although extracardiac mechanisms have been invoked (Houghton *et al*., 1994), chest symptoms may well be caused by coronary vasoconstriction, which has been observed after sumatriptan both *in vivo* (MacIntyre *et al*., 1993) and *in vitro* (Bax & Saxena, 1993; Chester *et al*., 1993; Connor *et al*., 1989; MaassenVanDenBrink *et al*., 1998). In some cases, the use of sumatriptan, like that of ergotamine (Galer *et al*., 1991; Yasue *et al*., 1981), was even associated with myocardial infarction (O'Connor & Gladstone, 1995; Ottervanger *et al*., 1993) and cardiac arrest (Kelly, 1995). 'Second generation' sumatriptan-like antimigraine drugs are aimed at, in addition to achieving high efficacy and long duration of action, avoiding coronary vasoconstrictor activity (Saxena *et al*., 1996). However, these drugs also contract human isolated coronary artery and seem to have a similar coronary side-effect potential as sumatriptan (MaassenVanDenBrink *et al*., 1998; MaassenVanDenBrink *et al*., 1999; Parsons *et al*., 1997).

Due to concerns about cardiac side effects, it would be highly desirable to develop antimigraine drugs that act via a mechanism not involving 5-$HT_{1B/1D}$ receptors. Indeed, the mechanistically novel benzopyran SB-220453 (Chan *et al*., 1999) has no significant affinity at 5-$HT_{1B/1D}$ receptors, nor does it show any activity in a large number of receptor-, ion channel- and enzyme-assays (Chan *et al*., 1999). SB-220453 exhibits a high affinity for a selective, but structurally unknown binding site in the human brain (Upton *et al*., 1999) and may be active in the treatment of migraine via blockade of excessive cortical excitability. SB-220453 inhibits neurogenic inflammation in rat brain meninges (Chan *et al*., 1999), nitric oxide release associated with cortical spreading depression as well as carotid vasodilatation induced by trigeminal nerve stimulation in the cat (Read *et al*., 1999; Upton *et al*., 1999). In the present study, we investigated the effects of SB-220453 on a number of human isolated blood vessels (coronary artery, saphenous vein and middle meningeal artery) as well as human isolated atrial and ventricular cardiac trabeculae. Sumatriptan was used for comparison.

6.2 Patients and methods

Preparation of tissue

Human isolated coronary artery

Right epicardial coronary arteries were obtained from six heart beating organ donors (2 male, 4 female; age 37-63 years), who died of non-cardiac disorders (5 of cerebrovascular accident, 1 head trauma) less than 24 h before the tissue was taken to the laboratory. Hearts, provided by the Rotterdam Heart Valve Bank (Bio Implant Services / Eurotransplant Foundation) after removal of the aortic and pulmonary valves for transplantation purposes, were stored at 0ºC to 4ºC in a sterile organ-protecting solution immediately after circulatory arrest. After arrival at the laboratory, the right coronary artery was removed and placed in a cold, oxygenated Krebs buffer solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 8.3; pH 7.4. The artery was kept overnight before the experiment.

On the following day, the artery was cut into segments of 3-4 mm length, excluding distinct, macroscopically visible atherosclerotic lesions. The vessel segments were mounted in 15-ml organ baths filled with oxygenated Krebs buffer solution at 37°C. After equilibration for at least 30 min and a wash every 15 min, the vessel segments were stretched to a stable tension of about 15 mN. Changes in tissue tension were measured using an isometric transducer (Harvard, South Nattick, Massachusetts, USA) and recorded on a flatbed recorder (Servogor 124, Goerz, Neudorf, Austria).

Human isolated saphenous vein

Human saphenous veins were obtained postoperatively from four patients (2 male, 2 female; 68-79 years) undergoing coronary bypass surgery. The tissue was immediately placed in cold saline and was brought to the laboratory within 15 min. Subsequently, the vein was cleaned of connective tissue and placed in a cold, oxygenated Krebs buffer solution (for composition, see above). After overnight storage, the vein was cut into segments of 3-4 mm length. The vessel segments were mounted in 15-ml organ baths filled with oxygenated Krebs buffer solution at 37°C. After equilibration for at least 30 min and a wash every 15 min, the vessel segments were stretched to a stable tension of about 10 mN. Contractions were measured with an isometric transducer (Harvard, South

Nattick, Massachusetts, USA) and recorded on a flatbed recorder (Servogor 124, Goerz, Neudorf, Austria).

Human isolated middle meningeal artery

Human middle meningeal arteries were obtained from ten patients (3 male, 7 female; 30-71 years) undergoing craniotomy during neurosurgical procedures. In such patients, a part of the skull is temporarily removed to gain access to the brain and a small redundant portion of a branch of the middle meningeal artery is usually found attached to the dural sheath covering the removed piece of the skull. After careful removal from the dura mater, this arterial piece was placed in cold saline and brought to the laboratory immediately. Upon arrival at the laboratory, the artery was cleaned of connective tissue and was placed in cold oxygenated Krebs buffer solution of the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 1.25, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.1; pH 7.4. Vessel segments of 4 mm length were and mounted in 10-ml organ baths filled with oxygenated Krebs buffer solution at 37°C. After equilibration for at least 30 min and a wash every 15 min, the vessel segments were stretched to a stable tension of about 4 mN. Contractions of the artery were measured with an isometric force displacement transducer and recorded using the IOX 1.103 software (both: EMKA Technology, Paris, France).

To prevent prostaglandin synthesis, the cyclo-oxygenase inhibitor indomethacin (0.1 μM) was added to the Krebs solution. The experiments were performed within 2 h after surgery.

Human atrial and ventricular cardiac trabeculae

As described above (coronary artery section), hearts were obtained from five heart beating organ donors (2 male, 3 female; age 36-57 years), who died of non-cardiac disorders (all cerebrovascular accident). Immediately after arrival at the laboratory, right atrial and left ventricular trabeculae of approximately 1 mm thickness were carefully dissected and mounted in a 15-ml organ bath in the same Krebs buffer solution as used for the coronary artery and saphenous vein. The trabeculae were paced at 1 Hz using electrical field stimulation (5 ms, 15-20 V) delivered by a Grass S6 Square Wave Stimulator (Quincy, MA, USA). Resting tension was set to 7.5 mN and 20 mN for atrial and ventricular tissue, respectively. Changes in contraction were recorded with a Harvard force transducer

(South Nattick, MA, USA) on a flatbed recorder (Servogor 124, Goerz, Neudorf, Austria). The preparation was allowed to stabilise during 1 h with a wash every 15 min (Du *et al*., 1994).

Experimental protocol

Human isolated coronary artery and saphenous vein

Segments were exposed to K^+ (30 mM) twice. The functional integrity of the endothelium was verified by observing relaxation to substance P (1 nM, coronary artery) or bradykinin (1 μM, saphenous vein, substance P is nearly inactive in this blood vessel) after precontraction with prostaglandin $F_{2\alpha}$ (1 μM). After washout, the tissue was exposed to K^+ (100 mM) to determine the maximal contractile response to K^+. After a 30-min incubation period, a concentration response curve to sumatriptan (dissolved in distilled water or dimethylsulfoxide; DMSO) or SB-220453 (dissolved in DMSO) was constructed. In four of the experiments in coronary artery, a concentration response curve to these agonists was also constructed after a 30-min incubation with the stable thromboxane A_2 analogue U46619 (3-10 nM, the lowest concentration, determined in half logarithmic steps, eliciting a contraction ≥10% of K^+-induced contraction). A paired parallel set-up (i.e. all compounds were tested in different segments obtained from the same artery) was used for coronary artery experiments. In the saphenous vein, a paired parallel crossover design was used (i.e. similar as in coronary artery, but, in addition, a second concentration response curve was constructed to SB-220453 after sumatriptan, or to sumatriptan after SB-220453 after a 30-60 min washout period). Contractions were expressed as a percentage of contraction to 100 mM K^+.

Human isolated middle meningeal artery

Since the addition of K^+ frequently increased basal tone (unpublished observations), vessel segments were exposed to prostaglandin $F_{2\alpha}$ (0.1 μM) two to three times to 'prime' the tissue for stable contractions. Subsequently, the segments were contracted with prostaglandin $F_{2\alpha}$ (1 μM) and the functional integrity of endothelium was assessed by observing relaxation to substance P (10 nM). Due to the limited number of vessel segments that could be obtained from one patient, middle meningeal artery experiments were performed in an unpaired design, i.e. concentration response curves to sumatriptan (dissolved in distilled water) or SB-220453 (dissolved in DMSO) were constructed in

different arterial segments, which were in most cases obtained from different patients. In addition, a concentration response curve to sumatriptan (dissolved in DMSO) was constructed following a 30-60 min washout period after the concentration response curve to SB-220453. Contractions were expressed as a percentage of contraction to 1 μM prostaglandin $F_{2\alpha}$.

Atrial and ventricular cardiac trabeculae

A concentration response curve to noradrenaline (10 nM – 10 μM) was constructed to verify the viability of the tissue. Trabeculae yielding less than 0.25 mN response to 10 μM noradrenaline were excluded from further analysis. After washout, a concentration response curve to sumatriptan (dissolved in distilled water or DMSO) or SB-220453 (dissolved in DMSO) was constructed. In four out of five experiments, concentration response curves were also obtained after precontraction with noradrenaline (10 μM). Since this precontraction was not always stable for a period long enough to allow construction of a complete concentration response curve (1 nM - 100 μM) to the agonists, not all concentrations were studied in these experiments. Changes in contraction were expressed as percentage of increase in contraction to 10 μM noradrenaline).

Compounds

Sumatriptan succinate was a kind gift from GlaxoWellcome (Dr. H.E. Connor, Ware, Hertfordshire, UK). SB-220453 ((-)-*cis*-6-acetyl-4*S*-(3-chloro-4-fluoro-benzoyl amino) 3,4-dihydro-2,2-dimethyl-2*H*-benzo[b]pyran-3*S*-ol) was kindly provided by SmithKline Beecham (Dr. A.A. Parsons, Harlow, Essex, UK). U46619 (9,11-dideoxy-11α,9α-epoxy, methanoprostaglandin $F_{2\alpha}$), prostaglandin $F_{2\alpha}$ (Tris salt), bradykinin acetate, substance P acetate and dimethylsulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, Missouri, U.S.A.). Indomethacin was obtained from the pharmacy of Erasmus University Medical Centre Rotterdam (Rotterdam, The Netherlands). The chemicals used for the Krebs buffer solutions were purchased from Merck (Darmstadt, Germany).

Sumatriptan was dissolved in distilled water or, where indicated, in DMSO and further diluted in distilled water. Indomethacin and SB-220453 were dissolved in DMSO and further diluted in distilled water. The other compounds were dissolved in distilled water.

Analysis of data

Concentration response curves analyzed using GraphPad software (GraphPad software Inc., San Diego, California, USA) to determine pEC_{50} values (negative logarithm of the concentration eliciting 50% of the maximal contractile response, E_{max}). When a plateau in the concentration response curve was not reached, the response observed with the highest concentration used (100 μM) was considered as E_{max}.

At 100 μM, SB-220453 and sumatriptan (dissolved in DMSO) induced a small relaxation of the blood vessels, which was not easily quantifiable. This relaxant response, which was also observed with equivalent amounts of the solvent DMSO, has been ignored. For the experiments in the presence of U46619, the precontraction induced by U46619 was subtracted from the concentration response curves obtained with the agonists.

All data in the text and illustrations are presented as mean±s.e.mean, with *n* representing the number of different subjects or, when specifically mentioned, the number of vessel segments or trabeculae. Differences between pEC_{50} and E_{max} values of the compounds were evaluated with Tukey's test, once an analysis of variance (ANOVA) for paired (coronary artery, saphenous vein, myocardial trabeculae) or unpaired data (middle meningeal artery) had revealed that the samples represented different populations. Values of $P<0.05$ were considered to indicate significant differences.

Ethical approval

The Ethical Committee of the Erasmus University Medical Centre Rotterdam approved this study.

6.3 Results

Human isolated coronary artery

All coronary artery segments relaxed after substance P (1 nM). The response amounted to 48±15% (range: 9-107%) of the precontraction (22±6 mN) to prostaglandin $F_{2\alpha}$ (1 μM). Contraction to 100 mM K^+ was 55±6 mN (*n*=6).

Sumatriptan induced a concentration-dependent contraction, which was independent of the solvent (distilled water and DMSO) used; E_{max}: 12±3% and 7±1% of K^+-induced contraction, respectively, pEC_{50}: 6.0±0.2 and 6.4±0.2, respectively. SB-220453 induced no response (Figure 6.1, *left panel*). After precontraction with U46619 (17±3% of K^+-induced contraction), the E_{max} to sumatriptan was substantially

augmented in two out of the four experiments. However, the mean E_{max} (39±24% for sumatriptan dissolved in distilled water and 38±17% for sumatriptan dissolved in DMSO) was, as reported previously (MaassenVanDenBrink *et al*., 1996), not significantly different from values in these four experiments in the absence of U46619 (16±3% for sumatriptan dissolved in distilled water and 9±1% for sumatriptan dissolved in DMSO). The pEC_{50} of sumatriptan in the presence of U46619 (6.7±0.3 and 6.6±0.3 for sumatriptan dissolved in distilled water and DMSO, respectively) was also not significantly increased, when compared to those in the absence of U46619 (6.2±0.3% for sumatriptan dissolved in distilled water and 6.5±0.2% for sumatriptan dissolved in DMSO). Similar to the experiments performed on quiescent arteries, SB-220453 failed to contract the coronary artery in the presence of U46619 (Figure 6.1, *right panel*).

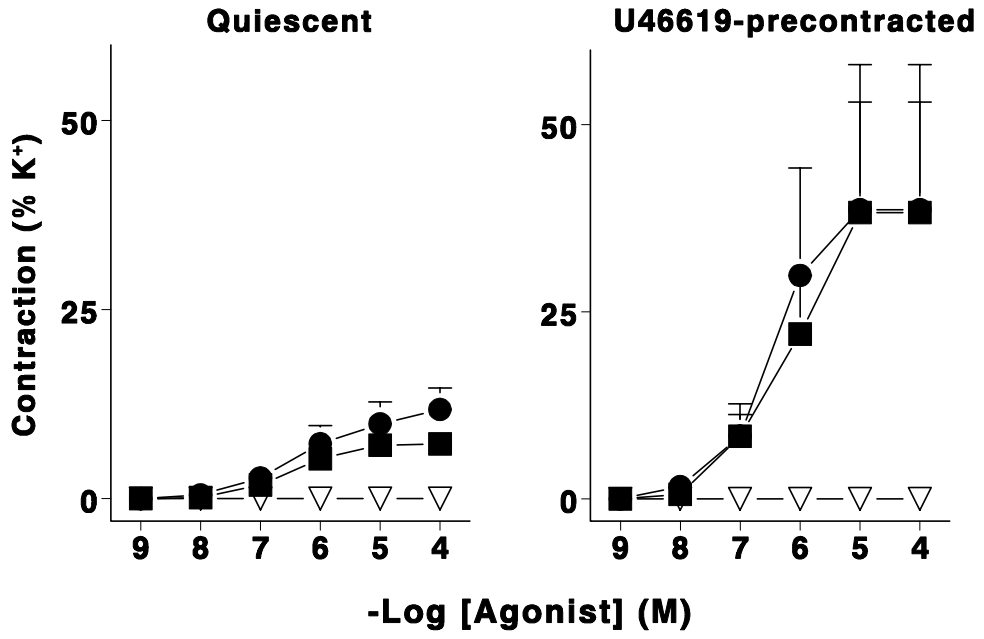

Figure 6.1 Concentration response curves in human isolated coronary arteries to sumatriptan (dissolved in distilled water, ● or DMSO, ■) and SB-220453 (∇, dissolved in DMSO). Experiments were performed in the absence of U46619 (*left panel, n*=6) or after precontraction with U46619 (3-10 nM; *right panel, n*=4, except sumatriptan dissolved in distilled water *n*=3). The precontraction induced by U46619 was 17±3%, *n*=15 vessel segments.

Human isolated saphenous vein

Saphenous vein segments relaxed after bradykinin (1 μM), the response amounting to 49±24% (range: 10-107%) of the precontraction (6±3 mN) induced by prostaglandin $F_{2\alpha}$ (1 μM). Contraction to 100 mM K^+ was 10±2 mN (*n*=4).

In all experiments, sumatriptan (dissolved in DMSO) induced a concentration-dependent contraction (E_{max}: 52±5%, pEC_{50}: 6.3±0.1). SB-220453 did not induce a contraction in any of the concentrations used (Figure 6.2, *left panel*). The concentration response curves to sumatriptan that were constructed after the concentration response curve to SB-220453 did not differ from those constructed before SB-220453 (E_{max}: 59±10%; pEC_{50}: 6.2±0.2). Also after the concentration response curve to sumatriptan, SB-220453 failed to contract the saphenous vein (Figure 6.2, *right panel*).

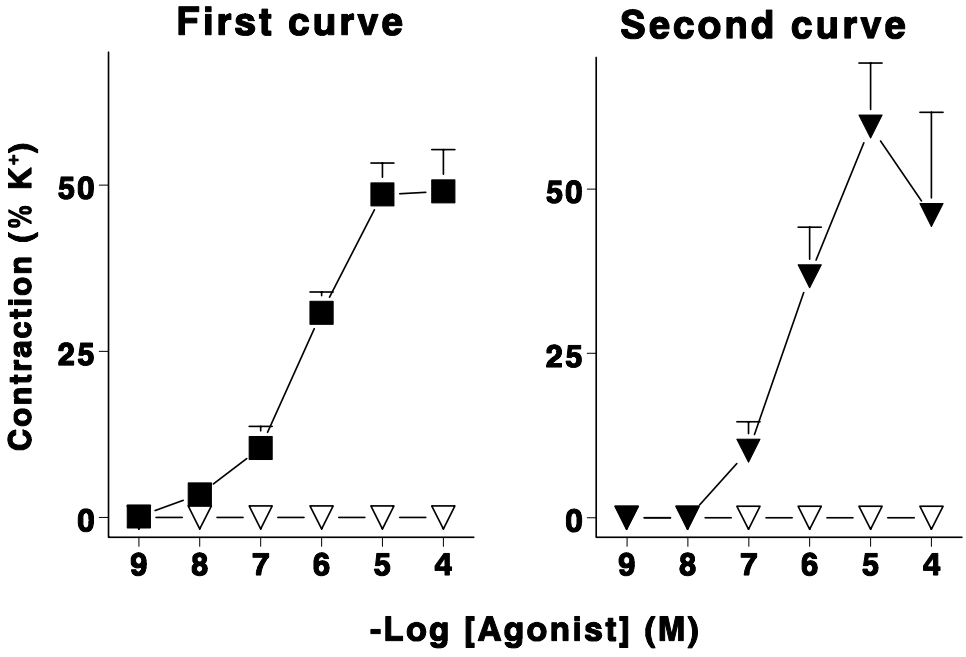

Figure 6.2 Concentration response curves in human isolated saphenous vein to sumatriptan (■) and SB-220453 (∇; both dissolved in DMSO; *n*=4). Whereas sumatriptan induced a concentration-dependent contraction in all experiments, SB-220453 had no effect (*left panel*). The second concentration response curves (*right panel*) to sumatriptan after SB-220453 (▼) as well as to SB-220453 after sumatriptan (∇; *n*=4) did not differ from the first curve.

Human isolated middle meningeal artery

Middle meningeal artery segments relaxed to substance P (10 nM) with 44±7% (range: 19-76%) of the precontraction (8±2 mN) induced by prostaglandin $F_{2\alpha}$ (1 μM, *n*=10).

Sumatriptan (dissolved in distilled water) induced a concentration-dependent contraction in all experiments (E_{max}: 105±18%, pEC_{50}: 6.9±0.2), whereas SB-220453 induced no contraction (Figure 6.3, *left panel*). After construction of the concentration response curves to SB-220453, the contraction to sumatriptan (dissolved in DMSO) did not differ significantly from the that to sumatriptan dissolved in distilled water (E_{max}: 101±2%; pEC_{50}: 6.8±0.4, Figure 6.3, *right panel*).

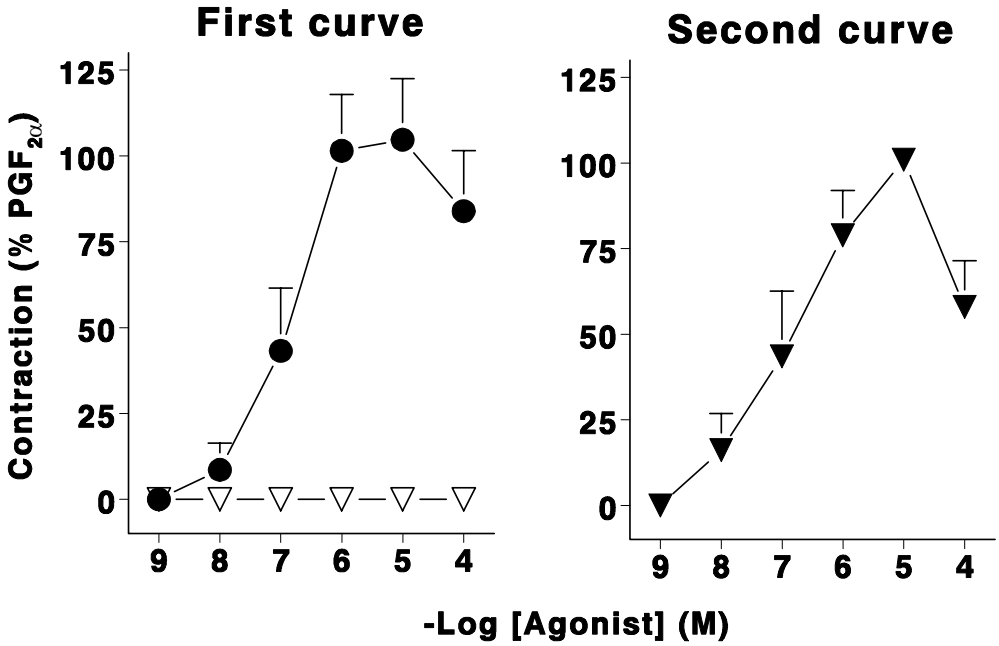

Figure 6.3 Concentration response curves in human isolated middle meningeal artery to sumatriptan (dissolved in distilled water, ●; *n*=5) and SB-220453 (∇, dissolved in DMSO; *n*=4; *left panel*). The second concentration response curves (*right panel*) to sumatriptan (dissolved in DMSO) after SB-220453 (▼, *n*=4) did not differ from the first curve.

