CRANIAL CIRCULATORY EFFECTS OF ANTIMIGRAINE DRUGS: AN EXPERIMENTAL STUDY IN THE PIG

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CRANIAL CIRCULATORY EFFECTS OF ANTIMIGRAINE DRUGS: AN EXPERIMENTAL STUDY IN THE PIG

(EFFECTEN VAN ANTIMIGRAINE MIDDELEN
OP DE CRANIËLE CIRCULATIE:
EEN EXPERIMENTELE STUDIE IN VARKENS)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
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Voor mijn ouders

CONTENTS

This thesis is based on the following articles:

1. Den Boer MO, Villalón CM, Heiligers JPC, Humphrey PPA, Saxena PR (1991) Role of 5-HT_1-like receptors in the reduction of porcine cranial arteriovenous anastomotic shunting by sumatriptan. Br J Pharmacol 102:323-30

2. Den Boer MO, Villalón CM, Saxena PR (1992) 5-HT_1-like receptor mediated changes in porcine carotid haemodynamics: are 5-HT_{1D}-receptors involved? Naunyn-Schmiedeberg's Arch Pharmacol 345:509-15

3. Den Boer MO, Heiligers JPC, Saxena PR (1991) Carotid vascular effects of ergotamine and dihydroergotamine in the pig: no exclusive mediation via 5-HT receptors. Br J Pharmacol 104:183-9

4. Den Boer MO, Somers JAE, Saxena PR (1992) Lack of effect of the antimigraine drugs sumatriptan, ergotamine and dihydroergotamine on arteriovenous anastomotic shunting in the dura mater of the pig. Br J Pharmacol 107:577-83

5. Den Boer MO, Somers JAE, Saxena PR (1992) Comparative effects of the antimigraine drugs sumatriptan and ergotamine on the distribution of cardiac output in anaesthetized pigs. Cephalalgia 12:206-13

6. Den Boer MO, Van Woerkens LJ, Somers JAE, Duncker DJ, Lachmann B, Saxena PR, Verdouw PD. Development of a novel anesthesia in swine to preserve the tone in arteriovenous anastomoses and to study their regulation. Submitted.

Chapter 1

General Introduction

This thesis is mainly concerned with an investigation in anaesthetized pigs of the vascular effects of sumatriptan, ergotamine and dihydroergotamine, drugs used in the treatment of the acute migraine attack. There are several reasons for performing this investigation.

Firstly, a disturbance within the cranial blood vessels has long been implicated in the pathogenesis of migraine. Such a vascular theory was first mentioned in 1684 by Thomas Willis, following the discovery of the blood circulation by Harvey. Until the middle of this century, an experimental basis for the vascular theory lacked, but it was strengthened by the experiments of Graham & Wolff (1938), who demonstrated increased pulsations of the superficial temporal artery in a proportion of their patients. More recently the primarily vascular theory has been severely criticized by many researchers in the field and a primarily neurological theory has been adopted. This theory involves hyperactivity of the pain-conducting trigeminal nerve, leading to pain, but also dilatation of the blood vessels innervated by this nerve (Markowitz et al., 1988). Therefore, even the leading neurological theory of this moment implies vascular involvement in the migraine attack, even if not the primary cause.

Secondly the antimigraine drugs mentioned above are powerful vasoactive agents. They usually constrict the carotid blood vessels, especially when they had been dilated before (Saxena & De Vlaam-Schluter, 1974). It is, therefore, quite possible that a vasoconstrictor action of these drugs contributes to the antimigraine activity. On the other hand, it is now proposed, albeit not yet definitely proven, that a direct effect of these drugs on the trigeminal nerve innervating the dura mater is a factor in their antimigraine efficacy (Markowitz et al., 1988; Buzzi & Moskowitz, 1990). A direct vascular action could, however, even explain the effects of these drugs on the dura mater.

It is not known which blood vessels are mainly involved in migraine. The pharmacological profile of the antimigraine drugs could provide a clue. The fact that they are potent constrictors of arteries and arteriovenous anastomoses, but not of arterioles (Müller-Schweinitzer & Weidmann, 1978; Saxena, 1978; this thesis) could point to involvement of arteries or arteriovenous anastomoses in migraine. For both some experimental evidence is present (Heyck, 1969; Friberg et al., 1991)

The present thesis is aimed at contributing to the knowledge of the vascular pharmacologic profile of these antimigraine drugs. The effect of these drugs on arteriovenous anastomoses and arterioles both in the head and in the body (the effect on arteries is beyond the scope of this thesis) was determined with injection of radioactive microspheres in anaesthetized pigs. Furthermore, by use of suitable pharmacologic agents an attempt was made to characterize the receptors involved in these vascular effects. By a good pharmacological characterization it might, in future, be possible to develop more specific antimigraine agents. Lastly, an attempt was made to determine the neurotransmitter which may be responsible for the tonic contraction of the arteriovenous anastomoses.