Human atrial and ventricular cardiac trabeculae

As we reported earlier (Du *et al*., 1994), baseline contractile force was significantly lower in the atrial (0.64±0.13 mN, *n*=17 trabeculae) than in ventricular (3.04±0.45 mN, *n*=20 trabeculae) tissue. In both tissues, noradrenaline (10 nM - 10 μM) increased contractile

force in a concentration-dependent manner. After exposure to 10 μM noradrenaline, the force of contraction increased to 2.43±0.36 mN (*n*=17 trabeculae) and 5.02±0.43 mN (*n*=20 trabeculae) in the atrial and ventricular trabeculae, respectively.

Neither SB-220453, nor sumatriptan displayed a positive inotropic effect on atrial and ventricular trabeculae (Figure 6.4). At concentrations ≥10 μM, SB-220453 and sumatriptan (dissolved in DMSO) induced a negative inotropic effect in both atrial (sumatriptan: 37±32% of contraction to 10 μM noradrenaline, SB-220453: 34±29%) and ventricular (sumatriptan: 18±9%, SB-220453: 55±21%) trabeculae, which were not different for these compounds. This negative inotropic effect may be assigned to the solvent DMSO, since it was not observed with sumatriptan dissolved in distilled water (Figure 6.4). After a precontraction with noradrenaline (10 μM) also, none of the compounds induced a positive inotropic effect, while a negative effect on contractility was observed at concentrations ≥10 μM of SB-220453 and sumatriptan dissolved in DMSO (data not shown).

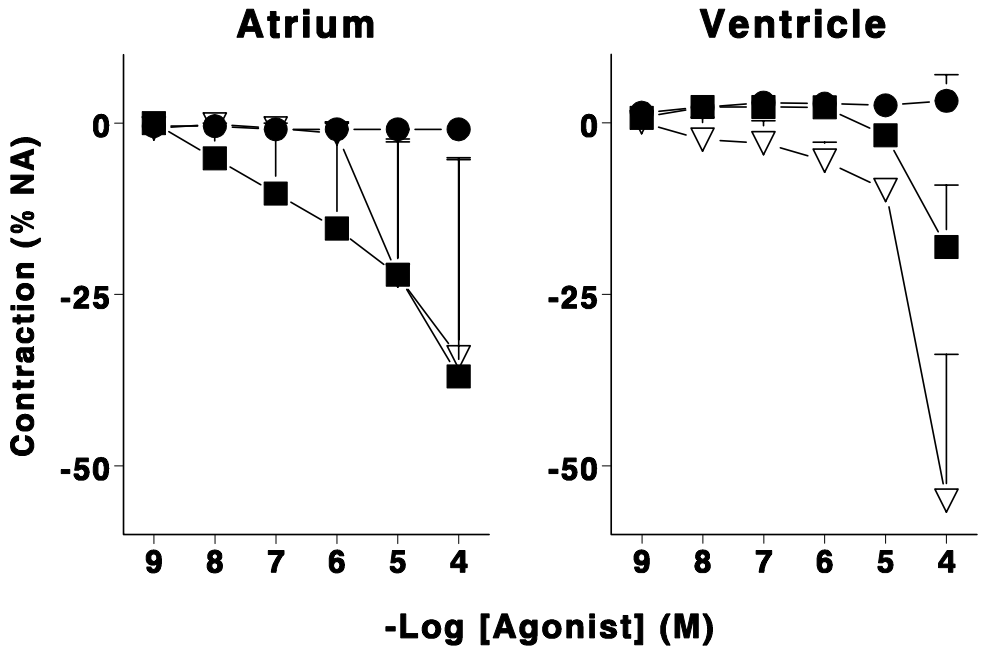

Figure 6.4 Concentration response curves in human isolated atrial (*left panel, n*=3-5) and ventricular (*right panel, n*=5) trabeculae to sumatriptan (dissolved in distilled water, ● or DMSO, ■) and SB-220453 (∇, dissolved in DMSO). NA, Noradrenaline.

6.4 Discussion

Human isolated blood vessels

SB-220453 did not induce any significant contraction of the human isolated coronary artery, saphenous vein or middle meningeal artery. In contrast, sumatriptan, investigated in parallel, produced marked contractions. Since contractions to some agonists are 'unmasked' or augmented in the presence of increased tension (Chester *et al*., 1993; MaassenVanDenBrink *et al*., 1996), we also investigated the coronary artery contraction in the presence of the thromboxane A_2 analogue U46619. Indeed, the contraction to sumatriptan was augmented in two of the coronary arteries investigated (in accordance with our previous findings (MaassenVanDenBrink *et al*., 1996)), but even in these precontracted segments, SB-220453 failed to elicit any contraction.

In all blood vessels, a slight relaxation was observed at the highest concentration of SB-220453 and sumatriptan (both dissolved in DMSO), but not with sumatriptan dissolved in distilled water. Therefore, this relaxation may be assigned to the solvent DMSO. However, despite this relaxant response, SB-220453 did not affect the concentration response curve to sumatriptan (see Figures 6.2 and 6.3).

It is known that the presence or absence of functional endothelium can influence contractile responses in blood vessels (MaassenVanDenBrink *et al*., 1999; Yang *et al*., 1991). In our study, the endothelial quality of the blood vessels varied, as is illustrated by the relaxation to substance P (coronary artery: 9-107%, middle meningeal artery: 19-76%) or bradykinin (saphenous vein: 10-107%). Because in none of the blood vessels SB-220453 induced any contraction, our results suggest that the lack of response to these compounds is not dependent on the quality of the endothelium.

Atrial and ventricular cardiac trabeculae

Apart from a negative inotropic effect associated with the solvent DMSO, no inotropic response was observed with SB-220453 or sumatriptan in both atrial and ventricular cardiac trabeculae. Sumatriptan did not display any effect on cardiac trabecular contractility despite expression of both 5-HT_{1B} and 5-HT_{1D} mRNA in human atrium and ventricle (Nilsson *et al*., 1999). However, the present results are consistent with the observed lack of hemodynamic effects of sumatriptan in patients, where no negative or positive inotropic effects were demonstrated (Hood *et al*., 1997). These studies are also in accordance with *in vitro* studies performed on guinea pig (Lattimer *et al*., 1993; Le Grand

et al., 1998) and rabbit (Paterna *et al*., 1995) cardiac tissue. In fact, the role of 5-HT_{1B} and 5-HT_{1D} receptors on the human heart is still poorly understood (Saxena & Villalón, 1991), although the 5-HT_{1D} receptor has been reported to mediate inhibition of noradrenaline release in human atrium (Molderings *et al*., 1996).

In conclusion, our results do not provide any evidence for human blood vessel contraction or altered myocardial contractility in response to SB-220453. Since SB-220453 did not contract the middle meningeal artery, we conclude that potential therapeutic efficacy is independent of cerebral vasoconstriction. SB-220453 did not contract coronary artery, saphenous vein or cardiac trabeculae, and is therefore likely to be devoid of adverse cardiovascular side effects observed with serotonergic agonists.

Acknowledgements

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Part III

Receptors/mechanisms involved in antimigraine drug mediated responses

Et quand le jour arrive
Je deviendrais le ciel
et je deviendrais la mer
et la mer va venir m'embrasser
Pour que j'aille à la maison
Rien ne pourra plus m'arrêter maintenant

NIN - La Mer

Chapter 7

Pharmacological analysis of contractile effects of eletriptan and sumatriptan on human isolated blood vessels

Summary - Eletriptan, a second-generation triptan with high affinity for 5-$HT_{1B/1D}$ receptors, is highly effective in migraine, with or without aura. We compared the effects of eletriptan and sumatriptan on the human isolated middle meningeal and coronary arteries and saphenous vein, used as models for therapeutic efficacy and potential side effects, and have investigated the role of 5-$HT_{1B/1D}$ receptors in contractions induced by these triptans. Concentration-response curves to eletriptan and sumatriptan were constructed in the absence or presence of a selective 5-$HT_{1B/1D}$ receptor antagonist, GR125743 (N-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl) benzamide). All three blood vessels constricted in response to eletriptan and sumatriptan, but the middle meningeal artery relaxed following the highest concentration (100 μM) of eletriptan. In the middle meningeal artery, GR125743 antagonised the contractions induced by both eletriptan (pEC_{50}: 7.34±0.13) and sumatriptan (pEC_{50}: 6.91±0.17) to a similar degree (pA_2: 8.81±0.17 and 8.64±0.21, respectively). In the human coronary artery and saphenous vein, sumatriptan-induced contractions (pEC_{50}: 6.24±0.14 and 6.19±0.12, respectively) were also potently antagonised by GR125743 (pA_2: 8.18±0.27 and 8.34±0.12, respectively). The eletriptan-induced contractions of the human saphenous vein (pEC_{50}: 6.09±0.13) were antagonised less effectively by GR125743 (pK_B: 7.73±0.18), and those of the human coronary artery (pEC_{50}: 5.54±0.22) remained unaffected by GR125743 up to a concentration of 100 nM. These results suggest that (i) based on the differences in pEC_{50} values, the cranioselectivity of eletriptan (63-fold) is higher than that of sumatriptan (5-fold) in coronary artery, (ii) the contractile effects of sumatriptan and eletriptan (lower concentrations) in the three blood vessels are mediated via the 5-HT_{1B} receptor, and (iii) additional mechanisms seem to be involved in coronary artery and saphenous vein contractions and middle meningeal artery relaxation following high concentrations of eletriptan.

Based on: Van den Broek, R.W.M., MaassenVanDenBrink, A., de Vries, R., Bogers, A.J.J.C., Stegmann, A.P., Avezaat, C.J. & Saxena, P.R. (2000). Pharmacological analysis of contractile effects of eletriptan and sumatriptan on human isolated blood vessels. *Eur. J. Pharmacol.*, **407,** 165-73.

7.1 Introduction

Sumatriptan, the first of a new class of compounds with an agonist action at 5-$HT_{1B/1D}$ receptors, is effective in the acute treatment of migraine and cluster headaches (Saxena & Tfelt-Hansen, 2000). The high efficacy profile as well as some shortcomings (low oral bioavailability, high headache recurrence and chest symptoms) has prompted the development of several second-generation triptans, including eletriptan. Eletriptan displays a higher affinity than sumatriptan for the 5-HT_{1B} (pK_i: 8.00 and 7.37, respectively) as well as 5-HT_{1D} (pK_i: 8.94 and 8.04, respectively) receptor (Napier *et al*., 1999). Like sumatriptan, eletriptan constricts porcine carotid arteriovenous anastomoses *in vivo* (Willems *et al*., 1998) and canine saphenous vein and basilar artery *in vitro* (Gupta *et al*., 1999) and inhibits c-fos expression in trigeminal nucleus caudalis following stimulation of the superior sagittal sinus in the cat (Goadsby & Hoskin, 1999). Oral administration of the highest dose of eletriptan (80 mg) has been shown to be superior to sumatriptan (100 mg) in the treatment of migraine in three head-to-head clinical trials (Goadsby *et al*., 2000; Saxena & Tfelt-Hansen, 2000).

It is now generally accepted that the therapeutic action of sumatriptan and other triptans is mainly due to constriction of dilated intra- and extra-cranial blood vessels, although other mechanisms interfering with the trigeminovascular system may also be involved (De Vries *et al*., 1999; Saxena & Tfelt-Hansen, 2000). Indeed, the triptans potently contract human isolated middle meningeal (Longmore *et al*., 1998; Razzaque *et al*., 1999), temporal (Verheggen *et al*., 1996), middle cerebral (Hamel & Bouchard, 1991; Jansen *et al*., 1992) and basilar (Parsons *et al*., 1998) arteries. However, albeit less profound, the triptans also have the ability to contract non-cranial blood vessels, such as human isolated pulmonary (MacLean *et al*., 1996) and coronary (Chester *et al*., 1990; Connor *et al*., 1989; MaassenVanDenBrink *et al*., 1998) arteries. Constriction of these blood vessels may lead to cardiovascular adverse events, including myocardial ischaemia and infarction in predisposed individuals (Kelly, 1995; Main *et al*., 1998; O'Connor & Gladstone, 1995; Ottervanger *et al*., 1997).

In the present study, we have investigated the contractile effects of eletriptan, in comparison to those of sumatriptan, on human isolated blood vessels used as models with relevance to therapeutic efficacy (middle meningeal artery), coronary adverse events (coronary artery) and peripheral vasoconstriction (saphenous vein). Furthermore, we used the competitive 5-$HT_{1B/1D}$ receptor antagonist GR125743 (N-[4-methoxy-

3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)benzamide) (Domenech *et al*., 1997; Pauwels, 1997) in an attempt to characterise the 5-HT receptors mediating contractions to eletriptan and sumatriptan.

7.2 Material and methods

Tissue collection

Human isolated middle meningeal artery

Human middle meningeal arteries were obtained from 10 patients (4 male, 6 female; age 30-69 years) undergoing craniotomy at the neurosurgical unit of the University Hospital 'Dijkzigt', Erasmus University Medical Centre, Rotterdam. During the surgical procedure, a part of the skull is temporarily removed and the dura mater, together with a small piece of the middle meningeal artery, is intentionally cut to obtain access to the brain. This piece of the artery was placed in a plastic tube filled with ice-cold (0-4 °C) physiological saline and immediately transported to the laboratory. Upon arrival at the laboratory, the artery was placed in a cold oxygenated modified Krebs bicarbonate solution of the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 1.25, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.1; pH 7.4. The cyclo-oxygenase inhibitor indomethacin (0.1 μM) was added to the Krebs solution to prevent prostaglandin synthesis. Excess tissue surrounding the artery was carefully removed and no attempt was made to remove the endothelium. The middle meningeal artery was used within 2 h of surgery.

Human isolated coronary artery and saphenous vein

The human right epicardial coronary artery was obtained from 11 ‘heart-beating’ organ donors (6 male, 5 female; 17-52 years), who died of non-cardiac disorders (cerebrovascular accidents, 8; brain trauma, 3). The hearts were provided by the Heart Valve Bank, Rotterdam, The Netherlands after donor mediation by Bio Implant Services Foundation/Eurotransplant Foundation, Leiden, The Netherlands; for details, see MaassenVanDenBrink *et al.* (1998). Leftover human saphenous vein was obtained postoperatively from 13 patients (7 male, 6 female; age 34-81 years) undergoing coronary bypass surgery. Saphenous vein was immediately placed in cold saline and was brought to the laboratory within 15 min.

Upon arrival in the laboratory, the right coronary artery and saphenous vein were cleaned from the surrounding tissue and placed in a cold, oxygenated Krebs bicarbonate

solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 8.3; pH 7.4. No attempt was made to remove the endothelium. The artery and vein were stored overnight in cold oxygenated Krebs solution and were used the following day.

Experimental protocol

Human isolated middle meningeal artery

The middle meningeal artery was cut into circular 3 to 4 mm long segments, which were mounted on metal prongs in 10-ml organ baths containing Krebs bicarbonate solution, aerated with 95% O_2 and 5% CO_2 and maintained at a temperature of 37 ^oC. Changes in isometric tension were measured by a force displacement transducer and recorded with IOX 1.203h software (both, EMKA Technology, Paris, France). Segments were stretched to a passive tension of 4 mN and were allowed to stabilise at this level for 60 min (with replacement of Krebs solution every 15 min). All segments were then exposed 2-3 times to 0.1 μM prostaglandin $F_{2\alpha}$ to demonstrate reproducibility of the evoked contractions. Subsequently, the segments were pre-contracted with prostaglandin $F_{2\alpha}$ (1 μM) and the relaxation response to substance P (10 nM) was used to evaluate the presence of a functional endothelium. After washing, the segments were allowed to equilibrate for 60 min, with replacement of buffer every 15 min. The segments were then used in a paired parallel experimental set-up, incubating with the 5-$HT_{1B/1D}$ receptor antagonist GR125743 (10 nM and 30 nM) or vehicle for 30 min. Subsequently, a single cumulative concentration response curve to either eletriptan or sumatriptan (both 1 nM to 100 μM) was constructed in each segment.

Human isolated coronary artery and saphenous vein

Vessels were cut into ring segments of approximately 4 mm length and were suspended on stainless steel hooks in 15-ml organ baths containing Krebs bicarbonate solution, aerated with 95% O_2 and 5% CO_2 and maintained at a temperature of 37 ^oC. Vessel segments containing macroscopically visible atherosclerotic lesions were not used. Changes in tension were recorded using a Harvard isometric transducer. The segments were allowed to equilibrate for at least 30 min and were washed every 15 min. Segments were stretched to a passive tension of 15 mN (coronary artery) or 10 mN (saphenous vein), respectively. All segments were then exposed to KCl (30 mM) twice, to demonstrate the

reproducibility of the evoked contractions. Subsequently, the functional integrity of the endothelium was verified by observing relaxation to substance P (coronary artery, 1 nM) or bradykinin (saphenous vein, 0.1 μM) after pre-contraction with prostaglandin $F_{2\alpha}$ (1 μM). After washout, the tissue was exposed to KCl (100 mM) to determine the maximal contractile response to KCl. The tissue was washed and then was allowed to equilibrate for another period of 30 min. After this equilibration period the segments were divided in a paired parallel experimental set-up and were incubated with the 5-$HT_{1B/1D}$ receptor antagonist GR125743 (coronary artery: 3 nM to 1 μM; saphenous vein: 3 nM to 30 nM) or with vehicle for 30 min. Subsequently, a single cumulative concentration response curve to eletriptan or sumatriptan (both 1 nM to 100 μM) was constructed in each segment.

Data analysis

Concentration response curves

Contractile responses were expressed as percentage of the contractile response to 1 μM prostaglandin $F_{2\alpha}$ (middle meningeal artery) or 100 mM KCl (coronary artery and saphenous vein). The occasional spontaneous phasic contractions observed in some coronary artery and saphenous vein segments were not considered in the calculations, but the respective plateau contraction levels were used. Initially, we calculated the mean value of the individual maximum contractile responses (E_{max}) to eletriptan or sumatriptan; in case the contractions did not reach a plateau in an individual experiment, the contraction induced by the highest concentration used (100 μM) was considered as E_{max}. We then analysed mean concentration response curves with a non-linear regression fitting technique for sigmoidal functions with variable slope using GraphPad Prism 3.0 (GraphPad Software Inc., San Diego, CA, USA) to obtain Hill slopes for the agonists in the absence or presence of different concentrations of GR125743. When mean E_{max} values and Hill slopes were not significantly different, we assumed that GR125743 behaved as a competitive antagonist. The whole data set was then transformed using SPSS 7.5 non-linear regression statistics (SPSS Inc., Chicago, IL, USA) into a dependent fitting model, where the E_{max} values were set to the respective agonist control E_{max}. This model enabled us to obtain valid S.E.M. and 95% confidence intervals of mean pEC_{50} values and dose ratios. Using the pEC_{50} values that were significantly different from their respective controls, Schild regression analysis was performed (SPSS Inc., Chicago, IL,

USA) to calculate antagonist pA_2 (≥3 concentrations of GR125743), apparent pA_2 (2 concentrations of GR125743 with slope set to unity) or pK_B (1 concentration of GR125743 with slope set to unity). When the ratio between EC_{50} values in absence or presence of antagonist was larger than 1, the dose ratio was calculated.

Statistical analysis

Differences between E_{max}, Hill slopes and pEC_{50} values of vehicle and antagonist-treated groups were analysed according to one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison t-test (GraphPad Software Inc., San Diego, CA, USA). Differences in potency, efficacy and logarithmically transformed dose ratios between the agonists were analysed according to Tukey's unpaired t-test (GraphPad Software Inc., San Diego, CA, USA). Cranioselectivity ratios were calculated as the inverse logarithmic difference between pEC_{50} values of agonists in middle meningeal artery and either coronary artery or saphenous vein. The 95% confidence limit (95% CI) of cranioselectivity ratios obtained with eletriptan and sumatriptan were calculated and compared as described by Steel and Torrie (1980). In all cases, statistical significance was assumed when $P<0.05$. Except for dose ratios and cranioselectivity ratios, where the geometric means with 95% CI are given, all other data are presented as mean±S.E.M.

Ethical approval

The Medical Ethics Committee of the Erasmus Medical Centre Rotterdam, dealing with the use of human material for scientific experiments, approved the protocols for this investigation.

Compounds

Bradykinin acetate, 5-hydroxytryptamine creatinine sulphate (serotonin; 5-HT), indomethacin hydrochloride, prostaglandin $F_{2\alpha}$ tris salt and substance P acetate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Eletriptan hydrogen bromide, sumatriptan succinate and GR125743 (N-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)benzamide) were kindly supplied by Pfizer (Sandwich, Kent, UK). Indomethacin was dissolved in 100% v/v dimethyl sulphoxide and further diluted in distilled water. All other compounds were dissolved in distilled water.

7.3 Results

Human middle meningeal artery

Both eletriptan and sumatriptan contracted the middle meningeal artery in a concentration dependent manner (up to 10 μM) and with similar maxima (E_{max}: 94±8% and 106±17% of contraction to 1 μM prostaglandin $F_{2\alpha}$, respectively), potency (pEC_{50}: 7.34±0.13 and 6.91±0.17, respectively) and Hill slopes (0.86±0.20 and 1.27±0.80, respectively) (Fig. 7.1, Table 7.1). However, in contrast to sumatriptan, the highest concentration of eletriptan (100 μM) induced a marked vasorelaxation, which was not affected by GR125743. Incubation with GR125743 caused a parallel rightward shift, with no alteration in Hill slopes, in the concentration response curves to both eletriptan (contractile part) and sumatriptan; the maximum contractile responses observed with 10 μM were not significantly different after GR125743 compared to the respective control values. The pEC_{50} values and dose ratios (95% CI) of eletriptan after 10 nM and 30 nM GR125743 were 6.43±0.13 and 8.2 (4.3-19.4), and 5.48±0.14 and 72 (30-172), respectively. For sumatriptan the pEC_{50} values and dose ratios (95% CI) yielded 6.11±0.17 and 6.4 (2.1-19.8) and 5.27±0.18 and 44 (14-137), for 10 nM and 30 nM GR125743, respectively. Schild regression analysis revealed similar apparent pA_2 values of GR125743 against eletriptan (8.81±0.17) and sumatriptan (8.64±0.21) (Table 7.1).

Human coronary artery

The human coronary artery contracted in response to eletriptan and sumatriptan with equal maxima (E_{max}: 17±4% and 12±2% of contraction to 100 mM KCl, respectively) and Hill slopes (0.73±0.26 and 0.96±0.31, respectively). The potency of eletriptan was significantly lower than that of sumatriptan (pEC_{50}: 5.54±0.22 and 6.24±0.14, respectively) (Fig. 7.2; Table 7.1). GR125743 (3 nM-1 μM) did not affect the maxima (E_{max}) and Hill slopes of eletriptan and at concentrations up to 100 nM GR125743 there was no shift in the concentration response curves to eletriptan (data not shown). Due to a non-sigmoidal nature of the concentration response curve to eletriptan following high concentrations (≥300 nM) of GR125743 (Fig. 7.2, *left panel*), pEC_{50} values and dose ratios of eletriptan and pA_2 of GR125743 were not determined (see Methods). The concentration response curve to sumatriptan was shifted in a parallel manner, with no alteration of Hill slopes, by GR125743 (Fig. 7.2, *right panel*), yielding a significant

decrease in potency (pEC_{50}: 5.44±0.15, 4.71±0.15, 4.66±0.18 and 3.92±0.20 after 30 nM, 100 nM, 300 nM and 1 μM GR125743, respectively, with corresponding dose ratios (95% CI) of 6.3 (2.5-16), 34 (13-87), 38 (14-107) and 209 (70-624), respectively). Schild regression analysis revealed a pA_2 value of 8.18±0.27 for GR125743 against sumatriptan with a slope of 0.89±0.15, which was not significantly different from unity. The dose ratios for eletriptan (2.2) and sumatriptan (6.3) after 30 nM GR125743 were similar, whereas at 100 nM GR125743 the dose ratio for eletriptan (2.7) was significantly lower than for sumatriptan (34).

Human saphenous vein

Eletriptan and sumatriptan contracted the saphenous vein with equal maxima (E_{max}: 71±10% and 80±6% of contraction to 100 mM KCl, respectively), potency (pEC_{50}: 6.09±0.13 and 6.19±0.12, respectively) and Hill slopes (0.64±0.13 and 0.79±0.14, respectively) (Fig. 7.3, Table 7.1). GR125743 (3-30 nM) did not affect the maxima (E_{max}) and Hill slopes of eletriptan and sumatriptan (Fig. 7.3). The Hill slope of eletriptan control was significantly different from unity, whereas those in presence of antagonist were not. The concentration response curves to eletriptan were shifted to the right only after incubation with 30 nM GR125743 (pEC_{50}: 5.56±0.11; dose ratio (95% CI): 3.4 (1.5-7.7)). A parallel rightward shift in the concentration response curves to sumatriptan was observed with GR125743 (10 and 30 nM), yielding a significant decrease in potency (pEC_{50}: 5.66±0.10 and 4.93±0.10, respectively, with corresponding dose ratios (95% CI) of 3.3 (1.6-7.0) and 18 (9-34), respectively). Schild regression analysis revealed an apparent pA_2 value of 8.34±0.12 for GR125743 against sumatriptan, which was similar to the pA_2 value found in both middle meningeal and coronary artery (Table 7.1). The pK_B of GR125743 obtained at 30 nM against eletriptan (7.73±0.18) appears to be somewhat lower compared to the apparent pA_2 value found against sumatriptan (8.34±0.12). This is also reflected in the fact that the dose ratio obtained at 30 nM GR125743 for eletriptan (3.4) was significantly lower than that for sumatriptan (18).

Cranioselectivity of eletriptan and sumatriptan

The mean cranioselectivity ratios (inverse logarithm of the difference between the pEC_{50} value in the middle meningeal artery and that in the coronary artery or saphenous vein) and their respective 95% CI are shown in Table 7.1. Compared to coronary artery and

saphenous vein, both eletriptan (63- and 18-fold, respectively) and sumatriptan (5-fold each) were significantly more selective for the meningeal artery. The cranioselectivity for eletriptan in the coronary was significantly higher than that for sumatriptan.

7.4 Discussion

In the present study, we investigated the effects of eletriptan and sumatriptan on the human isolated middle meningeal artery as a model for anti-migraine activity, and coronary artery and saphenous vein as models for peripheral vascular side-effect potential. In addition, the role of 5-$HT_{1B/1D}$ receptors in the vasoconstrictor responses to the two triptans was evaluated using GR125743, a potent and competitive 5-$HT_{1B/1D}$ receptor antagonist (Domenech *et al*., 1997; Pauwels, 1997).

Efficacy profile of eletriptan and sumatriptan in blood vessels

It is well known that acutely acting antimigraine drugs, such as the triptans, have a vasoconstrictor effect, particularly on cranial vessels (De Vries *et al*., 1999; Saxena & Tfelt-Hansen, 2000). Indeed, eletriptan contracted human isolated blood vessels used in this investigation with a maximum response similar to that of sumatriptan (see E_{max} values in Table 7.1). This is in accordance with our previous study in human vessels, where 5-HT also showed a similar E_{max} (MaassenVanDenBrink *et al*., 1999), but contrasts with observations in the canine basilar artery and saphenous vein, where eletriptan behaved as a partial agonist (Gupta *et al*., 1999). However, it must be pointed out that in our experiments the efficacy of eletriptan was not studied after receptor alkylation, thus not ruling out the partial agonist nature of eletriptan in human blood vessels.

Unlike sumatriptan, the highest concentration of eletriptan (100 μM) elicited a profound relaxation of the human middle meningeal. The exact reason for this difference between eletriptan and sumatriptan is not known. However, a possible explanation could be that at such high concentrations, which are well beyond the clinically relevant free (i.e. protein unbound) plasma C_{max} values of around 30 and 65-90 nM obtained after 40 and 80 mg oral eletriptan, respectively (Personal communication A.D. McHarg, Milton *et al*., 1998), the contraction elicited by eletriptan was overruled by vasorelaxation mediated by the 5-HT_7 receptor (Eglen *et al*., 1997; Saxena *et al*., 1998; Terron & Falcon-Neri, 1999). The 5-HT_7 receptor mRNA has been detected in human meningeal vessels (Schmuck *et al*., 1996) and at this receptor eletriptan (pK_i: 6.70±0.06) has a higher affinity than

sumatriptan (pK_i: 5.86±0.11) (Napier *et al*., 1999). Interestingly, eletriptan relaxed neither the coronary artery nor saphenous vein; the 5-HT_7 receptor mRNA is poorly expressed in the human coronary artery (Nilsson *et al*., 1999).

Effects of GR125743 on contraction elicited by eletriptan and sumatriptan

The contractions elicited by sumatriptan were competitively antagonised by GR125743 in the human middle meningeal and coronary arteries as well as the saphenous vein with similar pA_2 values (8.64, 8.18 and 8.34, respectively; Table 7.1). These values resemble the affinity estimates of GR125743 at 5-$HT_{1B/1D}$ receptors in radioligand binding (pKi: 8.2-9.0, Audinot *et al*., 1997; Domenech *et al*., 1997) and functional studies against sumatriptan-induced contractions of the human isolated middle meningeal artery (pKB: 9.1, Razzaque *et al*., 1999). The results reveal that sumatriptan acts on the same 5-HT receptor (most probably 5-HT_{1B}, Kaumann *et al*., 1993) in all three blood vessels studied. Indeed, elegant *in situ* hybridisation and immunohistochemistry experiments have demonstrated that the 5-HT_{1B} but not 5-HT_{1D} receptor mRNA and protein are present in the smooth muscles of human middle meningeal and coronary arteries (Bouchelet *et al*., 1996; Longmore *et al*., 1998; Longmore *et al*., 1997; Nilsson *et al*., 1999; Nilsson *et al*., 1999). In addition, selective 5-HT_{1D} and 5-HT_{1F} receptor agonists failed to constrict the human cerebral arteries, excluding a role for these receptors in vasomotor responses (Bouchelet & Hamel, 1999; Cohen & Schenck, 1999; Ennis *et al*., 1998). Finally, the selective 5-HT_{1B} receptor antagonist SB224289 (2,3,6,7-tetrahydro-1'-methyl-5-[2'-methyl-4'(5-methyl-1,2,4-oxadiazo-l-3-yl) biphenyl-4-carbonyl]furo[2,3f]indole-3-spiro-4'-piperidine hydrochloride) antagonises sumatriptan-induced vasoconstrictor responses (De Vries *et al*., 1999; Verheggen *et al*., 1998). Thus, there is now overwhelming evidence that the 5-HT_{1B} (not 5-HT_{1D}) receptor mediates vasoconstrictor responses to the triptans.

The contraction of the middle meningeal artery by eletriptan was antagonised by GR125743 with a pA_2 (8.81; Table 7.1) that was similar to that against sumatriptan-induced contraction in this artery and eletriptan-induced contractions in the dog isolated basilar artery and saphenous vein (pA_2 values: 9.1 and 9.4, respectively) (Gupta *et al*., 1999). However, in the coronary artery and saphenous vein, GR125743 appeared to block eletriptan-induced contractions in a slightly different manner compared to those by sumatriptan. In the coronary artery, higher concentrations of GR125743

(≥300 nM) revealed a non-sigmoidal nature of the concentration response curves to eletriptan, whereas lower concentrations (≤100 nM) of GR125743 failed to block eletriptan-induced contractions (Fig. 7.2). In the saphenous vein, the Hill slope of eletriptan was significantly different from unity, indicating the involvement of more than one mechanism and, accordingly, GR125743 caused a rightward shift in the concentration response curve to eletriptan only with the highest concentration used (30 nM; Fig. 7.3). Moreover, the dose ratio of eletriptan following 30 nM GR125743 (3.4) was significantly lower than that of sumatriptan (18). The discrepancy between antagonism by GR125743 against eletriptan-induced contraction in the coronary artery and saphenous vein and the results obtained in dog basilar artery and saphenous vein (Gupta *et al*., 1999) may well be species related or could be based on methodological differences, as we have not used the mixed 5-$HT_{2/7}$ receptor antagonist mesulergine in our experiments.

Overall, these data suggest that the sumatriptan-induced contraction in the three vessels examined is mediated by the 5-HT_{1B} receptor. Furthermore, the contractile effects of eletriptan at lower concentrations is largely mediated by the 5-HT_{1B} receptor but an additional receptor/mechanism, unaffected by GR125743, contributes to some extent in the contractions of the human coronary artery and saphenous vein elicited by high concentrations of eletriptan. This is indirectly supported by our experiments in the anaesthetised pigs, where we reported that, unlike sumatriptan (De Vries *et al*., 1996), the eletriptan-induced constriction of carotid arteriovenous anastomoses was not completely blocked by GR127935, another potent 5-$HT_{1B/1D}$ receptor antagonist (Willems *et al*., 1998). The exact nature of this additional mechanism involved in eletriptan-induced contraction is unknown, but it is conceivable that a part of the contraction to high concentrations of eletriptan, particularly after blockade of the 5-HT_{1B} receptor by GR125743, may be mediated via the 5-HT_{2A} receptor. In contrast to the human middle meningeal artery that predominantly possesses 5-HT_{1B} receptor population (Jansen *et al*., 1992; Razzaque *et al*., 1999), it is known that both human coronary artery and saphenous vein have a mixed population of 5-HT_{1B} and 5-HT_2 receptors (Bax *et al*., 1993; Bax *et al*., 1992; Connor *et al*., 1989; Ishida *et al*., 1999; Nilsson *et al*., 1999). We must, however, concede that eletriptan does not differ from sumatriptan with regard to the affinity at the 5-HT_{2A} receptor (pK_i: both <5.5, Napier *et al*., 1999).

Cranioselectivity of eletriptan and sumatriptan

Eletriptan and sumatriptan contracted the human isolated middle meningeal artery with equal efficacy (E_{max}: 94±8 and 106±17% of the contraction to 1 μM prostaglandin $F_{2\alpha}$, respectively) and potency (pEC_{50}: 7.34±0.13 and 6.91±0.17, respectively). The two drugs exhibited cranioselectivity because they were significantly more potent in contracting the middle meningeal artery than coronary artery (pEC_{50}: 5.54±0.22 and 6.24±0.14, respectively) or saphenous vein (pEC_{50}: 6.09±0.13 and 6.19±0.12, respectively; Table 7.1). Eletriptan and sumatriptan were equipotent in the saphenous vein, but in the coronary artery, as we found earlier with another 5-$HT_{1B/1D}$ receptor agonist GMC2021 (3-[2-(dimethylanimo)ethyl]-5-[(trifluoromethyl)sulfonyl]oxy][1H]indoleoxalate) (Saxena *et al*., 1996), eletriptan was significantly less potent than sumatriptan. This latter finding appears at variance with the higher binding affinity of eletriptan for the 5-HT_{1B} receptor compared to sumatriptan (Napier *et al*., 1999), but may involve agonist-specific differences in stimulus-effector coupling (Kenakin, 1987). It can perhaps be argued that the lower potency of eletriptan on the coronary artery may be due to the involvement of the non-5-$HT_{1B/1D}$ mechanism for which eletriptan has a low affinity (see section 7.2). However, we believe that the contraction elicited via a high affinity site (in this case, the 5-HT_{1B} receptor) would overrule that via a low affinity site.

Based on the difference in pEC_{50} values, sumatriptan proved 5-fold (95% CI: 2-13) more selective for the meningeal artery compared to both coronary artery and saphenous vein. This cranioselectivity ratio of eletriptan in the coronary artery (63, 95% CI 20-200) was significantly higher compared to that of sumatriptan (P<0.01), whereas in the saphenous vein the cranioselectivity of eletriptan, being 18-fold (95% CI: 8-41), was not different from that of sumatriptan (P=0.06). Admittedly, the data obtained with eletriptan are complicated by two factors. Firstly, the coronary artery contraction has a non-5-HT_{1B} receptor component. However, it is the resultant coronary artery contraction and not its underlying mechanism that is important for potential coronary side effects. Secondly, the relaxant response in the middle meningeal artery observed with eletriptan (100 μM) could well reduce its E_{max} and, thus, overestimate its pEC_{50}, compared to sumatriptan. Our results, however, show that the maximal contraction (and Hill slope) by eletriptan is similar to that of sumatriptan as well as 5-HT (see Table 1, MaassenVanDenBrink *et al*., 1999). Although, the relaxant response manifests at the highest concentration, it cannot be excluded that this response is latently present throughout the concentration range so that

the whole concentration-response curve may have been shifted to the right. In this case, it is equally possible that we may have underestimated the pEC_{50} (and cranioselectivity) of eletriptan.

The cranio-coronary selectivity of eletriptan, together with the clinical data showing that the highest dose of eletriptan (80 mg) has a superior efficacy, onset of action and patient acceptability in the acute treatment of migraine when compared with oral sumatriptan (100 mg) (Goadsby *et al*., 2000), speak in favour of eletriptan. However, it cannot be overemphasised that eletriptan has the capacity to constrict the human coronary artery and, therefore, like the other triptans, must remain contraindicated in patients with coronary artery disease.

Table 7.1 Functional parameters of the agonists eletriptan and sumatriptan, and the antagonist GR125743 in human isolated blood vessels.

	Middle meningeal artery		Coronary artery		Saphenous vein	
	Eletriptan	Sumatriptan	Eletriptan	Sumatriptan	Eletriptan	Sumatriptan
E_{max}	94±8	106±17	17±4[a]	12±2[a]	71±10	80±6
pEC_{50}	7.34±0.13[b]	6.91±0.17[b]	5.54±0.22[c]	6.24±0.14	6.09±0.13	6.19±0.12
Cranioselectivity ratio						
Mean (95% CI)d			63 (20 – 199)[c,e]	5 (2-13)[e]	18 (8-41)[e]	5 (2-13)[e]
pA_2 GR125743	8.81±0.17	8.64±0.21	ND	8.18±0.27	7.73±0.18[f]	8.34±0.12

Data are presented as means±S.E.M. or as 95% CI (n=4-9). The E_{max} values of eletriptan and sumatriptan are presented as percent of contraction elicited by either 1 μM prostaglandin $F_{2\alpha}$ (middle meningeal artery) or 100 mM KCl (coronary artery and saphenous vein).
ND, Not determined because of non-sigmoidal nature of the concentration response curve.
[a]E_{max} eletriptan and sumatriptan significantly lower than the respective E_{max} in the saphenous vein (P<0.05).
[b]pEC_{50} value significantly higher compared to the respective pEC_{50} values in the coronary artery and saphenous vein (eletriptan P<0.01, sumatriptan P<0.05).
[c]Significantly different from the respective value of sumatriptan (P<0.05).
[d]Cranioselectivity ratio = Inverse logarithm (pEC_{50} (middle meningeal artery) – pEC_{50} (coronary artery or saphenous vein)).
[e]Significantly different from 1 (P<0.05).
[f]pK_B value.

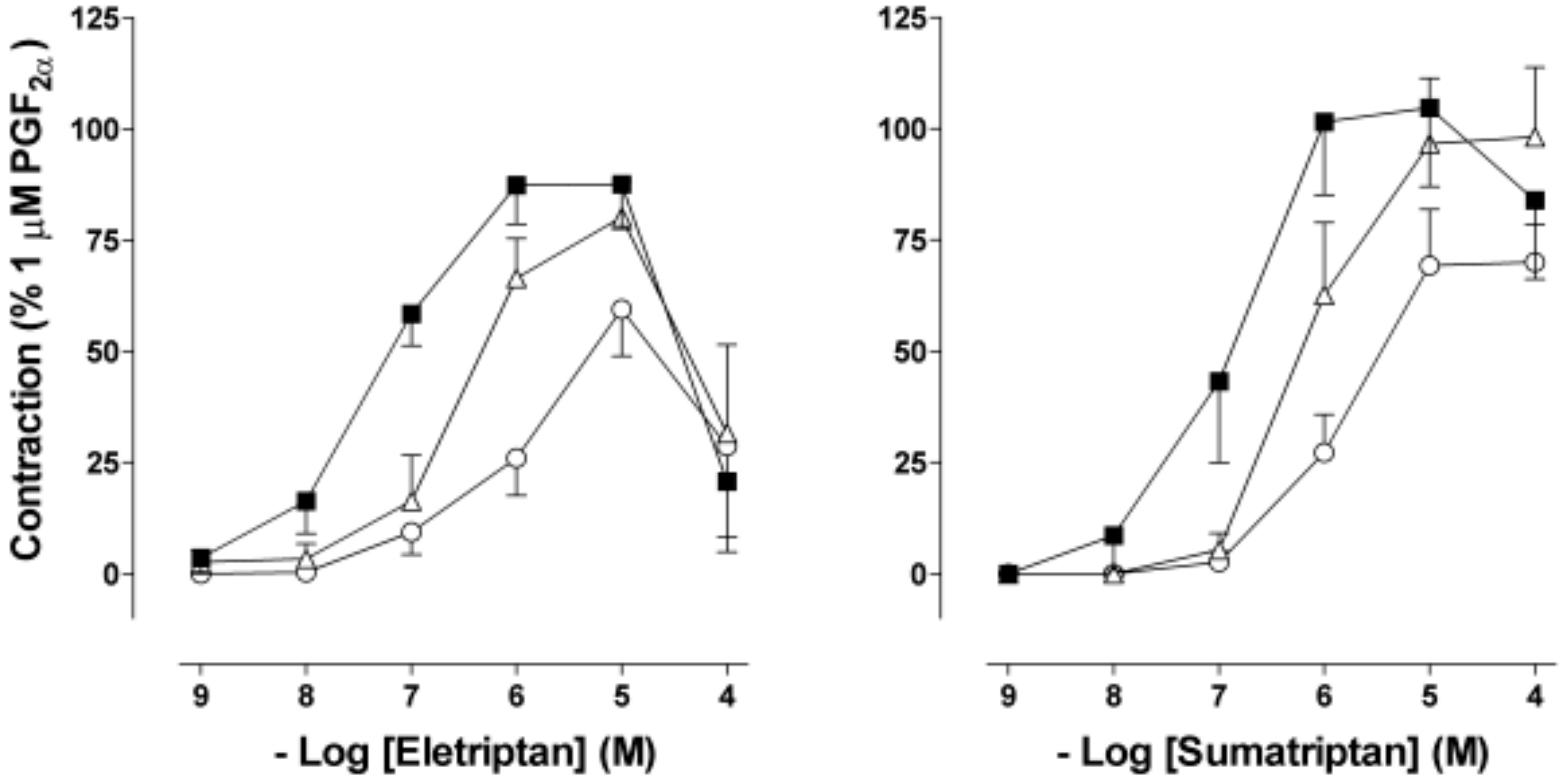

Figure 7.1 Cumulative concentration response curves to eletriptan (*left panel*; n=5) and sumatriptan (*right panel*; n=5) in the human isolated middle meningeal artery in the absence (■) or presence of GR125743 (Δ, 10 nM or ○, 30 nM). Symbols and vertical bars represent the means and S.E.M. $PGF_{2\alpha}$= prostaglandin $F_{2\alpha}$.

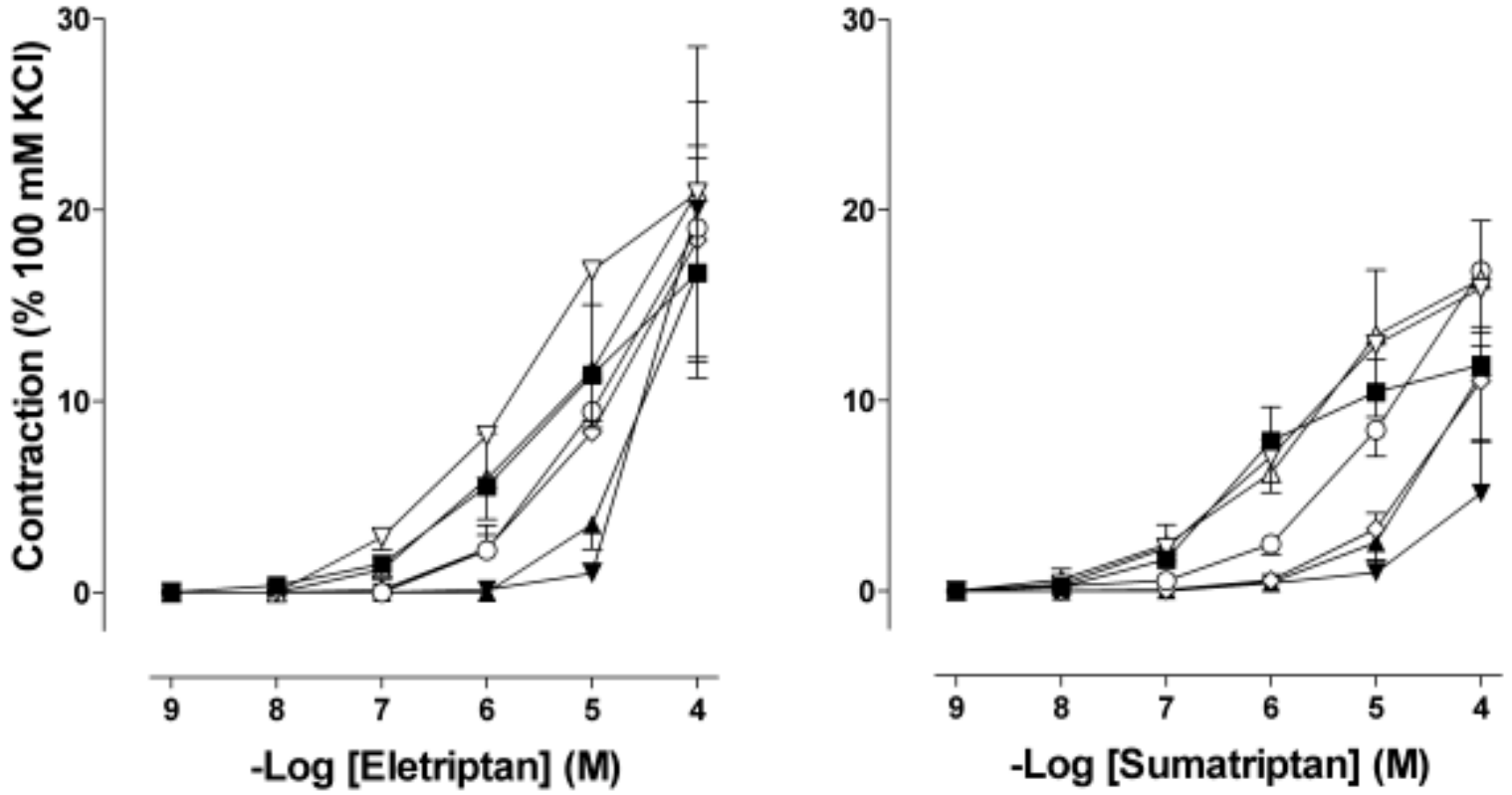

Figure 7.2 Cumulative concentration response curves to eletriptan (*left panel*; n=4-8) and sumatriptan (*right panel*; n=4-9) in the human isolated coronary artery in the absence (■) or presence of GR125743 (▽, 3 nM; Δ, 10 nM; ○, 30 nM; ◇, 100 nM; ▲, 300 nM or ▼, 1 μM). Symbols and vertical bars represent the mean and S.E.M.

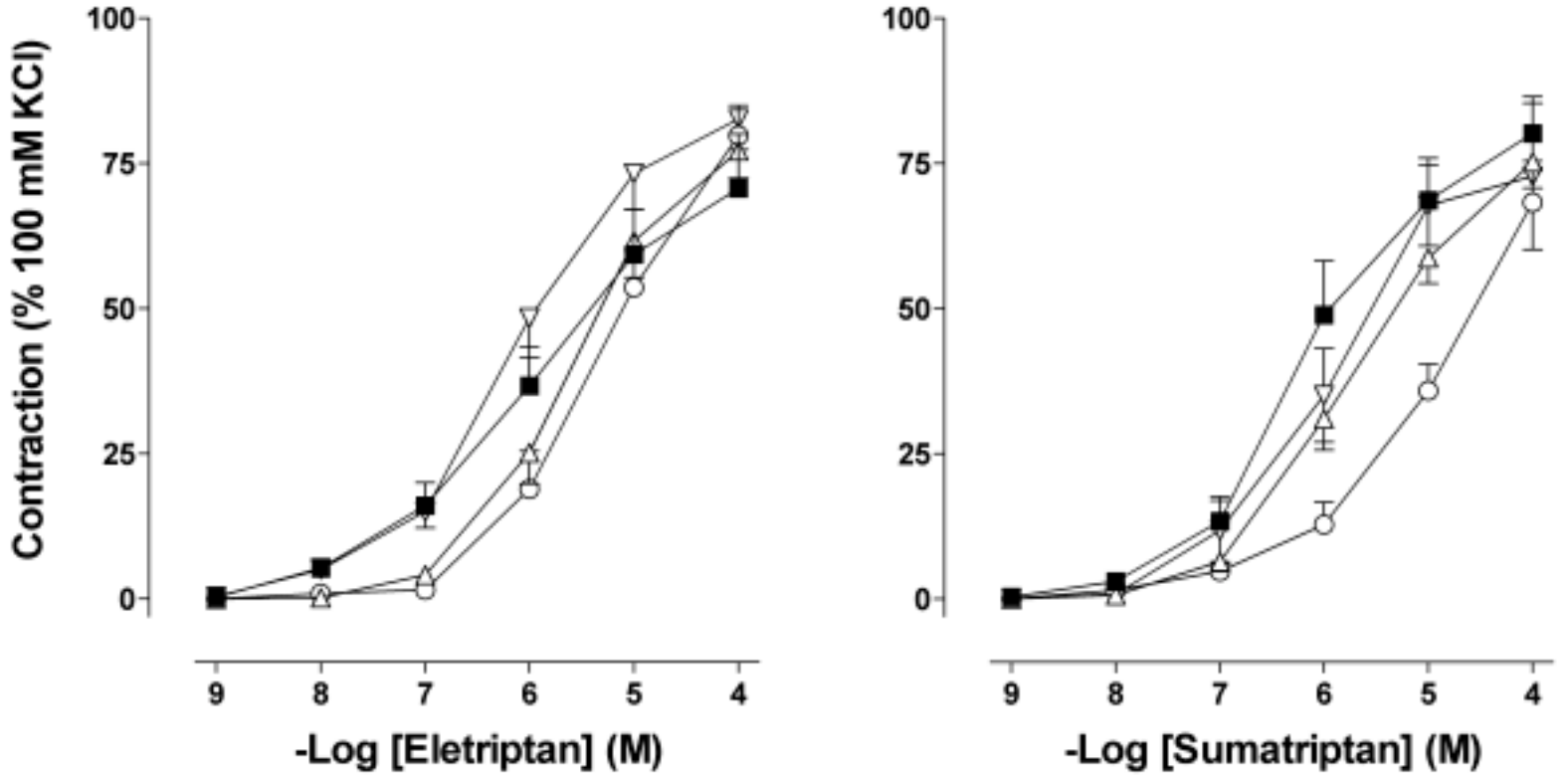

Figure 7.3 Cumulative concentration response curves to eletriptan (*left panel*; n=6) and sumatriptan (*right panel*; n=6) in the human isolated saphenous vein in the absence (■) or presence of GR125743 (∇, 3 nM; Δ, 10 nM or ○, 30 nM). Symbols and vertical bars represent the mean and S.E.M.

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Chapter 8

Characterisation of sumatriptan-induced contractions in human isolated blood vessels using selective 5-HT_{1B} and 5-HT_{1D} receptor antagonists and *in situ* hybridisation

Summary - The 5-$HT_{1B/1D}$ receptor agonist sumatriptan is effective in aborting acute attacks of migraine and is known to cause constriction of cranial arteries as well as some peripheral blood vessels. The present study set out to investigate whether 5-HT_{1B} and/or 5-HT_{1D} receptors mediate contractions of the human isolated middle meningeal and temporal arteries (models for antimigraine efficacy) and coronary artery and saphenous vein (models for side-effect potential). Concentration-response curves were made with sumatriptan (1 nM-100 μM) in blood vessels in the absence or presence of selective antagonists at 5-HT_{1B} (SB224289) and 5-HT_{1D} (BRL15572) receptors. SB224289 antagonised sumatriptan-induced contractions in all blood vessels, although the antagonism profile was different amongst these blood vessels. In the temporal artery, SB224289 abolished contraction to sumatriptan, whereas in the middle meningeal artery and saphenous vein, sumatriptan-induced contractions were blocked in an insurmountable fashion. Moreover, SB224289 acted as a weak surmountable antagonist in the coronary artery (pK_B: 6.4±0.2). In contrast, BRL15572 had little or no effect on sumatriptan-induced contractions in the four blood vessels investigated. *In situ* hybridisation revealed the expression of 5-HT_{1B} receptor mRNA in the smooth muscle as well as endothelial cells of the blood vessels, whereas the mRNA for the 5-HT_{1D} receptor was only very weakly expressed. These results show that the 5-HT_{1B} receptor is primarily involved in sumatriptan-induced contractions of human cranial as well as peripheral blood vessels.

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8.1 Introduction

Sumatriptan, the first of the 5-$HT_{1B/1D}$ receptor agonists, is highly effective in aborting migraine headaches (Goadsby, 1998; Tfelt-Hansen *et al*., 2000). It is believed that migraine headache results from dilation of extracerebral cranial blood vessels and that sumatriptan, as well as other triptans, constrict these dilated vessels (Humphrey & Feniuk, 1991; Tfelt-Hansen *et al*., 2000). Although the triptans are known to be cranioselective, they all have the propensity to constrict the coronary artery (Longmore *et al*., 1998; MaassenVanDenBrink *et al*., 2000; Parsons *et al*., 1998; Van den Broek *et al*., 2000). Constriction of the coronary artery may lead to cardiovascular adverse events, including myocardial ischaemia and infarction in predisposed individuals (Ottervanger *et al*., 1997).

The triptans are high affinity agonists at 5-HT_{1B} and 5-HT_{1D} receptors, but their vasoconstrictor effect seems to be mediated via the 5-HT_{1B} receptor (Tfelt-Hansen *et al*., 2000). Using RT-PCR techniques, it has also been shown that the 5-HT_{1B} receptor mRNA is predominant over 5-HT_{1D} receptor mRNA in the human middle cerebral (Bouchelet *et al*., 1996; Hamel *et al*., 1993), middle meningeal (Schmuck *et al*., 1996), temporal (Verheggen *et al*., 1998) and coronary (Bouchelet *et al*., 2000; Nilsson *et al*., 1999) arteries. The 5-HT_{1B} receptor protein has also been localised in the smooth muscle layer as well as endothelium of the human middle cerebral (Nilsson *et al*., 1999), middle meningeal (Longmore *et al*., 1998; Longmore *et al*., 1997) and coronary (Longmore *et al*., 1998; Longmore *et al*., 1997; Nilsson *et al*., 1999) arteries, where the 5-HT_{1D} receptor protein is not, or poorly, expressed. Functional pharmacological *in vitro* studies suggest that sumatriptan behaves as a full agonist in blood vessels (Bax *et al*., 1992; Jansen *et al*., 1992; Kaumann *et al*., 1994; Kaumann *et al*., 1993; Verheggen *et al*., 1996), which do not contract in response to selective 5-HT_{1D} receptor agonists (Bouchelet *et al*., 2000; Ennis *et al*., 1998).

A more direct evidence for a 5-HT_{1B} receptor-mediated vasoconstriction to sumatriptan can be obtained from antagonist studies. Until now, studies in the human isolated middle meningeal (Jansen *et al*., 1992; Razzaque *et al*., 1999; Van den Broek *et al*., 2000) and coronary (Bax *et al*., 1993; Kaumann *et al*., 1994; MaassenVanDenBrink *et al*., 2000; Van den Broek *et al*., 2000) arteries and saphenous vein (Bax *et al*., 1992), have used non-selective 5-$HT_{1B/1D}$ receptor antagonists (GR127935, GR55562 and GR125743). Recently, SB224289 and BRL15572 have been introduced as selective 5-HT_{1B} and 5-HT_{1D} receptor antagonists, respectively (Schlicker *et al*., 1997; Selkirk *et al*., 1998).

The use of these compounds revealed that sumatriptan-induced contractions in human isolated temporal (Verheggen *et al*., 1998) and small pulmonary (Morecroft *et al*., 1999) arteries as well as canine (De Vries *et al*., 1998) and porcine (De Vries *et al*., 1999) carotid vascular beds are mediated via the 5-HT_{1B} receptor. Using both functional *in vitro* and *in situ* hybridisation techniques, we investigated the role of 5-HT_{1B} and 5-HT_{1D} receptors in mediating contractions of the human isolated middle meningeal and temporal arteries (models for therapeutic efficacy in migraine) and coronary artery and saphenous vein (models for peripheral side-effect potential) (MaassenVanDenBrink *et al*., 1998; MaassenVanDenBrink *et al*., 2000; Van den Broek *et al*., 2000).

8.2 Material and methods

Tissue collection

The middle meningeal (4 male, 5 female; age 30-72 years) and temporal (3 female; age 45-59 years) arteries and saphenous vein (8 male, 1 female; age 45-78 years) were obtained postoperatively from patients undergoing craniotomy (middle meningeal and temporal artery: 8 aneurysms; 4 meningiomas) or coronary artery bypass grafting (saphenous vein) at the Erasmus University Medical Centre, Rotterdam, The Netherlands. The blood vessels were placed in a propylene tube filled with ice-cold (0-4 °C) physiological saline, transported immediately to the laboratory and used within 2 hr of surgery.

The right epicardial coronary artery was obtained from 9 heart beating organ donors (3 male, 6 female; 37-64 years) who died of non-cardiac disorders (6 cerebrovascular accident, 2 cerebral infarction, 1 head trauma). The Rotterdam Heart Valve Bank, Rotterdam, The Netherlands provided the hearts, after donor mediation by Bio Implant Services Foundation/Eurotransplant Foundation, Leiden, The Netherlands. The vessel was stored overnight in a modified Krebs bicarbonate solution (see below) and used the next day.

Organ bath experiments

Measurement of vascular contractions. The methods used were similar to those described in detail earlier (MaassenVanDenBrink et al., 2000; Van den Broek et al., 2000). Briefly, approximately 4-mm segments, obtained from pieces of the middle meningeal (n=6), temporal (n=3), coronary (n=6) arteries and saphenous vein (n=6), were mounted on metal

prongs in organ baths, containing a modified Krebs bicarbonate solution (pH 7.4; 37 ^{o}C), aerated with 95% O_2 and 5% CO_2. The composition (mM) of the Krebs bicarbonate solution was NaCl 119, KCl 4.7, $CaCl_2$ 1.25 (or 2.5 for coronary artery and saphenous vein), $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.1 (or 8.3 for coronary artery and saphenous vein). The cyclo-oxygenase inhibitor indomethacin (0.1 μM) was added to the Krebs solution to prevent prostaglandin synthesis. In addition, the Krebs solution was enriched with the muscarinic receptor antagonist, atropine, the histamine H_1 receptor antagonist mepyramine, the mixed 5-$HT_{2/7}$ receptor antagonist mesulergine, the α_1-adrenoceptor antagonist prazosin, the inhibitor of neuronal uptake$_1$, imipramine (all 1 μM) and the inhibitor of extra-neuronal uptake$_2$, corticosterone (10 μM) to exclude the putative involvement of these receptors/mechanisms. Changes in isometric tension were registered on recording set-ups from either EMKA Technology (Paris, France) for the middle meningeal and temporal arteries or Harvard Apparatus (South Natick, MA, USA) for the coronary artery and saphenous vein. The segments were allowed to equilibrate for at least 30 min, stretched to a passive tension of 4 mN (middle meningeal and temporal arteries), 15 mN (coronary artery) or 10 mN (saphenous vein). Since sumatriptan is metabolised by monoamine oxidase (Dixon et al., 1994), we then treated the segments with pargyline (100 μM) for 15 min to prevent its possible breakdown. After washing, all segments were exposed 2-3 times to either 0.1 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; middle meningeal and temporal arteries) or 30 mM KCl (coronary artery and saphenous vein) to demonstrate the reproducibility of the evoked contractions. Subsequently, the relaxation response to 10 nM substance P (1 nM in case of coronary artery or 0.1 μM bradykinin in the case of saphenous vein) in vessel segments pre-contracted with $PGF_{2\alpha}$ (1 μM) was used to verify the functional integrity of the endothelium.

After washing, the segments were allowed to equilibrate for 60 min, with replacement of the Krebs solution every 15 min. The segments were then studied in a paired parallel experimental set-up (MaassenVanDenBrink *et al*., 2000; Van den Broek *et al*., 2000), where a single concentration response curve to sumatriptan (1 nM-100 μM) was constructed in each segment incubated for 60 min with either the vehicle, SB224289, BRL15572 or both antagonists together. Since only 3 segments could be obtained from the human temporal artery, addition of both antagonists together was omitted. In view of the affinity of SB224289 and BRL15572 at recombinant h5-HT_{1B} (pK_i: 8.0 and 6.1, respectively) and h5-HT_{1D} receptors (pK_i: 6.2 and 7.9, respectively) (Schlicker *et al*.,

1997; Selkirk *et al*., 1998), we initially employed the two antagonists in a concentration range of 10-30 μM in the coronary artery (without the cocktail of inhibitors as mentioned above) and saphenous vein. Since in these concentrations neither antagonist affected the concentration response curves of sumatriptan, all experiments were performed with 1 μM of SB224289 and BRL15572.

Data presentation

Contractile responses were expressed as percentage of the contractile response to 1 μM $PGF_{2\alpha}$ (temporal and middle meningeal arteries) or 100 mM KCl (coronary artery and saphenous vein). The occasional spontaneous phasic contractions, observed in some coronary artery and saphenous vein segments, were ignored where measuring contractions. When the concentration contraction curve to sumatriptan did not attain a plateau, the contraction with its highest concentration (100 μM) was considered as the apparent maximum contraction (E_{max}). Initially, the mean value of E_{max} of sumatriptan observed in individual experiments was calculated. The mean concentration response curves were analysed with a non-linear regression fitting technique for sigmoidal functions with variable slope using GraphPad Prism 3.0 (GraphPad Software Inc., San Diego, CA, USA) to calculate potency (pEC_{50}) and Hill slopes for the agonists in the absence or presence of antagonists. When mean (apparent) E_{max} and Hill slopes were not significantly different in control and antagonist experiments, a surmountable antagonism was assumed. The whole data set was then transformed using SPSS 7.5 non-linear regression statistics (SPSS Inc., Chicago, IL, USA) into a dependent fitting model, where the mean maximal-induced contraction was set to the respective agonist control value. In case of a parallel rightward shift, a Schild regression analysis was performed with a slope set to unity to calculate the pK_B value. Due to the insurmountable behaviour of SB224289 in the middle meningeal artery and saphenous vein, pK_B values could not be calculated. Instead, we calculated the negative logarithm of the mean concentration of sumatriptan eliciting a contraction equivalent to 25% of E_{max} in the individual control experiments ($pEC_{25\%}$) in the absence or presence of antagonists. This parameter enabled us to compare the properties of the antagonists in these vascular preparations against low concentrations of sumatriptan.

Statistical analysis

Differences between the (apparent) E_{max} of sumatriptan in the absence or presence of antagonists were analysed with a paired t-test, using GraphPad Prism 3.0 (GraphPad Software Inc., San Diego, CA, USA). In case the E_{max} values in the absence of antagonists were similar to those in the presence of antagonist, the differences in Hill slopes and pEC_{50} values of mean concentration response curves between vehicle and antagonist groups were analysed with a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test. In case E_{max} values were different between the respective groups, we performed a two-way repeated measurement analysis of variance (RM-ANOVA) followed by Bonferroni's multiple comparison t-test to evaluate the effect of treatments (i.e. control or antagonist) at repetitive concentrations of sumatriptan. Differences between the $pEC_{25\%}$ values of sumatriptan in absence or presence of antagonists were calculated according to paired t-test. In all cases statistical significance was assumed when $P<0.05$. Data are presented as mean±s.e. mean.

Molecular biological experiments

In situ hybridisation

5-HT_{1B} and 5-HT_{1D} receptor mRNAs were localised employing non-radioactive *in situ* hybridisation on the human middle meningeal artery, coronary artery and saphenous vein (n=3 each). After cleaning the surrounding tissue, the blood vessels were fixed for 24 h in 4% paraformaldehyde dissolved in phosphate buffered saline. Chinese hamster ovary (CHO) cells expressing either the human recombinant 5-HT_{1B} or 5-HT_{1D} receptor served as positive controls. After dehydration with increasing percentage of ethanol in phosphate buffered saline, the blood vessels were embedded in paraffin and 5-µm thick sections were cut with a microtome (model HM325, Microm GmbH, Walldorf, Germany). The sections were mounted on superfrost plus® glass slides (Menzel-Glaser, Braunschweig, Germany) and dried at 37 °C for 48 h.

Recombinant plasmid DNAs encoding the human 5-HT_{1B} (Genbank accession number D10995, nucleotide 40-390, length 350 bp) or 5-HT_{1D} (Genbank accession number M81589; nucleotide 1-400, length 400 bp) receptor were employed for the preparation of non-radioactive cRNA probes. Linearised DNA templates were transcribed to synthesise the sense and antisense cRNAs probes, using T_7 or SP_6 RNA polymerase as per protocol described for the DIG-RNA labelling kit. DIG-labelled cRNA probes were

quantified by dot blotting and using serial dilutions of standard DIG-labelled control RNA supplied in the kit. Treatment of tissue sections and cells and subsequent hybridisation was performed as described earlier (de Boer *et al*., 1998). The tissues were hybridised with 25 ng of cRNA probe per slide for 16 h at 55 °C and the DIG-labelled hybrids were detected by incubation with antidigoxigenin antibody (1:2000 dilution) conjugated to alkaline phosphatase for 2.5 h at room temperature. The immunodetection of DIG-labelled hybrids was done using 4-nitroblue tetrazolium chloride (NBT) as chromogen and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) as coupling agent. Slides were counter-stained with nuclear red solution, dehydrated with ethanol gradients and mounted with Euparal (Chroma-Gesellschaft, Schmid GmbH, Köngen, Germany). Cells and tissue sections were visualised under a light microscope (model Leica DM RBE, Leica NL, BV, Rijswijk, The Netherlands) and photographed using a CCD video camera (Sony DXC-950, Sony Corporation, Japan). The sense riboprobes were included as negative controls, which showed no or little staining compared to antisense riboprobes.

Endothelial staining

Serial sections of 5-µm thickness were processed for immunohistochemical localisation of endothelial cell marker, CD31. Sections were deparaffinised, rehydrated and incubated with pronase (1 mg/ml) at 37 °C for 10 min prior to incubation with specific purified mouse monoclonal antibodies raised against human CD31 (Neomarkers, Union City, CA, USA). To block non-specific binding, sections were incubated with 10% normal goat serum diluted in 5% bovine serum albumin in phosphate buffered saline (pH = 7.4). Subsequently, sections were incubated overnight at 4 °C with primary antibodies, CD31 (1:75 v/v). Incubation for 30 min with secondary biotinylated anti-immunoglobulins (Multilink®, 1:75 v/v, Biogenex, San Ramon, USA) and tertiary complex of streptavidin conjugated to Alkaline Phosphatase (Label® 1:50 v/v, Biogenex, San Ramon, USA) were used to enhance the detection sensitivity. Colour was developed using New Fuchsine as chromogen, while endogenous alkaline phosphatase activity was inhibited by 0.01 M levamisole (Sigma, St Louis, USA). Slides were counterstained with Mayer's hematoxylin. Positive controls consisted of human cardiac tissue where intense staining was seen in the endothelium of all blood vessels. The optimal dilutions of primary antibody were identified by examining the intensity of staining obtained with a series of dilutions, which gave specific and easily visible signal on paraffin sections of control tissues. Slides were mounted and

visualised using light-microscopy. Negative controls consisted of omission of the primary antibody.

Ethical approval

The Medical Ethics Committee of the Erasmus University Medical Centre Rotterdam, dealing with the use of the human material for scientific experiments, approved the protocols for this investigation.

Compounds and kits

For pharmacological study, the following compounds were used: atropine sulphate, bradykinin acetate, corticosterone, 5-hydroxytryptamine creatinine sulphate (serotonin; 5-HT), imipramine hydrochloride, indomethacin hydrochloride, mepyramine maleate, pargyline hydrochloride, prostaglandin $F_{2\alpha}$ tris salt ($PGF_{2\alpha}$) and substance P acetate (all purchased from Sigma Chemical Co., St. Louis, MO, USA), mesulergine hydrochloride (gift: Novartis AG, Basel, Switzerland), prazosin hydrochloride (gift: Pfizer, Sandwich, UK), sumatriptan succinate, SB224289 and BRL15572 (gift: SmithKline Beecham Pharmaceuticals, Harlow, Essex, UK; courtesy: Dr. A.A. Parsons). Stock solutions of corticosterone (100 mM), indomethacin, mesulergine, prazosin, SB224289 and BRL15572 (all 10 mM) were dissolved in 100% v/v dimethyl sulphoxide and further diluted in distilled water. All other compounds were dissolved in distilled water.

For *in situ* hybridisation, the materials used were: acetic anhydride, diethyl pyrocarbonate, levamisol, maleic acid, triethanolamine and xylene (Sigma Chemical Co., St. Louis, MO, USA), antidigoxigenin-AP Fab fragments, blocking reagent for nucleic hybridisation, DIG-RNA labelling kit, glycine and RNase T_1 (Boehringer Mannheim, Almere, The Netherlands), bovine serum albumin, dextran sulphate, ethylene diaminetetraacetic acid, ficoll, hering sperm DNA, phenol and Tris-HCl (Amersham Pharmacia Biotech Benelux, Roosendaal, The Netherlands). BCIP, formamide, NBT, proteinase K, sodium citrate and yeast tRNA (Life Technologies, Breda, The Netherlands), DNase, RNase inhibitors and Triton-X-100 (Promega Benelux, Leiden, The Netherlands) and RNase away solution (Molecular Bio-products, San Diego, CA, USA).

8.3 Results

Relaxation responses to substance P and bradykinin

The relaxation to substance P (10 nM) amounted to 29% (range: 17-44%, n=6) and 42% (range: 9-86%, n=3) of precontraction with 1 μM $PGF_{2\alpha}$ in the middle meningeal and temporal arteries, respectively. In the coronary artery, relaxation to substance P (1 nM) was 46% (range: 30-66%, n=6) of precontraction to 1 μM $PGF_{2\alpha}$. In the saphenous vein, relaxation to bradykinin (1 μM) was 54% (range: 26-82%, n=6) of precontraction to 1 μM $PGF_{2\alpha}$.

Effects of sumatriptan on cranial arteries

Concentration response curves to sumatriptan in the middle meningeal and temporal arteries are depicted in Fig. 8.1. In both blood vessels, sumatriptan elicited a concentration-dependent contraction. The E_{max} values (efficacy) of sumatriptan in the middle meningeal and temporal arteries were 83±15% and 68±28% of the contraction to 1 μM $PGF_{2\alpha}$, respectively and the pEC_{50} values (potency) amounted to 6.7±0.2 and 6.7±0.3, respectively (Table 8.1).

The responses to sumatriptan in both cranial arteries were antagonised by the 5-HT_{1B} receptor antagonist SB224289 (1 μM) and the magnitude of antagonism did not correlate with the functional integrity of the endothelium (substance P response). Since SB224289 decreased the E_{max} of sumatriptan, the antagonism was apparently insurmountable and pEC_{50} values of sumatriptan were not determined (Fig. 8.1, Table 8.1). The 5-HT_{1D} receptor antagonist BRL15572 (1 μM) had no effect on either the E_{max} or pEC_{50} values of sumatriptan. Also, no additional antagonism was observed with the combination of the two antagonists (Fig. 8.1, Table 8.1).

Due to the insurmountable antagonism of sumatriptan by SB224289, pK_B values could not be estimated. Alternatively, we calculated the negative logarithm of sumatriptan concentration eliciting a response equivalent to 25% of sumatriptan control E_{max} ($pEC_{25\%}$) in the absence or presence of the antagonists in the middle meningeal artery; the temporal artery data was not further processed as SB224289 virtually abolished sumatriptan-induced contractions (Table 8.2). While BRL15572 was ineffective, the $pEC_{25\%}$ of sumatriptan was significantly decreased by SB224289. No additional antagonism was observed with the combination of the two antagonists (Table 8.2).

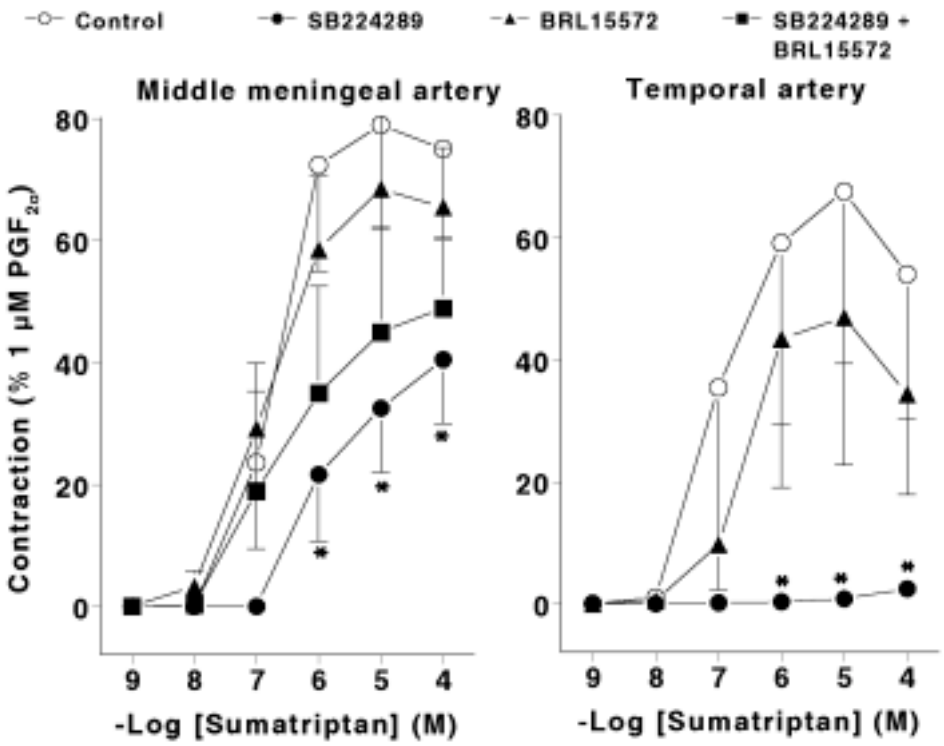

Figure 8.1 Cumulative concentration response curves to sumatriptan in the human isolated middle meningeal (n=5-6) and temporal (n=3) arteries in the absence (control; ○) or presence of SB224289 (1 µM; ●), BRL15572 (1 µM; ▲) or both antagonists (1 µM each; ■). Symbols and vertical bars represent the means ± S.E.M. *, Significant difference from control contraction elicited by respective concentrations of sumatriptan (Bonferroni multiple comparison t-test, P<0.05)

Effect of sumatriptan on peripheral vessels

Sumatriptan also contracted the human coronary artery and saphenous vein in a concentration-dependent manner. The pEC_{50} and (apparent) E_{max} of sumatriptan were, respectively, 5.7±0.1 and 13±2% of the response to 100 mM KCl in the coronary artery and 6.1±0.1 and 62±1% of the response to 100 mM KCl in the saphenous vein (Fig. 8.2, Table 8.1).

In the coronary artery, SB224289 caused a small parallel rightward shift in the concentration response curve to sumatriptan, yielding a significant decrease in the pEC_{50} (5.0±0.1) with no change in the apparent E_{max} (11±3% of the response to 100 mM KCl) of sumatriptan. Schild regression analysis revealed a pK_B value of 6.4±0.2 for SB224289 against sumatriptan. The shift in the concentration response curve of sumatriptan by

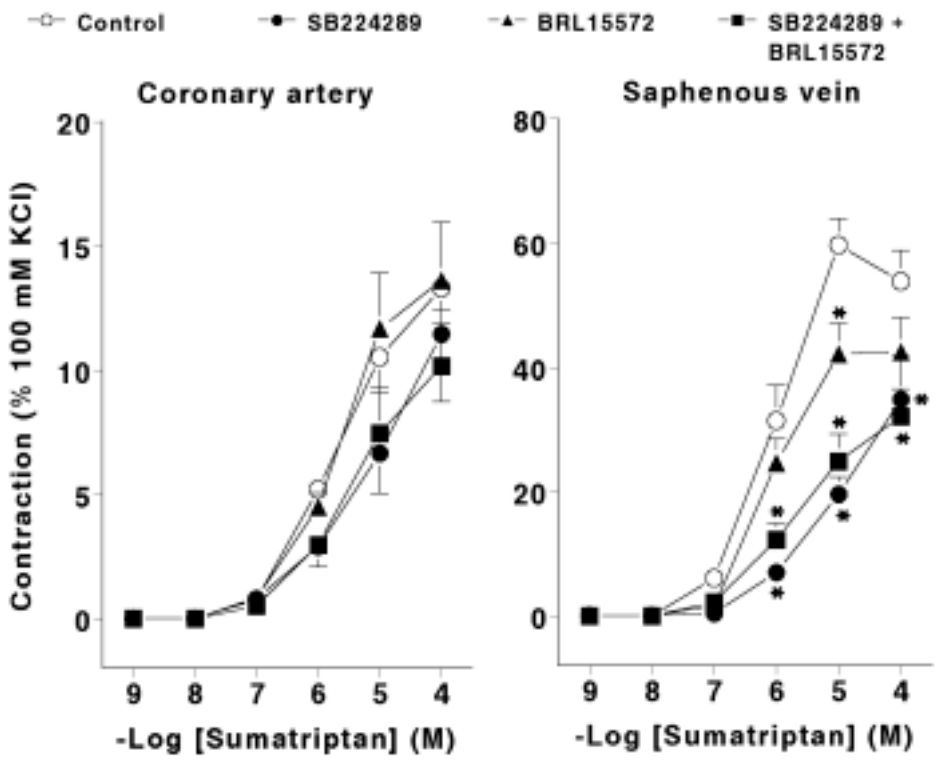

Figure 8.2 Cumulative concentration response curves to sumatriptan in the human isolated coronary artery (n=5-6) and saphenous vein (n=4-6) in the absence (control; ○) or presence of SB224289 (1 μM; ●), BRL15572 (1 μM; ▲) or both antagonists (1 μM each; ■). Symbols and vertical bars represent the means ± S.E.M. *, Significant difference from control contraction elicited by respective concentrations of sumatriptan (Bonferroni multiple comparison t-test, $P<0.05$).

SB224289 was independent of the functional integrity of the endothelium. BRL15572 did not have any effect on sumatriptan-induced contraction. Incubation with both antagonists together resulted in a small parallel rightward shift as was noticed with SB224289 alone (Fig. 8.2, Table 8.1).

In the saphenous vein, the response to sumatriptan was antagonised by SB224289 (1 μM). Although the contraction to sumatriptan increased with concentration, the response did not reach a plateau with the highest concentration used and, therefore, pEC_{50} and pK_B values after SB224289 were not determined. As observed in the other blood vessels, the antagonism of SB224289 was independent of the functional integrity of the endothelium. Incubation with BRL15572 (1 μM) resulted in a slight attenuation of the response to 10 μM sumatriptan as well as its E_{max}, but no change was observed in the pEC_{50} value of sumatriptan. Also, no additional antagonism was observed with the

combination of the two antagonists (Fig. 8.2, Table 8.1). The $pEC_{25\%}$ of sumatriptan was not affected by BRL15572, but was reduced after SB224289 alone or in combination with BRL15572 (Table 8.2).

In situ hybridisation

The specificity of mRNA signals and standardisation of the *in situ* hybridisation conditions were first established by the use of specific sense and antisense probes in CHO cells expressing either the human 5-HT_{1B} or 5-HT_{1D} receptor. Strong cytoplasmic and nuclear mRNA expression as a dark purple/blue colour for respective antisense riboprobes was observed in these cells, whereas sense riboprobes depicted no staining. Furthermore, the sense riboprobes did not show any specific mRNA expression assessed as purple/blue staining in the tissue samples included in this study. If at all staining with sense riboprobes was detected, it was always far less than the staining with antisense probes (data not shown). Cellular localisation of mRNAs for the 5-HT_{1B} and 5-HT_{1D} receptors in the human middle meningeal and coronary arteries and saphenous vein is depicted in Fig. 8.3. In the middle meningeal artery, hybridisation with antisense riboprobe showed 5-HT_{1B} receptor mRNA signals in the medial smooth muscle layer as well as in the endothelial cells, whereas adventitial cells showed only a faint staining. Although not so intense, the coronary artery and saphenous vein also expressed the 5-HT_{1B} receptor mRNA in the vascular smooth muscle cells. Specific expression for the 5-HT_{1B} receptor was seen in the luminal endothelial cells in the case of coronary artery. In order to verify the *in situ* hybridisation data for the endothelial cell expression of 5-HT_{1B} receptor mRNA, CD31 immunohistochemistry was performed and it confirmed a cytoplasmic endothelial cell staining (Fig. 8.3). In contrast to the 5-HT_{1B} receptor mRNA, no signals were detected for the 5-HT_{1D} receptor mRNA in both smooth muscle and endothelial cells in the middle meningeal artery and saphenous vein. However, a weak staining for the 5-HT_{1D} receptor mRNA was noticed in some endothelial cells in the coronary artery and in adventitial cells in the case of middle meningeal as well as coronary arteries (Fig. 8.3).

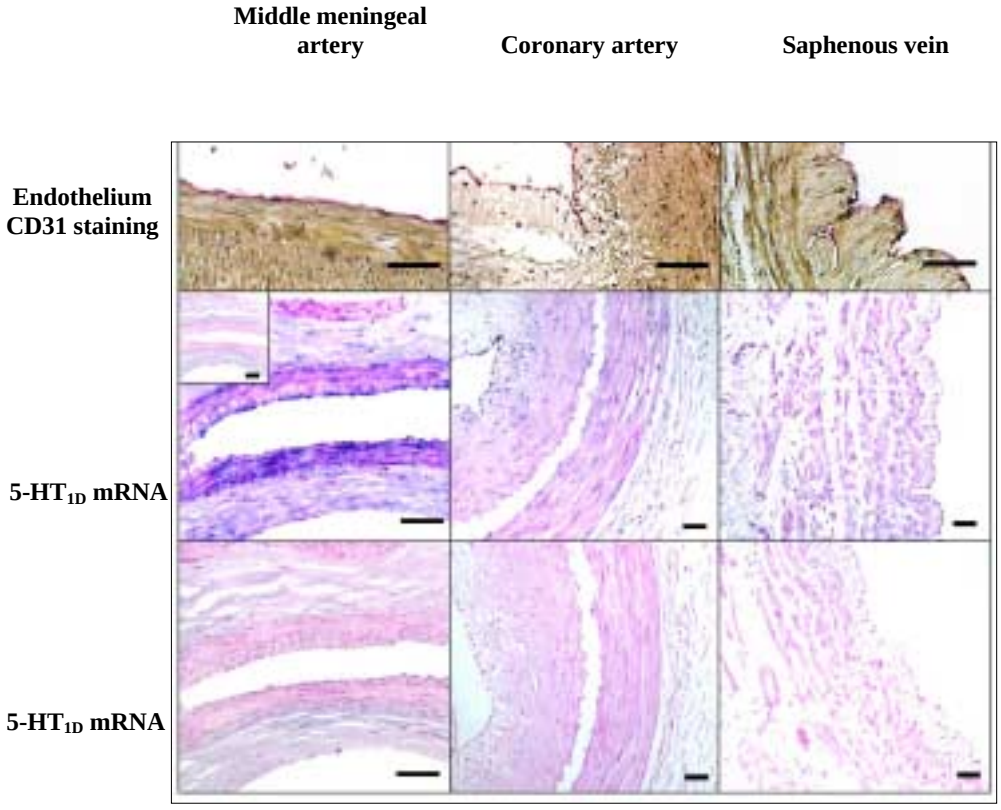

Figure 8.3 Photographs of immunohistochemical staining with CD31 endothelial marker (top panels) and *in situ* hybridisation with DIG-labelled cRNA probes for h5-HT_{1B} (middle panels) and h5-HT_{1D} (lower panels) receptor mRNAs in the human middle meningeal and coronary arteries and saphenous vein. The reddish colour in the top panels denotes endothelial cells, while the purple precipitates in the middle panels correspond to 5-HT_{1B} receptor mRNA signals. The 5-HT_{1D} receptor mRNA (lower panels) as well as the sense riboprobes, as exemplified by the left middle panel inset, did not show signals in the three blood vessels. Scale bar = 50 μm.

Table 8.1 E_{max} and pEC_{50} values of sumatriptan in contracting human isolated blood vessels in the absence or presence of antagonists

Antagonist	Middle meningeal artery		Temporal artery		Coronary artery		Saphenous vein	
	E_{max} (%)	pEC_{50}	E_{max} (%)	pEC_{50}	E_{max} (%)	pEC_{50}	E_{max} (%)	pEC_{50}
None	83±15	6.7±0.2	68±28	6.7±0.3	13±2	5.7±0.1	62±4	6.1±0.1
SB224289 (1 μM)	40±11*	ND	2±1*	ND	11±3	5.0±0.1*	35±7*	ND
BRL15572 (1 μM)	72±10	6.8±0.2	47±24	6.5±0.2	14±2	5.7±0.1	44±7*	6.1±0.1
SB224289 (1 μM) + BRL15572 (1 μM)	53±14	ND	-	-	10±2	5.0±0.2*	32±4*	ND

Data are presented as means±s.e.m. (n=3-6). E_{max} is presented as % of contraction elicited by either 1 μM $PGF_{2\alpha}$ (middle meningeal and temporal artery) or 100 mM KCl (coronary artery and saphenous vein). ND, Not determined because of insurmountable nature of the antagonism; -, not investigated. *, Significantly different from sumatriptan control ($P<0.05$).

Table 8.2 $pEC_{25\%}$ of sumatriptan in contracting the middle meningeal artery and saphenous vein in the absence or presence of antagonists

Antagonist	Middle meningeal artery	Coronary artery	Saphenous vein
None	6.8±0.2	6.3±0.1	6.4±0.1
SB224289 (1 μM)	5.6±0.4*	5.7±0.3	5.3±0.2*
BRL15572 (1 μM)	7.0±0.5	6.1±0.1	6.3±0.1
SB224289 (1 μM) + BRL15572 (1 μM)	6.0±0.5	5.7±0.2*	5.7±0.3*

Data are presented as mean±s.e.m. (n=4-6). $pEC_{25\%}$ represents the negative logarithm of the concentrations of sumatriptan eliciting a contraction equivalent to 25% of individual control E_{max} (middle meningeal artery, 20.8±3.8% of the response to 1 μM $PGF_{2\alpha}$; coronary artery, 3.3±0.4% of the response to 100 mM KCl; saphenous vein, 15.2±0.9% of the response to 100 mM KCl). * Significantly different from sumatriptan control ($P<0.05$).

8.4 Discussion

Craniovascular selectivity of sumatriptan

Sumatriptan contracted in a concentration-dependent manner both cranial and peripheral blood vessels, used as models for antimigraine activity (middle meningeal and temporal artery) and possible peripheral side-effect potential (coronary artery and saphenous vein), respectively. The potency of sumatriptan was higher at the middle meningeal and temporal arteries (pEC_{50}: 6.7 each) than at the coronary artery (pEC_{50}: 5.7) and saphenous vein (pEC_{50}: 6.1). Compared to the other vessels, the efficacy (E_{max}) of sumatriptan in the coronary artery was clearly lower (see Table 8.1). These data, which confirm the cranioselectivity of sumatriptan, are in accordance with other studies dealing with middle meningeal (Jansen *et al*., 1992; Longmore *et al*., 1998; MaassenVanDenBrink *et al*., 2000; Razzaque *et al*., 1999), temporal (Jansen *et al*., 1992; Verheggen *et al*., 1998) and coronary (Connor *et al*., 1989; Kaumann *et al*., 1994; MaassenVanDenBrink *et al*., 1998; MaassenVanDenBrink *et al*., 2000; Nilsson *et al*., 1999; Van den Broek *et al*., 2000) arteries and saphenous vein (Bax *et al*., 1992; MaassenVanDenBrink *et al*., 2000; Van den Broek *et al*., 2000).

Receptors mediating sumatriptan-induced contractions

The data obtained in *in vitro* functional studies show that the 5-HT_{1B} receptor antagonist SB224289 clearly attenuated sumatriptan-induced contractions, while the 5-HT_{1D} receptor antagonist BRL15572 had little effect on the concentration response curves to sumatriptan; the combination of SB224289 and BRL15572 did not reveal any additional antagonism. These results establish that the 5-HT_{1B} receptor mediates the contractile effects of sumatriptan in both cranial and peripheral blood vessels, while the 5-HT_{1D} receptor does not seem to play any role. The latter conclusion is also supported by the fact that selective 5-HT_{1D} receptor agonists (PNU-109291 and L775,606) did not contract isolated blood vessels (Bouchelet *et al*., 2000; Ennis *et al*., 1998; Longmore *et al*., 2000) and, interestingly, one such compound (PNU-142633) was also found ineffective in migraine (Cutler *et al*., 2000). Based on the high affinity of sumatriptan for the 5-HT_{1F} receptor (pK_i: 7.9, Leysen *et al*., 1996), it has been argued that this receptor might play a role in the therapeutic action of sumatriptan (Johnson *et al*., 1997). Although we cannot rule this out, the 5-HT_{1F} receptor is not involved in vasoconstriction, since selective 5-HT_{1F} receptor agonists (LY344864 and LY334370) show no vasoconstrictor effect

(Bouchelet *et al*., 2000; Cohen & Schenck, 1999; Shepheard *et al*., 1999). In addition, Verheggen et al. (1998) have suggested that in the presence of SB224289 high concentrations of sumatriptan can elicit contractions of the human isolated temporal artery via the 5-HT_{2A} receptor. This was not the case in the present studies, probably because we included the mixed 5-$HT_{2/7}$ receptor antagonist mesulergine (pK_B: 9.1 and 8.2, respectively, Hoyer *et al*., 1994) in the Krebs solution. However, the 5-HT_2 receptor antagonist ketanserin was unable to block sumatriptan-induced contractions in the human middle meningeal (Jansen *et al*., 1992) and coronary (Connor *et al*., 1989; Kaumann *et al*., 1994) arteries as well as the saphenous vein (Bax *et al*., 1992).

It may be noted that SB224289 antagonised the responses to sumatriptan in an insurmountable manner in the middle meningeal artery, whereas in the temporal artery the contractions were virtually abolished. In the coronary artery, there was a weak competitive (surmountable) antagonism, whilst in the saphenous vein an intermediate antagonistic response was observed. The nature of the difference in the antagonistic behaviour of SB224289 in these blood vessels is not clear. A possible explanation (Schutz & Freissmuth, 1992) could be that SB224289, which acts as an inverse agonist in cells expressing recombinant 5-HT_{1B} receptors (Gaster *et al*., 1998), may also do so at constitutive 5-HT_{1B} receptors. Another explanation for the observed differences could be the influence of receptor density and receptor reserve, but, in view of considerably higher 5-HT_{1B} receptor density in meningeal artery as compared to coronary artery (Longmore *et al*., 1997), we should have observed a surmountable antagonism in the middle meningeal artery and insurmountable antagonism in the coronary artery. Finally, in view of the localisation of the 5-HT_{1B} receptor on the endothelium (see below), the effect of SB224289 may be influenced to a different degree by endothelial factors (either contractile or relaxing) released upon activation of the 5-HT_{1B} receptor. Whatever the mechanism, differences in the nature of antagonism have also been observed between sumatriptan and the non-selective 5-$HT_{1B/1D}$ receptor antagonist GR127935 (Bouchelet *et al*., 2000; Razzaque *et al*., 1999).

It is surprising that SB224289 proved to be a weak antagonist in our experiments as its pK_B (6.4±0.2) against sumatriptan in the coronary artery resembled more its pK_i (6.2) at the 5-HT_{1D} receptor and was far less than its pK_i at the 5-HT_{1B} receptor (8.2) (Schlicker *et al*., 1997; Selkirk *et al*., 1998). However, if 5-HT_{1D} receptors were involved, we would have found BRL15572 to be an even more potent antagonist than SB224289;

this was obviously not the case. The involvement of 5-HT_{1B} receptor in the sumatriptan-induced coronary artery contraction is also supported by previous investigations using non-selective 5-$HT_{1B/1D}$ receptor antagonists (Bax *et al*., 1993; Connor *et al*., 1989; MaassenVanDenBrink *et al*., 2000; Nilsson *et al*., 1999; Van den Broek *et al*., 2000). However, the weaker antagonism by SB224289 than these non-selective antagonists suggests that an additional mechanism is partly responsible for the coronary contraction induced by sumatriptan. The nature of this additional mechanism is not known, but it cannot be related to 5-HT_2 receptors in view of the presence of mesulergine in the organ bath. Certainly, this additional mechanism is of interest in regard to the sumatriptan related cardiac side effects and may prove relevant in future antimigraine drug development.

Vascular localisation of 5-HT_{1B} and 5-HT_{1D} receptor mRNA

Although RT-PCR studies have shown the presence of 5-HT_{1B} receptor mRNA in the human middle meningeal (Schmuck *et al*., 1996) and coronary (Bouchelet *et al*., 2000; Nilsson *et al*., 1999) arteries, the cellular localisation of 5-HT receptor subtypes at mRNA level has not been demonstrated. Our results of *in situ* hybridisation showed the mRNA expression of 5-HT_{1B} receptor in both smooth muscle and endothelium, whereas the expression of 5-HT_{1D} receptor mRNA was weak, if any, in the three human blood vessels investigated (Fig. 8.3). These results are in agreement with the 5-HT_{1B} and 5-HT_{1D} receptor protein localisation in coronary and middle meningeal arteries (Longmore *et al*., 1998; Longmore *et al*., 1997; Nilsson *et al*., 1999). The localisation of 5-HT_{1B} receptor mRNA within the smooth muscle cells of these blood vessels, and the lack of 5-HT_{1D} receptor mRNA, strongly suggest that contraction to sumatriptan is mediated via the 5-HT_{1B} receptor rather than the 5-HT_{1D} receptor. Although the exact role of the endothelial 5-HT_{1B} receptor in these vessels is not well understood, we cannot rule out the release of relaxing and/or contractile substances upon stimulation

Conclusion

In conclusion, our data show that sumatriptan contracts the human middle meningeal, temporal and coronary arteries and saphenous vein via the 5-HT_{1B} receptor, but not 5-HT_{1D} receptor. In the human coronary artery, the contraction may also be mediated by an unknown, SB224289-resistant, mechanism.

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Part IV

General discussion and synopsis

Chapter 9

General Discussion

9.1 Human isolated middle meningeal artery in relation to therapeutic efficacy of antimigraine drugs

The main objective of this thesis was to study the effects of current and prospective antimigraine drugs *in vitro*, with the emphasis on the therapeutic site of action: the cranial vasculature. Even though the underlying mechanisms for the pathophysiology of migraine attacks still remain a 'black box', several findings suggest a predominant role for the cranial vasculature, either primary (Humphrey, 1991) or secondary in nature (Goadsby and Hoskin, 1998). As discussed in previous chapters, the most probable cause of a migraine headache is dilatation of cranial arteries. The 5-$HT_{1B/1D}$ receptor agonist sumatriptan was designed to selectively reverse this craniovascular dilatation (Humphrey et al., 1988; Humphrey et al., 1989) via vasoconstriction leading to subsequent relief of migraine headache. Not surprisingly, sumatriptan and the since then developed 'second-generation' triptans all are effective in migraine relief (Limmroth and Diener, 1998; Deleu and Hanssens, 2000; Roon, 2000). Ever since the introduction of sumatriptan the research in migraine accelerated, leading to better insights in its pathophysiology. One such a pathway is characterising the mode of action of these antimigraine drugs in the isolated middle meningeal artery, used as a predictive therapeutic model. Using organ bath techniques we have investigated sumatriptan, eletriptan and donitriptan using isometric contraction measurements in isolated artery segments. As was observed *in vivo* for sumatriptan (Henkes et al., 1996), these compounds potently constricted the human isolated middle meningeal artery, as is the case with other triptans (see Table 1.4).

To validate whether cranial vasoconstriction is the main mechanism of the therapeutic action of triptans we re-analysed concentration response curves to triptans in the middle meningeal artery, obtained from previous published studies. First we recalculated the concentration response curves to various second-generation triptans as percentage of maximal contraction to sumatriptan, which was derived from the same study as the respective compound (with E_{max} values of sumatriptan fixed at 100%). Secondly, we predicted the relative magnitude of contraction from the concentration response curves at free plasma concentrations (i.e. plasma protein unbound; free C_{max}) of the compounds for dosages currently on the market. Finally, we calculated the predicted contraction at its

respective free C_{max} and correlated the predicted contractions to the therapeutic gain (defined as reduction of pain from severe or moderate to mild or none, 2 hours postdosing) of the triptans versus placebo. To make a valid comparison between predicted contractions by triptans we have considered the fact that some triptans may have pharmacologically active metabolites. This is particularly the case with zolmitriptan since its main metabolite, *N*-desmethyl zolmitriptan is present in a 1:2 concentration ratio at C_{max}, (Seaber et al., 1996; Peck et al., 1998), which is twice as potent in mediating human isolated cerebral artery contraction (Nilsson et al., 1999a). With the assumption that zolmitriptan and its active metabolite have a similar efficacy, equilibrium and plasma protein binding the predicted contraction by zolmitriptan would be at twice the concentration of its free C_{max}. It should be mentioned that active metabolites of rizatriptan and eletriptan have been reported (Cooper et al., 1999; Goldberg et al., 2000) but their concentrations are rather low (<10% of original dosage) and therefore, they are unlikely to cause a significant additional pharmacological effect. As depicted in Figure 9.1, all triptans constricted the middle meningeal artery at free C_{max} to a similar extent (between 50-75%), except for zolmitriptan (25-40%). To correlate predicted contraction in middle meningeal artery *in vitro* with clinical observations we used the well defined clinical endpoint of therapeutic gain over placebo at two hours postdosing (International Headache Society Committee on Clinical Trials in Migraine, 1991), since all triptans included have a T_{max} lower than two hours (Table 1.3). As can be seen in Figure 9.2, predicted contraction in middle meningeal artery correlated well with the therapeutic gain of the respective triptan, which strongly indicates that the therapeutic efficacy of the triptans is mediated by cranial vasoconstriction. Interestingly, this conclusion is strengthened by the low therapeutic gain of the two dosages of zolmitriptan (2.5 and 5 mg), which resulted in low predicted contractions of the middle meningeal artery.

As discussed in Chapter 3, it appears that the 5-HT_{1B} receptor is the predominant receptor mediating middle meningeal artery contraction and thus, the therapeutic effects by the triptans. Although most triptans display a high affinity for the 5-HT_{1D} as well as the 5-HT_{1F} receptor, several lines of pharmacological evidence confirm that the vasoconstrictor activity of the triptans is mediated by the 5-HT_{1B} receptor. First of all we showed that the non-selective 5-$HT_{1B/1D}$ receptor antagonist GR125743 and the selective 5-HT_{1B} receptor antagonist SB224289 blocked sumatriptan-induced contractions, whereas the selective 5-HT_{1D} receptor antagonist BRL15572 was without effect. Secondly, molecular techniques revealed dense 5-HT_{1B} receptor mRNA (Chapter 8) and protein

(Longmore et al., 1997; Longmore et al., 1998) present in middle meningeal artery whereas little or no 5-HT_{1D} receptor mRNA (Chapter 8) and protein (Longmore et al., 1997; Longmore et al., 1998) was detected. Thirdly, several triptans (i.e. alniditan, donitriptan and IS-159) display a low affinity for the 5-HT_{1F} receptor (see Table 1.2), but still constricted cranial blood vessels (Chapter 5 and Bouchelet et al., 2000). Fourthly, specific 5-HT_{1D} (PNU109291) and 5-HT_{1F} (LY334370) receptor agonists were devoid of vasoconstrictor activity *in vitro* (Bouchelet et al., 2000). Therefore, for the therapeutic efficacy of the triptans, 5-HT_{1D} and 5-HT_{1F} receptor activity seems not to be required. Nonetheless, several other mechanisms (such as those affecting the trigeminovascular system or cortical spreading depression), which do not require the 5-HT_{1B} receptor, have also been implicated in migraine relief. As will be discussed below, the 5-HT_{1F} receptor agonist LY334370 may provide migraine relief, but needs further study. As for the cortical spreading inhibitor SB220453, no vasoconstrictive properties in human isolated middle meningeal artery were observed (Chapter 6). However, by inhibiting cortical spreading depression, SB220453 could prevent the subsequent cranial vasodilatation.

9.2 Human isolated peripheral blood vessels in relation to side-effect potential of antimigraine drugs

As previously discussed, sumatriptan has the propensity to cause side effects, such as chest pain, in a substantial percentage of migraine patients (Brown et al., 1991; Plosker and McTavish, 1994). Most probably the side effects are caused by constriction of peripheral blood vessels such as the coronary artery. Indeed, sumatriptan constricted this artery both *in vivo* and *in vitro*, which, together with the observed cardiac side effects, led to its contraindication in migraine patients with coronary artery diseases. Apart from the success of sumatriptan, these cardiovascular effects prompted several pharmaceutical companies to develop safer triptans. To predict whether these compounds will constrict coronary arteries *in vivo*, these compounds can be studied in the human isolated coronary artery. In parallel with measuring contraction in middle meningeal artery we measured contraction to sumatriptan, eletriptan and donitriptan in human isolated coronary artery. Overall, these compounds constricted this artery with a potency that is lower than in middle meningeal artery, confirming that these compounds are cranioselective. Using the concentration response curves we predicted that these antimigraine drugs will only cause a small constriction of the coronary artery *in vivo* at clinical free plasma concentrations, as

is the case for other triptans investigated in this preparation (MaassenVanDenBrink et al., 1998). However, these results, together with the chest-related adverse events observed in clinical trials, point out that all triptans still remain contraindicated in patients with coronary artery diseases. As described in Part III, we also investigated via which receptors the triptans induced constriction in human isolated coronary artery. The most probable candidate is the 5-HT_{1B} receptor, which (in contrast to the 5-HT_{1D} receptor) is expressed in the smooth muscle and endothelium (Nilsson et al., 1999b), although far less abundant than in the middle meningeal artery. The low 5-HT_{1B} receptor expression is most probably the reason that all triptans have a similar lower intrinsic efficacy as compared to 5-HT, which induces an additional vasoconstriction via the ample expressed 5-HT_{2A} receptor (Connor et al., 1989; Bax et al., 1993; Kaumann et al., 1994). Interestingly, donitriptan, which has a moderate affinity for the 5-HT_{2A} receptor (John et al., 1999), displays a higher intrinsic efficacy than sumatriptan. This intrinsic activity was reduced to the sumatriptan level after incubation with the 5-HT_2 receptor antagonist ketanserin (see Chapter 5). Our data confirmed the role for the 5-HT_{1B} receptor in sumatriptan-induced coronary artery constriction since this response was blocked by the non-selective 5-$HT_{1B/1D}$ receptor antagonist GR125743 and partly by the 5-HT_{1B} receptor antagonist SB224289, while the 5-HT_{1D} receptor antagonist BRL15572 was without effect. However, our results suggest that apart from the 5-HT_{1B} receptor, other mechanisms may be involved in triptan-induced coronary artery contraction as was observed for eletriptan (Chapter 7) and sumatriptan (Chapter 8).

Besides the isolated coronary artery we also used the human isolated saphenous vein as a model for peripheral blood vessel constriction. In general, the investigated triptans revealed a potency profile similar to the results found in coronary artery, whereas the efficacy profile showed a closer resemblance to the middle meningeal artery.

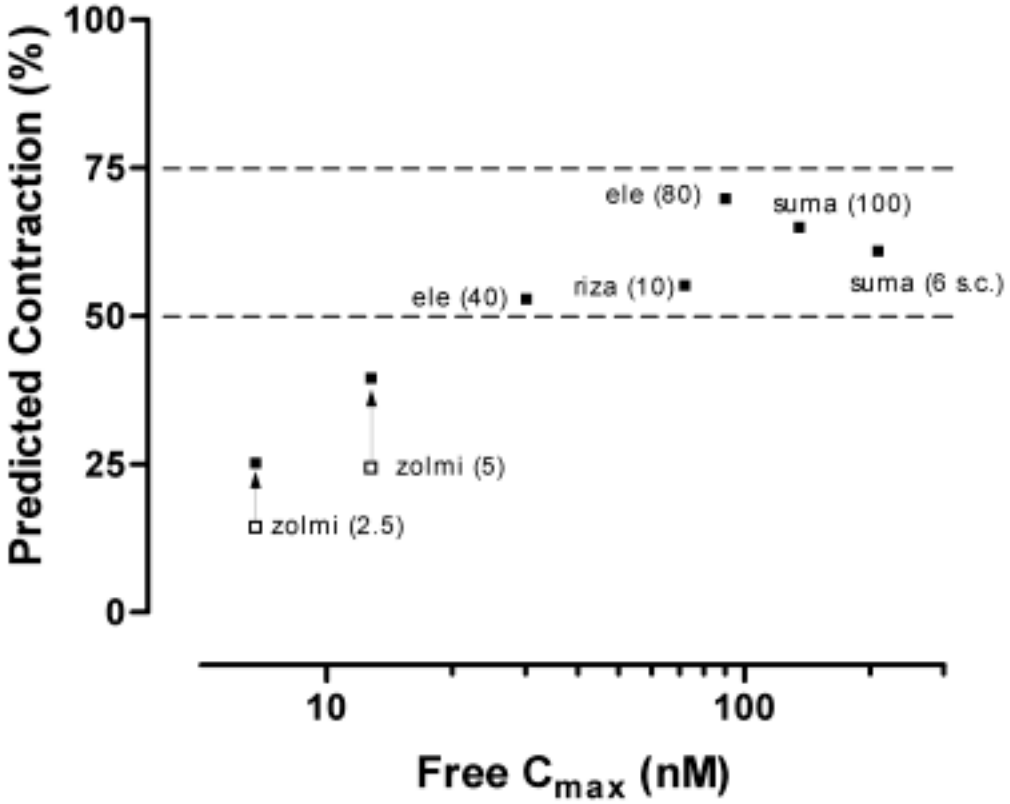

Figure 9.1 Predicted contraction in middle meningeal artery of various triptans dosages at their respective free plasma concentrations in human. Predicted contractions were calculated from concentration response curves in middle meningeal artery (Longmore et al., 1998; Razzaque et al., 1999; Maassen VanDenBrink et al., 2000b), for calculation see Chapter 9.1. Free plasma concentrations (free C_{max}) were obtained from MaassenVanDenBrink et al., 1998; MaassenVanDenBrink et al., 2000a. Data points represent individual triptans with oral dosage in mg between brackets. s.c.: subcutaneous. In case of zolmitriptan the predicted contraction is given with (■) and without (□) participation of the active metabolite. Dotted lines represent the predicted contraction window.

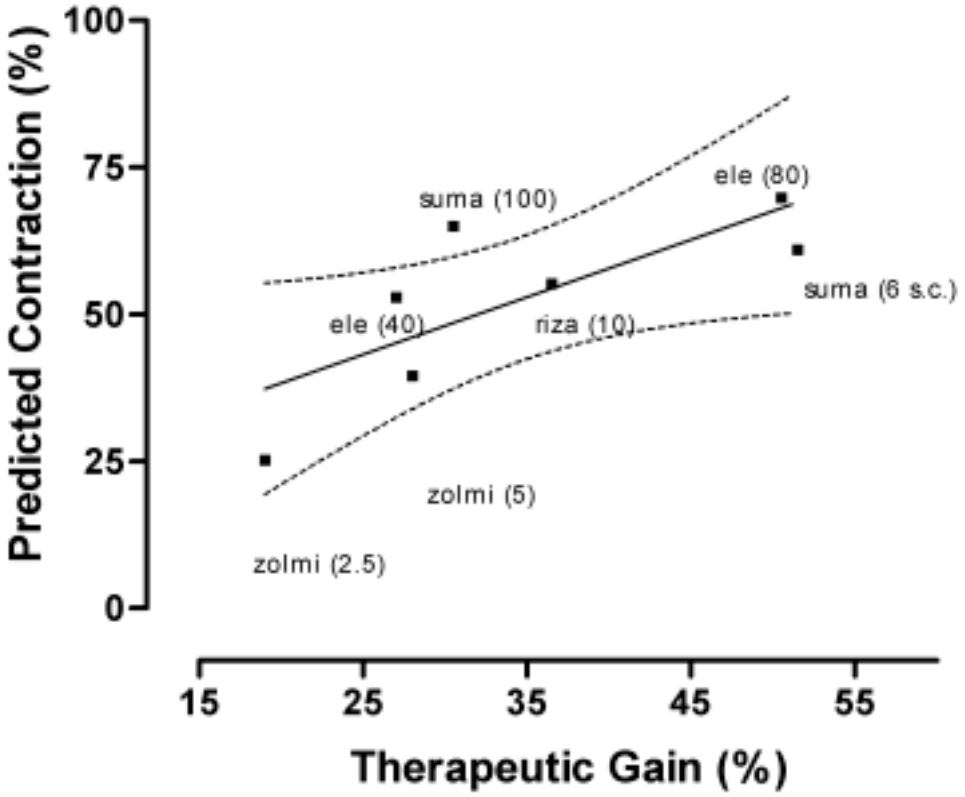

Figure 9.2 Correlation analysis of predicted contraction in middle meningeal artery of various triptans dosages and their respective therapeutic gain. Predicted contractions were calculated from concentration response curves in middle meningeal artery (Longmore et al., 1998; Razzaque et al., 1999; MaassenVanDenBrink et al., 2000b), for calculation see Chapter 9.1. Therapeutic gain (defined as reduction of pain from severe or moderate to mild or none 2 hours postdosing) of the triptans versus placebo, obtained from Deleu and Hanssens, 2000; Millson et al., 2000. Data points represent individual triptans with oral dosage in mg between brackets. s.c.: subcutaneous. Pearson's correlation analysis revealed a significant correlation, p=0.04 with r=0.77).

9.3 Limitations of *in vitro* blood vessel models

Experiments described in this thesis were performed in organ baths, where segments of the blood vessels were suspended for isometric tension measurements under conditions where the *in vivo* situation is most closely mimicked. These models *per se* are straightforward methods to predict antimigraine efficacy and side-effect potential as well as to resolve the mechanisms involved in contraction mediated by these drugs. Obviously, extrapolation of the results obtained from these *in vitro* studies to the clinical situation introduces uncertainties and limitations. First of all the material itself does not necessarily reflect the

in vivo situation in man due to the lack of systemic hemodynamic responses, such as blood pressure changes, in isolated preparations. Secondly, in the organ bath set-up, the drugs reach both intraluminal and extraluminal cells whereas *in vivo*, only the intraluminal side of the blood vessel is directly exposed. One possible way to avoid extraluminal exposure is to use perfused blood vessel segments. Another disadvantage could be the source of the material since storage and transportation can damage the tissue. To minimise this risk we opted for post-operative material, which was transported immediately to the laboratory in sterile, ice-cold, physiological buffers and processed as soon as possible. Since post-operative coronary artery material often contains atherosclerotic lesions we used the right epicardial coronary arteries from heart-beating organ donors, which were obtained within 24 hours after explantation. Before starting experiments we have examined whether the blood vessels are intact by challenging them first with substances such as KCl or $PGF_{2\alpha}$. Intact segments were then tested for the functional integrity of the endothelium by observing relaxations to endothelium-dependent compounds, such as substance P or bradykinin. Despite the limitations mentioned above, experiments on human isolated blood vessels provide unique possibilities for more detailed and in depth investigations than would otherwise be possible in patients. One such advantage is the possibility to investigate a number of segments from the same preparation in parallel.

9.4 Interpretation of concentration response curves

With our experimental set-up we can measure contractions to current and prospective antimigraine drugs in blood vessel segments important for therapeutic activity and side-effect potential. Since sumatriptan was the first triptan with 5-HT_{1B} receptor agonist activity, the newly developed triptans or other possible antimigraine compounds were compared against this 'golden standard'. As described in Chapter 3, concentration response curves can be constructed after applying cumulative concentrations of the drugs in question. If possible, by applying different drugs to different segments from the same preparation pharmacologically important parameters such as efficacy and potency of a drug can be compared in a paired manner. The primary goal in these types of experiments is to predict what these drugs will do in migraine patients and not per se via which mechanisms (i.e. via which receptors) these drugs act. Hence, the definition of potency is: that concentration of the drug eliciting half-maximal overall effect, and not necessarily the potency of a compound at a certain receptor (see Chapter 3). In this case we calculated

cranioselectivity ratios by taking the ratio of potency in middle meningeal artery and coronary artery. Interestingly, eletriptan was found to be slightly more cranioselective than sumatriptan since it had a lower potency in the coronary artery than sumatriptan, while it was equally potent in middle meningeal artery. A few concerns, regarding these results in coronary artery, were raised when these results were published (see Chapters 4 and 7). These concerns were mainly based on the misconception that the potencies in question were compared on the basis of receptor classification and not for the overall effects *per se*. It was questioned whether the concentration response curves of sumatriptan and eletriptan should be compared since the curves to eletriptan did not reach a plateau and the curves themselves were indistinguishable (see Chapter 4.6, letter to Neurology). Since our experiments were performed in parallel we believe that comparisons between two drugs can be made, in our case by constructing concentration response curves with an E_{max} set to the maximum-induced contraction by the drug at the highest concentration of agonist used (i.e. 'fixed' E_{max}). Furthermore we recalculated the potency and efficacy using the concentration response curves to eletriptan and sumatriptan. By fitting concentration response curves to both drugs into a different model, without setting the E_{max} to the level of maximum-induced contraction of the agonist (i.e. 'free' E_{max}), we did not observe any differences in efficacy between eletriptan and sumatriptan. Still, even if the efficacy of both compounds would differ, a comparison of potencies is still valid since this parameter is discernible from efficacy. Another issue that can be raised is consistency of the results throughout the different studies. As depicted in Table 9.1, only marginal differences in potency of sumatriptan were observed within the middle meningeal or the coronary artery in the studies included in this thesis. However, these small differences can lead to a large variation in cranioselectivity ratios. Therefore, to compare the cranioselectivity ratio of newer triptans to sumatriptan (Chapters 5 and 7) a head-to-head parallel comparison is required to justify the conclusions. In any case, conclusions based on cranioselectivity ratios alone should be made with caution since these drugs still contract the coronary artery at therapeutic concentrations.

Table 9.1 Sumatriptan potency (pEC_{50}) in middle meningeal and coronary artery and respective cranioselectivity ratio obtained from studies included in this thesis.

Middle meningeal artery pEC_{50}	Coronary artery pEC_{50}	Cranioselectivity ratio (95%CI)	Chapter
7.26±011	6.06±0.21	16 (5-47)	4
7.41±0.08	5.71±0.16	50 (22-113)	5
6.9±0.2	6.0±0.2	8 (2-28)	6
6.91±0.12	6.24±0.14	5 (2-13)	7
6.7±0.2	5.7±0.1	10 (4-28)	8

An additional way of using concentration response curves is to predict the contraction of various blood vessels and relate them to the clinical situation. By interpolating the concentration response curves the level of contraction *in vitro* at clinical free plasma concentrations can be calculated and correlated to therapeutic efficacy or potential side-effects *in vivo*. Certainly, one should be cautious with extrapolating *in vitro* findings to the clinical situation, but the results obtained can be of help in the evaluation of antimigraine drugs. As described above, we predicted the contraction at therapeutic concentrations for the second-generation triptans in comparison with that of sumatriptan using the isolated middle meningeal artery. Overall the results indicated that, although there are differences in potency and efficacy, all compounds, except zolmitriptan, yielded similar predicted contractions at therapeutic concentrations. Similarly, we predicted side-effect potential *in vivo* using *in vitro* coronary artery contraction. Overall it becomes clear that the triptans investigated in this thesis have a small propensity to constrict the coronary artery at therapeutic concentrations as was observed with other triptans previously described. All triptans therefore, remain contraindicated in patients with coronary artery diseases.

9.5 Implication for future research

The results described in this thesis do raise questions that can be of interest for future research. Our data, and those of others, show that vasoconstriction to triptans is predominantly mediated via the 5-HT_{1B} receptor. Since this receptor is most abundant in the cranial vasculature these compounds are cranioselective, but there is a catch. Although not as densely present in the coronary artery, the 5-HT_{1B} receptor is active at therapeutic concentrations and may cause unwanted coronary artery related side effects. What is clear from our results is that a higher affinity for the 5-HT_{1B} receptor does not necessarily mean that the compound is more cranioselective, as was found with donitriptan (Chapter 5). One notable finding was that eletriptan, which has a higher affinity for the 5-HT_{1B} receptor as compared to sumatriptan, had a significant lower potency in coronary artery (Chapters 4 and 7). Furthermore, although the contraction to eletriptan was potently blocked in middle meningeal artery by the 5-$HT_{1B/1D}$ receptor antagonist GR125743, this antagonist failed to block eletriptan-induced contractions at the expected concentration in coronary artery (Chapter 7). In contrast, this antagonist potently blocked sumatriptan-induced contractions in both middle meningeal and coronary arteries. Hence, further investigations into this yet unknown vasoconstrictor mechanism could clarify this controversy. Since all triptans to date have a high affinity for the 5-HT_{1B} receptor it is unlikely that such compounds will not affect the cardiovasculature and remain contraindicated in patients with coronary artery syndromes. Thus, it would be of value to search for cranioselective compounds that do not affect peripheral blood vessels. On the other hand, it may be argued whether cranial vasoconstriction by triptans *per se* is required for therapeutic efficacy since all triptans are also known to affect peripheral and central trigeminal neuronal pathways. As described in Chapter 2, activation of trigeminal afferents leads to vasodilatation and agonist activity at 5-HT_{1B}, 5-HT_{1D} and/or 5-HT_{1F} receptors can lead to inhibition of this process at concentrations that lack vasoconstriction. Furthermore, brain-penetrant triptans such as zolmitriptan, eletriptan and naratriptan are known to inhibit action potentials generated in the trigeminus nucleus caudalis, a possible site of action in migraine. As noted before, sumatriptan does not cross the blood brain barrier due to its low lipophilicity and poorly penetrates the brain, but further studies have to be fulfilled to see whether this blood brain barrier is disrupted during a migraine attack. In order to challenge the different hypotheses it could be of interest to appraise the

antimigraine activity of a non-brain-penetrant selective 5-HT_{1B} receptor agonist, but such a compound is not yet available.

9.6 Beyond the triptans

One of the key goals in designing antimigraine drugs is the success rate of the treatment. Interestingly, the highest antimigraine efficacy to date is observed with subcutaneous injections of sumatriptan (Dahlöf and Saiers, 1998; Tfelt-Hansen *et al*., 2000). Oral sumatriptan revealed a lower efficacy and the second-generation triptans only marginally improved this level, despite improved pharmacokinetics (Goadsby, 1998; Saxena and Tfelt-Hansen, 2000). To improve on efficacy using such serotonin agonists, the way forward is probably not by designing drugs with a selective affinity for a specific 5-HT_1 receptor subtype. The specific 5-HT_{1D} receptor agonist PNU142633 was ineffective in migraine, although better results might be expected if such compounds can penetrate the brain (McCall, 1999) or have a full efficacy (May & Goadsby, 2001). The selective 5-HT_{1F} receptor agonist LY334370 (a modified triptan, withdrawn because of liver toxicity) did show antimigraine efficacy but with no improvement over the second-generation triptans (Roon, 2000). No cardiovascular adverse effects were noted at clinical effective doses, but larger trials have to be performed to conclusively exclude cardiovascular symptoms. Interestingly, significant central nervous system adverse events were observed (Roon, 2000). Further research has to be performed since these agonists also have moderate affinity for the 5-HT_{1B} receptor, furthermore, no data are known regarding active metabolites, which are known to exist with other triptans (Seaber et al., 1996; Goldstein et al., 1997) and may have vasoconstrictor properties.

Overall, to achieve safer antimigraine efficacy, newly developed drugs should be designed to act via several mechanisms including the trigemino-vascular system and cranial artery constriction without effecting the peripheral vasculature. Further research should be employed, like clarifying if there could be a ‘cross-talk’ between the different 5-HT_1 receptor subtypes and its effectors (Berg et al., 1998), which can be important in further understanding of migraine pathophysiology and possible improvement in antimigraine efficacy.

Another important issue in designing future antimigraine drugs is consistency in treatment and prevention. One of the drawbacks of the triptans is the high recurrence of the headache after initial successful treatment. This recurrence can be explained by the

fact that these antimigraine drugs are agonists. When administering agonists one can expect desensitisation at the receptor level which could cause the recurrence of headache, moreover, desensitisation may lead to drug abuse (Bruynzeel et al., 1985; Freedman and Lefkowitz, 1996). A simple way to avoid this phenomenon is by designing antagonists that counter vasodilatation and thus, avoid a migraine headache. As described in Chapter 2, various prophylactic antimigraine drugs have some 5-HT_{2B} or 5-HT_7 receptor antagonistic properties. Consequently, it may be worth assessing whether selective 5-HT_{2B} or 5-HT_7 receptor antagonists are effective in the prevention of migraine. In this light, the human isolated middle meningeal artery can be used as a suitable model by designing experiments where these antagonists can block vasodilatory-induced responses. Another pathway to counter vasodilatation is by blocking the effects or release of the vasodilator neuropeptide CGRP, which is elevated during a migraine attack (see Chapter 2). Currently several new CGRP antagonists are under clinical investigation such as BIBN4096BS (Doods et al., 2000), Compound 1 (Edvinsson et al., 2001) and SB273779 (Aiyar et al., 2001). Interestingly, several new avenues are currently being explored that involve mediation of CGRP release from trigeminal neurones, which, upon stimulation, induce a vasodilatation of meningeal arteries and neurogenic inflammation (Williamson et al., 1997; Hargreaves and Shepheard, 1999). As was postulated for sumatriptan, which inhibits CGRP release via prejunctional 5-$HT_{1B/1D}$ receptors (Humphrey and Feniuk, 1991; Goadsby and Edvinsson, 1993), other receptors are involved in mediating CGRP release. One such example is the adenosine A_1 receptor, which, after activation with the highly selective A_1 receptor agonist GR79236, inhibits trigeminal neurones and their release of CGRP both *in vitro* and *in vivo* (Humphrey et al., 2001). Early indications from a human experimental study suggest that GR79236 may have antimigraine action by inhibiting nociceptive trigeminal neurones (Giffin et al., 2001). Another class of receptors that are also implicated in mediating CGRP release are the prostanoid receptors. Exposure to the prostaglandines PGE_2, carbaprostacyclin ($cPGI_2$) and PGD_2 results in increased CGRP release from trigeminal neurones of the rat mediated via the E-, I- and D- prostanoid receptors, respectively (Jenkins et al., 2001). These observations suggest that certain prostanoid receptor antagonists could be potential antimigraine drugs. Overall, inhibition of CGRP release is an interesting and challenging approach to unravel certain aspects of the pathophysiology of migraine. For this purpose, the human isolated middle meningeal artery can be used as a very suitable preparation since it is richly innervated with CGRP containing fibres. As discussed in Chapter 2, an

additional level of interest in the acute treatment of migraine is the trigeminus nucleus caudalis, which transduces intracranial nociceptive information to higher centres in the brain. Apart from the binding sites that recognise 5-HT_1 receptor ligands various other receptors, which also modulate *c-fos* expression in the trigeminus nucleus caudalis, have been identified (Mitsikostas and Sanchez del Rio, 2001). Particularly, the non-NMDA ionotropic glutamate GluR5 (an AMPA/kainate subtype) receptor appears to be an interesting new target. The GluR5 receptor antagonist LY293558 potently blocks electrically-induced *c-fos* expression in the rat trigeminus nucleus caudalis. A high intravenous dose (1.2 mg/kg) of LY292558 is effective in migraine patients (Ramadan et al., 2001) with no vasoconstrictor properties in rabbit saphenous vein (Johnson et al., 2001). It can be argued whether the role for this GluR5 receptor is specific for migraine since it is involved in general pain processing (Zhou et al., 1996). LY293558 is known as an analgesic by reducing spinal neurone sensitisation and is also effective for post-operative and clinical pain (Sang et al., 1998; Gilron et al., 2000).

Inhibition of cortical spreading depression is yet another way to target migraine, as described earlier, SB220453 is such a compound currently under clinical investigation. SB220453, after intravenous administration of glyceryltrinitrate, however, did not delay a migraine attack in migraine patients implicating no acute antimigraine activity (Tvedskov et al., 2001). Another way in developing antimigraine drugs is linking genetic factors to migraine. Several investigators have shown mutations on the α_{1A} subunit of a brain specific P/Q type calcium channel in patients with hemiplegic migraine (Ophoff et al., 1998; Terwindt et al., 1998). The full understanding of this calcium channelopathy is not know but at least one compound has been investigated with promising results. The P/Q type calcium channel blocker α-eudesmol potently inhibits presynaptic neuropeptide release from perivascular trigeminal terminals and attenuated neurogenic vasodilatation in rat (Asakura et al., 2000). Whether this compound will be effective in migraine remains to be elucidated. It should be noted though that in a substantial group of migraine patients no genetic mutations have been found. In any case, various different pathways are currently being explored, all with a different angle but with the same goal; the development of safer and better antimigraine drugs.

Chapter 10

Summary – Samenvatting

10.1 Summary

Chapter 1 discusses the clinical symptoms of migraine as well as the current antimigraine drugs available. Although the pathophysiology of migraine has not been fully elucidated, this neurological disorder can be divided in distinct phases. The initial trigger phase most probably involves a brainstem 'generator' which upon activation causes a reduction of cerebral blood flow. If this reduction of cerebral blood flow falls below a critical value a migraine aura can be experienced. In response to this blood flow reduction, vasodilatation of large cranial arteries occurs, inducing the headache phase.

This vasodilatation is one of the key targets in acute migraine treatment, ergot alkaloids were the first non-selective vasoconstrictors effective in migraine. With the aim of craniovascular selectivity, sumatriptan and its followers, the second-generation triptans, were designed to exclusively counter cranial vasodilatation. These, co-called 5-$HT_{1B/1D}$ receptor agonists are all effective in migraine with similar pharmacotherapeutic but different pharmacokinetics properties. Despite their success, all triptans to date have the propensity to contract the human coronary artery and are therefore, contraindicated in patients with coronary artery disease.

In **Chapter 2** the mechanisms of action of current and future antimigraine drugs are reviewed. Originally designed to act on postsynaptic vascular 5-HT_1 receptors, the triptans demonstrated high affinities for the 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} receptors. Various studies did show that the triptans constrict cranial blood vessels via the 5-HT_{1B} receptor and not via the 5-HT_{1D} or 5-HT_{1F} receptor. Other mechanisms of action of the triptans have been postulated, which include the trigeminovascular system. Activated trigeminal fibres release vasodilatory neuropeptides and the triptans are believed to block the subsequent plasma protein extravasation via the presynaptic 5-HT_{1D} receptor. However, plasma protein extravasation has only been demonstrated in animal models and selective blockers failed to demonstrate antimigraine activity in patients. Another suggested pathway in the action of the triptans lays within the central nervous system. Brain penetrant triptans inhibit action potentials from the trigeminus nucleus caudalis and thus, block the nociceptive information towards higher brain centres. The exact nature of

the receptor involved in this pathway needs to be further investigated, but a role for the 5-HT_{1D} or 5-HT_{1F} receptor is suggested. Interestingly, but still under debate, agonists with a selectivity towards the 5-HT_{1F} receptor, devoid of vasoconstrictive properties, are effective in migraine and active in the trigeminovascular models. Specific 5-HT_{1D} receptor agonists are inactive in migraine.

Preventing vasodilatation of cranial blood vessels can be a target in antimigraine therapy. Various receptors mediate relaxation in blood vessels and are implicated in migraine, the 5-HT_{2B} and 5-HT_7 receptors are such targets and antagonists against these receptors can be effective in migraine therapy. The role of the neuropeptide CGRP is also discussed, released during a migraine attack it dilates cranial arteries and relief of migraine headache by sumatriptan is accompanied by normalisation of CGRP levels. To prevent CGRP release or block the CGRP receptors may prevent or counter a migraine headache. Currently, several CGRP antagonists are under clinical investigation to elucidate antimigraine efficacy.

Yet another way to target migraine is by early interception of the cascade that leads to a migraine attack. Cortical spreading depression can lead to cranial vasodilatation and thus, by preventing this event a migraine attack can be blocked. The potent cortical spreading depression inhibitor SB220453 is under clinical investigation and the results can further unravel the importance of this phenomenon in migraine.

Chapter 3 provides an overview of the human blood vessels used as *in vitro* models in migraine. By using organ bath techniques and measuring mechanical responses to antimigraine drugs we can determine certain pharmacological parameters such as potency (pEC_{50}) and efficacy (E_{max}). The potency and efficacy of different antimigraine drugs (with particular interest in the triptans) can be compared or can reveal an insight in the receptors involved in the measured responses by using selective antagonists. Various blood vessels used as models for the therapeutic activity or side-effect potential of triptans are described. The human isolated middle meningeal artery is considered the most important *in vitro* model depicting the therapeutic site of action. Measuring potency and efficacy of current and newly developed triptans clearly indicates that the 5-HT_{1B} receptor mediates the contraction and thus, the therapeutic activity. Other cranial arteries such as the cerebral and temporal arteries reveal similar results. As further discussed, the triptans also have the propensity to constrict the coronary artery causing unwanted coronary related side effects. In order to predict whether antimigraine drugs cause coronary adverse

events, peripheral blood vessels, such as the isolated coronary artery and saphenous vein, can be studied. The results obtained clearly indicate that the triptans constrict the isolated coronary artery, albeit with a profound lower potency and efficacy as compared to cranial blood vessels. A well as was the case for contraction in cranial blood vessels, the triptans constrict the peripheral blood vessels most probably via the 5-HT_{1B} receptor. The differences between the therapeutic and side effect models can be explained by the fact the 5-HT_{1B} receptor is more densely expressed in the cranial vasculature as opposed to the peripheral blood vessels, supporting the craniovascular selectivity of the triptans. Despite this cranioselectivity, all triptans to date will remain contraindicated in patients with coronary artery disease.

The **aims** of the thesis therefore, were (*i*) to determine the effects of several current and prospective antimigraine drugs on human isolated blood vessels of relevance to therapeutic activity and side-effect potential, and (*ii*) to investigate and characterise which receptors/mechanisms are involved in vascular responses elicited by antimigraine drugs.

In **Chapter 4**, we investigated contraction to eletriptan, sumatriptan and 5-HT in blood vessel models predictive of clinical efficacy (human middle meningeal artery) and side-effect potential (human coronary artery and saphenous vein). By constructing cumulative concentration response curves, using organ bath techniques, we derived EC_{50} (potency) and E_{max} (efficacy) values. Using these parameters we predicted the contraction of eletriptan and sumatriptan at clinical plasma concentrations. The potency of eletriptan and sumatriptan was higher in middle meningeal artery than in coronary artery and saphenous vein, indicating cranioselectivity. In coronary artery the potency of eletriptan was lower as compared to sumatriptan, whereas the compounds were equipotent in middle meningeal artery and saphenous vein. The efficacy of eletriptan and sumatriptan was similar within tissues. The predicted contractions by sumatriptan (100 mg p.o.) and eletriptan (40 mg and 80 mg p.o.) at free plasma concentrations were similar in middle meningeal artery, but in the side-effect potential models they appear to be lower for 40 mg eletriptan than for sumatriptan. In conclusion, both eletriptan and sumatriptan contract middle meningeal artery at therapeutic concentrations more than coronary artery. While both drugs have a limited propensity to cause adverse coronary side effects in patients with healthy coronary arteries, they must remain contraindicated in patients with coronary artery disease.

In **Chapter 5**, we studied the contractile effects of donitriptan and sumatriptan on the human isolated blood vessels of relevance to therapeutic efficacy in migraine (middle meningeal artery) and coronary adverse events (coronary artery). Furthermore, using the concentration response curves in the middle meningeal artery, we have predicted the plasma concentration needed for therapeutic effect of donitriptan. Both triptans contracted the middle meningeal artery with similar efficacy, but the potency of donitriptan was significantly higher than that of sumatriptan. In the coronary artery, the contraction to donitriptan was biphasic with a significantly higher efficacy than sumatriptan, yielding two distinct pEC_{50} values. Incubation with the 5-HT_2 receptor antagonist ketanserin (10 μM) eliminated the low-affinity, high-efficacy component of the concentration response curve of donitriptan. Ketanserin was without effect on the sumatriptan-induced contraction. Both triptans had similar selectivity for the middle meningeal over coronary artery. The predicted therapeutic concentration for donitriptan amounted to ~4.5 nM, which is likely to induce only a small contraction of the coronary artery. In conclusion, the results suggest that donitriptan would be effective in aborting migraine attacks with similar coronary side-effect profile as sumatriptan.

The aim of the investigation in **Chapter 6** was to study the effects of the benzopyran derivative SB220453 in human isolated blood vessels important in migraine (middle meningeal, coronary artery and saphenous vein) and atrial and ventricular cardiac trabeculae. SB220453, inhibits the cortical spreading depression and is undergoing clinical evaluation in migraine, exhibits a high affinity for a selective, yet unknown, binding site in the human brain. Previous studies revealed that SB220453 inhibits nitric oxide release and cerebral vasodilatation induced by trigeminal nerve stimulation. While the 5-$HT_{1B/1D}$ receptor agonist sumatriptan induced a marked contraction in all blood vessels studied, SB220453 was devoid of any effect. In atrial and ventricular cardiac trabeculae, neither sumatriptan nor SB220453 displayed any inotropic effect. Since SB220453 did not contract the middle meningeal artery, we conclude that should SB220453 prove effective in migraine, its therapeutic efficacy, unlike that of sumatriptan, will be independent of cranial vasoconstriction. Because SB220453 also did not contract the coronary artery, saphenous vein or cardiac trabeculae, the compound is unlikely to display adverse cardiac side effects.

In **Chapter 7** we further characterised which receptor is involved in eletriptan-induced contraction in comparison to that of sumatriptan. We compared the effects of eletriptan and sumatriptan on the human isolated middle meningeal and coronary arteries and saphenous vein, used as models for therapeutic efficacy and potential side effects, and have investigated the role of 5-$HT_{1B/1D}$ receptors in contractions induced by these triptans. Concentration-response curves to eletriptan and sumatriptan were constructed in the absence or presence of a selective 5-$HT_{1B/1D}$ receptor antagonist, GR125743. All three blood vessels constricted in response to eletriptan and sumatriptan, but the middle meningeal artery relaxed following the highest concentration (100 μM) of eletriptan. In the middle meningeal artery, GR125743 antagonised the contractions induced by both eletriptan and sumatriptan to a similar degree (pA_2: 8.81±0.17 and 8.64±0.21, respectively). In the human coronary artery and saphenous vein, sumatriptan-induced contractions were also potently antagonised by GR125743 (pA_2: 8.18±0.27 and 8.34±0.12, respectively). The eletriptan-induced contractions of the human saphenous vein were antagonised less effectively by GR125743 (pK_B: 7.73±0.18), and those of the human coronary artery remained unaffected by GR125743 up to a concentration of 100 nM. In conclusion, these results suggest that based on the differences in pEC_{50} values, the cranioselectivity of eletriptan (63-fold) is higher than that of sumatriptan (5-fold) in coronary artery. Furthermore, the contractile effects of sumatriptan and eletriptan (lower concentrations) in the three blood vessels are mediated via the 5-HT_{1B} receptor, but additional mechanisms seem to be involved in coronary artery and saphenous vein contractions and middle meningeal artery relaxation following high concentrations of eletriptan.

Chapter 8 set out to investigate whether 5-HT_{1B} and/or 5-HT_{1D} receptors mediate contractions of the human isolated middle meningeal and temporal arteries (models for antimigraine efficacy) and coronary artery and saphenous vein (models for side-effect potential). Concentration-response curves were made with sumatriptan in blood vessels in the absence or presence of selective antagonists at 5-HT_{1B} (SB224289) and 5-HT_{1D} (BRL15572) receptors. SB224289 antagonised sumatriptan-induced contractions in all blood vessels, although the antagonism profile was different amongst these blood vessels. In the temporal artery, SB224289 abolished contraction to sumatriptan, whereas in the

middle meningeal artery and saphenous vein, sumatriptan-induced contractions were blocked in an insurmountable fashion. Moreover, SB224289 acted as a weak surmountable antagonist in coronary artery (pK_B: 6.4±0.2). In contrast, BRL15572 had little or no effect on sumatriptan-induced contractions in the four blood vessels investigated. *In situ* hybridisation revealed the expression of 5-HT_{1B} receptor mRNA in the smooth muscle as well as endothelial cells of the blood vessels, whereas the mRNA for the 5-HT_{1D} receptor was only very weakly expressed. These results show that the 5-HT_{1B} receptor is primarily involved in sumatriptan-induced contractions of human cranial arteries, but indicate that possible other mechanisms may as well be part of this contraction in peripheral blood vessels.

10.2 Samenvatting

Hoofdstuk 1 beschrijft de klinische symptomen van migraine, alsmede de huidige antimigraine middelen op de markt. De pathofysiologie van migraine is nog niet geheel bekend, maar deze neurologische ziekte kan worden opgedeeld in verschillende fases. De initiële fase bestaat uit een stimulans waarbij hoogst waarschijnlijk een hersenstam generator betrokken is, die na activatie een verlaging geeft van cerebrale bloedstromen. Als deze cerebrale bloedstroom lager wordt dan een bepaalde kritieke waarde kan er een migraine aura optreden. Als reactie op de verlaging van de bloedstroom gaan de grote craniale bloedvaten verwijden, die vervolgens de migraine hoofdpijn fase bewerkstelligen.

Deze dilatatie is een van de belangrijkste aanknooppunten in de acute antimigraine behandeling, ergot alkaloïden waren de eerste niet-selectieve vaatvernauwers effectief tegen migraine. Met als doel om een betere selectiviteit voor craniale bloedvaten te bewerkstelligen werd sumatriptan, en zijn opvolgers, de tweede generatie triptanen, ontwikkeld om deze craniale vasodilatatie tegen te gaan. Deze zogenaamde 5-$HT_{1B/1D}$ receptor agonisten zijn allen effectief tegen migraine met vergelijkbare farmacotherapeutische, maar verschillende farmacokinetische eigenschappen. Ondanks hun succes kunnen huidige triptanen ook de humane coronair arterie contraheren, waardoor zij gecontraïndiceerd zijn in patiënten met coronaire arterie ziekten.

In **Hoofdstuk 2** worden de werkingsmechanismen van huidige en toekomstige antimigraine middelen beschreven. Oorspronkelijk ontwikkeld om op postsynaptische vasculaire 5-HT_1 receptoren aan te pakken, is gebleken dat de triptanen voornamelijk een

hoge affiniteit voor de 5-HT_{1B}, 5-HT_{1D} en 5-HT_{1F} receptoren hebben. Uit verschillende studies is gebleken dat de triptanen craniale bloedvaten contraheren via de 5-HT_{1B} receptor, en niet via de 5-HT_{1D} of 5-HT_{1F} receptoren. Er zijn suggesties voor andere werkingsmechanismen van de triptanen waarbij het trigemino-vasculaire systeem betrokken is. Geactiveerde trigeminale zenuwvezels geven vasodilatoire neuropeptiden af en men denkt dat de triptanen de hierop volgende plasma eiwit extravasatie blokkeert via de presynaptische 5-HT_{1D} receptoren. Echter, plasma eiwit extravasatie is alleen aangetoond in proefdier modellen en selectieve blokkers zijn ineffectief gebleken tegen een migraine aanval. Een derde mogelijk werkingsmechanisme van de triptanen ligt binnenin het centrale zenuwstelsel. Triptanen die de bloed-hersen barrière kunnen passeren blokkeren actiepotentialen, die ontstaan in de trigeminus nucleus caudalis, waardoor ze de pijn transmissie naar hogere hersencentra remmen. Het is nog niet precies bekend welke receptoren dit laatste mechanisme mediëren, maar een rol voor de 5-HT_{1F} of 5-HT_{1D} receptor wordt gesuggereerd. Opvallend, maar nog steeds betwist, zijn de agonisten met affiniteit voor de 5-HT_{1F} receptor die, zonder vasoconstrictieve eigenschappen, effectief zijn in de trigeminovasculaire modellen en ook effectief in migraine. Specifieke 5-HT_{1D} receptor agonisten zijn ineffectief in migraine.

Het voorkomen van dilatatie van craniale bloedvaten kan een streefpunt zijn in antimigraine therapie. Verscheidene receptoren mediëren relaxatie van bloedvaten en zijn betrokken in migraine, zoals de 5-HT_{2B} en 5-HT_{7} receptoren, en antagonisten tegen deze receptoren kunnen effectief zijn tegen migraine. De rol van de neuropeptide CGRP wordt ook bediscussieerd, welke wordt afgegeven tijdens een migraine aanval, waarna het dilatatie van craniale bloedvaten veroorzaakt. Verlichting van de hoofdpijn door sumatriptan gaat samen met het normaliseren van de CGRP waarden. Het voorkomen van CGRP afgifte of het blokkeren van de CGRP receptoren zou een migraine aanval kunnen tegengaan of stoppen. Momenteel worden er verschillende CGRP antagonisten getest in de kliniek om antimigraine activiteit op te helderen.

Nog een andere manier om een migraine aanval tegen te gaan is door een vroege onderbreking van de cascade, die leidt tot de uiteindelijke migraine aanval. Corticale spreidingsdepressie kan leiden tot craniale vasodilatatie en dus door het voorkomen van dit fenomeen kan een opkomende migraine aanval geblokkeerd worden. De potente corticale spreidingsdepressie blokker SB220453 wordt momenteel onderzocht in de kliniek en de resultaten kunnen de rol van dit fenomeen in migraine verduidelijken.

Hoofdstuk 3 geeft een overzicht van de humane bloedvaten, die gebruikt worden als *in vitro* modellen in migraine. Door middel van het gebruik van orgaanbad technieken en het meten van responsen van antimigraine medicijnen kunnen we verschillende farmacologische parameters bepalen, zoals potency (pEC_{50}) en efficacy (E_{max}). De potency en efficacy van verschillende antimigraine middelen (met name de triptanen) kan vergeleken worden of inzichten verschaffen in de receptoren, die betrokken zijn bij de gemeten responsen, bijvoorbeeld door middel van het gebruik van selectieve antagonisten. Verscheidene bloedvaten die als therapeutische- of als mogelijke bijwerkingsmodellen gebruikt kunnen worden, worden beschreven in dit hoofdstuk. De geïsoleerde humane arteria meningea media wordt beschouwd als het belangrijkste therapeutische *in vitro* model. De gemeten potency en efficacy van huidige en nieuw ontwikkelde triptanen geeft een duidelijke indicatie dat de 5-HT_{1B} receptor verantwoordelijk is voor de contractie en dus de therapeutische werking. Andere craniale bloedvaten, zoals de cerebrale en temporale arteriën, vertonen vergelijkbare resultaten. Verder wordt nog bediscussieerd dat de triptanen de neiging hebben om de coronair arterie te contraheren, wat ongewenste coronair gerelateerde bijwerkingen geeft. Om te voorspellen of antimigraine medicijnen coronaire bijwerkingen veroorzaken kunnen perifere bloedvaten, zoals de geïsoleerde coronair arterie of vena saphena magna, gebruikt worden. De resultaten laten duidelijk zien dat de triptanen de geïsoleerde coronair arterie contraheren, alhoewel met een onmiskenbaar lagere potency en efficacy in vergelijking tot craniale bloedvaten. Zoals het in sterke mate gold voor de craniale bloedvaten, veroorzaken de triptanen contractie van perifere bloedvaten via de 5-HT_{1B} receptoren. De verschillen tussen de therapeutische- en bijwerkingsmodellen kunnen verklaard worden door het feit dat de 5-HT_{1B} receptor veel sterker tot expressie wordt gebracht in de craniale vasculatuur ten opzichte van de perifere bloedvaten, wat de stelling van cranioselectiviteit van de triptanen ondersteunt. Ondanks deze cranioselectiviteit zijn alle huidige triptanen gecontraïndiceerd in patiënten met coronair arterie ziekten.

Het **doel** van dit proefschrift was daarom tweeledig. Ten eerste wilden wij de effecten van bestaande en nieuw ontwikkelde antimigraine middelen bepalen met behulp van verschillende *in vitro* modellen relevant voor therapeutische activiteit en mogelijke bijwerkingen. Ten tweede wilden we de receptoren/mechanismen karakteriseren van de door deze antimigraine middelen gemedieerde responsen.

In **Hoofdstuk 4** onderzochten we de contractie van eletriptan, sumatriptan en 5-HT in bloedvaten, die gebruikt worden voor het bepalen van therapeutische activiteit (humane arteria meningea media) of mogelijke bijwerkingen (humane coronair arterie en vena saphena magna). Door middel van het construeren van concentratie respons curven, met behulp van orgaanbadjes, hebben we EC_{50} (potency) en E_{max} (efficacy) waarden bepaald. Met deze parameters hebben we de verwachte contractie van eletriptan en sumatriptan bepaald van klinische plasma concentraties. De potency van eletriptan en sumatriptan was hoger in de arteria meningea media in vergelijking tot die in de coronair arterie en vena saphena, dat cranioselectiviteit impliceert. In de coronair arterie was de potency van eletriptan lager dan die van sumatriptan, terwijl de middelen een gelijke potency hadden in de arteria meningea media en vena saphena. De efficacy van eletriptan en sumatriptan was gelijk binnen alle bloedvaten. De voorspelde contracties van sumatriptan (100 mg oraal) en eletriptan (40 mg en 80 mg oraal) bij vrije plasma concentraties waren gelijk, maar in de bijwerkingsmodellen leek het erop dat de voorspelde contractie lager was voor 40 mg eletriptan dan die voor sumatriptan. Samenvattend, zowel eletriptan als sumatriptan contraheren de arteria meningea media bij therapeutische concentraties meer dan de coronair arterie. Alhoewel beide medicijnen weinig neiging hebben om coronaire bijwerkingen te veroorzaken, zullen ze gecontraïndiceerd blijven in patiënten met coronaire arterie ziekten.

In **Hoofdstuk 5** hebben we de contraherende effecten van donitriptan en sumatriptan bestudeerd in de geïsoleerde humane bloedvaten, die relevant zijn voor de therapeutische effectiviteit (arteria meningea media), en coronaire bijwerkingen (coronair arterie) in migraine. Verder hebben we getracht om, met behulp van de concentratie respons curven in de arteria meningea media, de plasma concentratie van donitriptan te berekenen, waarbij klinische activiteit te verwachten is. Beide triptanen contraheerden de arteria meningea media met een zelfde efficacy, maar de potency van donitriptan was significant hoger dan die van sumatriptan. In de coronair arterie bestond de contractie door donitriptan uit twee fasen, elk met een eigen pEC_{50} waarde, met een significant hogere efficacy dan sumatriptan. Incubatie met de $5\text{-}HT_2$ receptor antagonist ketanserine (10 μM) elimineerde het ‘lage affiniteit, hoge efficacy’-gedeelte van de concentratie response curve van donitriptan. Ketanserine had geen effect op de sumatriptan geïnduceerde contracties. Beide triptanen hadden vergelijkbare selectiviteit voor de arteria meningea media ten

opzichte van de coronair arterie. De voorspelde therapeutische concentratie voor donitriptan bedroeg ~4.5 nM, die waarschijnlijk voor een kleine contractie zorgt in de coronair arterie. Samenvattend suggereren de resultaten dat donitriptan effectief zal zijn tegen migraine, waarbij een vergelijkbaar coronaire bijwerkingsprofiel als sumatriptan te verwachten is.

Het doel van de studie in **Hoofdstuk 6** was het bestuderen van de effecten van de benzopyran derivaat SB220453 in geïsoleerde humane bloedvaten belangrijk in migraine (arteria meningea media, coronair arterie en vena saphena magna), alsmede cardiale atriale en ventriculaire trabekels. SB220453, een corticale spreidingsdepressie blokker dat momenteel een klinische evaluatie in migraine ondergaat, heeft een hoge affiniteit voor een selectieve maar nog onbekende bindingsplaats in de humane hersenen. Eerdere studies hebben laten zien dat SB220453 zowel de afgifte van stikstof oxide, maar ook cerebrale vasodilatatie, na trigeminale zenuw stimulatie, remt. Terwijl de 5-$HT_{1B/1D}$ receptor agonist sumatriptan alle onderzochte bloedvaten duidelijk contraheerde, had SB220453 geen effect. In cardiale atriale en ventriculaire trabekels had noch sumatriptan, noch SB220453 enig inotroop effect. Omdat SB220453 de arteria meningea media niet contraheert kunnen wij concluderen dat, indien SB220453 effectief blijkt te zijn in migraine, het therapeutisch effect, in tegenstelling tot sumatriptan, onafhankelijk zal zijn van craniale vasoconstrictie. Omdat SB220453 ook de coronair arterie, de vena saphena en de cardiale trabekels niet contraheert, is het onwaarschijnlijk dat deze stof cardiale bijwerkingen veroorzaakt.

In **Hoofdstuk 7** hebben we een verdere karakterisatie gemaakt van de receptor die betrokken is bij de eletriptan geïnduceerde contractie en deze vergeleken met sumatriptan. We hebben de effecten van eletriptan en sumatriptan vergeleken in de geïsoleerde humane arteria meningea media, coronair arterie en vena saphena magna, die gebruikt worden als modellen voor therapeutische effectiviteit en mogelijke bijwerkingen. Verder hebben we de rol van de 5-$HT_{1B/1D}$ receptoren, die deze contractie mediëren, onderzocht. Concentratie response curven van eletriptan en sumatriptan werden geconstrueerd in af- en aanwezigheid van de selectieve 5-$HT_{1B/1D}$ receptor antagonist GR125743. Alledrie de bloedvaten contraheerden in respons op eletriptan en sumatriptan, maar in de arteria meningea media volgde er een relaxatie bij de hoogste concentratie (100 μM) van

eletriptan. In de arteria meningea media antagoneerde GR125743 zowel de eletriptan, alsmede de sumatriptan geïnduceerde contractie op een zelfde manier (pA_2: 8.81±0.17 and 8.64±0.21, respectievelijk). In de humane coronair arterie en vena saphena werd de sumatriptan geïnduceerde contractie ook potent geblokt door GR125743 (pA_2: 8.18±0.27 and 8.34±0.12, respectievelijk). De eletriptan geïnduceerde contractie in de vena saphena werd minder effectief geblokkeerd door GR125743 (pK_B: 7.73±0.18) en die in de coronair arterie bleven onaangetast door GR125743 tot en met een concentratie van 100 nM. Concluderend, suggereren de resultaten dat, gebaseerd op de verschillen in pEC_{50} waarden, eletriptan (63-keer) cranioselectiever is dan sumatriptan (5-keer) in de coronair arterie. Verder is gebleken dat de contractie geïnduceerde effecten van sumatriptan worden gemedieerd via de 5-HT_{1B} receptor, terwijl er aanvullende mechanismen betrokken zijn in de coronair arterie en vena saphena constrictie, en arteria meningea media relaxatie, na hoge concentraties eletriptan.

Hoofdstuk 8 had als doel te onderzoeken of de 5-HT_{1B} of 5-HT_{1D} receptor verantwoordelijk is voor het mediëren van geïsoleerde humane arteria meningea media en temporalis (modellen voor antimigraine effectiviteit), en coronair arterie en vena saphena magna (modellen voor mogelijke bijwerkingen). Concentratie response curven werden geconstrueerd met behulp van sumatriptan in de af- en aanwezigheid van selectieve antagonisten voor de 5-HT_{1B} (SB224298) en 5-HT_{1D} (BRL15572) receptoren. SB224289 antagoneerde sumatriptan geïnduceerde contracties in alle bloedvaten, alhoewel het soort antagonisme verschillend was tussen de bloedvaten. In de arteria temporalis werd sumatriptan volledig geblokkeerd door SB224289, terwijl in de arteria meningea media en vena saphena de sumatriptan geïnduceerde contractie niet competitief geblokkeerd was. Bovendien antagoneerde SB224289 op een zwakke niet-competitieve manier in de coronair arterie (pK_B: 6.4±0.2). In tegenstelling tot SB224289 had BRL15572 weinig tot geen effect op de sumatriptan geïnduceerde contracties in de vier bloedvaten die waren onderzocht. *In situ* hybridisatie toonde aan dat er expressie van 5-HT_{1B} receptor mRNA aanwezig was in zowel het gladde spierweefsel als het endotheel, terwijl er zeer weinig expressie gevonden werd voor de 5-HT_{1D} receptor. Deze resultaten laten zien dat de 5-HT_{1B} receptor primair betrokken is bij sumatriptan geïnduceerde contracties in humane craniale bloedvaten, maar suggereren dat er mogelijk andere mechanismen

medeverantwoordelijk zijn voor een gedeelte van deze contracties in de perifere bloedvaten.

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Curriculum Vitae

The author of this thesis was born in Rotterdam, The Netherlands, on the 5th of June 1969. After graduating from the Montessori Lyceum Rotterdam in 1989 he studied first Biology and then his MSc degree in Bio-Pharmaceutical Science at the Leiden University Medical Centre until 1997. During his studies he was involved in various research projects at the Medical Pharmacology Department (Dr. O.C. Meijer and Prof. dr. E.R. de Kloet). His graduation project was on *"The influence of corticosterone on 5-HT_{1A} receptor mediated behavioural responses in rats using the Morris Water maze"*. He joined the department of Pharmacology on the Erasmus University Medical Centre Rotterdam in 1997. Under the guidance of Prof. dr. P.R. Saxena and dr. A. Maassen van den Brink he worked on a project entitled "*Vascular effects of antimigraine drugs; pharmacology of human in vitro models in migraine"*. Since November 2001 he works as Plan Executive at the Department of Strategic Publication Planning, Excerpta Medica Medical Communications in Almere.

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List of Abbreviations

5-HIAA	5-hydroxyindole acetic acid
5-HT	5-hydroxytriptamine, serotonin
AMPA	alpha-amino-3-hydroxy-5-methyl-4-propionate
ANOVA	analysis of variance
BIBN4096BS	a CGRP antagonist
BMS181885	a discontinued triptan
BRL15572	a selective 5-HT_{1D} receptor antagonist
c-fos	an early expression gene
cAMP	cyclic adenosine monophosphate
CGRP	calcitonin gene related peptide
CHO cells	Chinese hamster ovary cells
CI	confidence interval
C_{max}	maximum plasma concentration
CP122288	a PPE inhibitor, restricted analogue of sumatriptan
$cPGI_2$	carbaprostacyclin I_2
DIG	digoxigenine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EC_{50}	concentration of agonist at half maximal effect
E_{max}	maximum induced effect of an agonist
F11356	donitriptan HCl
F12640	donitriptan mesylate
GMC2021	a discontinued triptan
GR125743	a reversible 5-$HT_{1B/1D}$ receptor antagonist
GR127935	an irreversible 5-$HT_{1B/1D}$ receptor antagonist
GR79236	an $adenosine_1$ receptor agonist
IHS	international headache society
IS-159	a novel triptan
L775,606	a 5-HT_{1D} receptor agonist
LY292558	a glutamate R5 receptor antagonist
LY334370	a 5-HT_{1F} receptor agonist
LY344864	a 5-HT_{1F} receptor agonist
mCPP	meta-chlorophenylpiperazine
(m)g	(milli)gram
min	minute
mm	millimeter
(m)N	(milli) Newton
(m)RNA	(messenger) ribonucleic acid
NA	noradrenaline
(n/µ/m)M	(nano/micro/milli) molar
NK-1	neurokinin 1
nH	Hill coefficient
NMDA	N-methyl-D-aspartate
NO	nitric oxide

NPY	neuropeptide Y
NSAIDs	non-steroid anti-inflammatory drugs
pA_2	negative logarithm of the concentration of antagonist to produce a two-fold shift to the right of an agonist concentration response curve.
$pEC_{25\%}$	negative logarithm of agonist concentration eliciting a response equivalent to 25% of (control) E_{max}
pEC_{50}	negative logarithm of EC_{50}
$PGF_{2\alpha}/E_2/D_2$	prostaglandin $F_{2\alpha}/E_2/D_2$
pK_i	negative logarithm of binding affinity constant
pK_B	negative logarithm of antagonist-receptor dissociation equilibrium constant
PNU-109291	a 5-HT_{1D} receptor agonist
PNU-142633	a 5-HT_{1D} receptor agonist
p.o.	per os (orally)
PPE	plasma protein extravasation
RNase	ribonuclease
RT-PCR	reverse transcriptase polymerase chain reaction
SB220453	a cortical spreading depression inhibitor
SB224289	a selective 5-HT_{1B} receptor antagonist
SB273779	a CGRP antagonist
s.c.	subcutaneous
s.e.mean	standard error of the mean
$T_{1/2}$	plasma half life time
T_{max}	time to reach maximal plasma concentration
U46619	tromboxane A2 analogue
VIP	vasoactive intestinal peptide