Before the presentation of the results of this investigation, chapters 2 through 4 will discuss in detail the field on which this thesis is based. In chapter 2 current views on the pathophysiology of migraine will be discussed. Chapter 3 is concerned with previous knowledge on the pharmacology of the antimigraine agents. In chapter 4 general features of the cranial circulation are discussed, with the existing differences between the human and porcine circulations. Chapter 6 will specify the aim of the thesis.

Chapter 2

Migraine and its Pathogenesis

The clinical picture

Migraine is a common disorder, afflicting 5-15 % of the adult population in western countries (Linet & Stewart, 1987). The clinical picture differs among individuals and can vary from quite simple, involving only a headache phase, to such complex forms as migraine preceded by hemiplegia. This complexity has caused a lack in uniformity in criteria, applied through the centuries, to discern migraine from other forms of headache. Such a lack of uniform criteria in diagnosing migraine is reflected in the way that patient populations were assembled for scientific studies concerning migraine. In order to increase uniformity and improve the comparability of studies, the International Headache Society has published criteria for diagnosing and classifying migraine (Headache Classification Committee, 1988; Table 1.).

Table 1. Diagnostic criteria proposed by the 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

Migraine with aura

A. At least two attacks fulfilling B
B. At leats 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 gradually over more than 4 min, or two or more symptoms occur in succession
   3. No aura symptom lasts more than 60 min. 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 min. (It may also begin before or simultaneously with the aura)

According to this classification, apart from some rare types, two common forms of migraine are distinguished: migraine without aura and migraine with aura. Thus, migraine is a syndrome characterized by attacks of moderate or severe headache lasting from 4-72 hours. Very rarely headache is absent after the aura, and this is nonetheless characterized as migraine with aura. During the headache the patient usually feels severely ill, dislikes movement, light or sound, and often has nausea and may even vomit. If aura symptoms are present, they usually precede the headache phase, but they may persist into this phase or even arise when the headache is already present. The aura symptoms are very diverse and may comprise sensory (a scintillating scotoma, a feeling of pins and needles) and motor (weakness, paralysis) phenomena. Characteristically the aura symptoms do not exceed 60 minutes.

The distinction in migraine with and without aura is not only important on clinical grounds. Some features of the migraine attack differ among the two groups. For instance, in migraine with aura there is usually a reduction of cerebral blood flow, which may last well into the headache phase (Olesen et al., 1981). In migraine without aura no change in cerebral blood flow is observed (Lauritzen & Olesen, 1984). Also differences in blood changes induced by the two forms are found: the platelet content of 5-hydroxytryptamine falls during a migraine attack without aura, but not during an attack with aura (Ferrari et al., 1989).

The pathogenesis of migraine-historical perspective

Hippocrates launched one of the first pathogenetic theories to explain both the aura and the headache of migraine. He postulated that vapours, rising from the liver to the head, were responsible for all symptoms of the migraine attack. While this theory has been completely abandoned, in 1664 Thomas Willis was the first to launch the vascular theory of migraine. He held congestion and dilatation of the cranial vasculature responsible for the headache of migraine. This was a genial hypothesis (although not based on experimental evidence), because oedema and dilatation of cranial blood vessels is nowadays still accepted as possible pathogenetic factors in migraine. In the nineteenth century a controversy arose between advocates of the vascular theory and those of the more recently introduced neurogenic theory. Again, experimental evidence lacked.

Graham & Wolff (1938) were the first to really perform experiments on migraine patients. They applied tambours to the temporal arteries of patients during migraine attacks and found increased pulsations of this vessel, which could be abolished by ergotamine or dihydroergotamine. Both digital compression of this vessel and ergotamine

abolished the headache. Furthermore, the same group demonstrated that the vasodilator amyl nitrite could abolish the migraine aura (Schumacher & Wolff, 1941). They thus proposed vasoconstriction as the cause of the aura and vasodilatation as the cause of the headache, thus laying a solid foundation for the vascular theory of migraine. The fact that more recent findings have severely questioned this pathogenetic model will be discussed below.

In the search for possible mechanisms of the vascular disturbances, different neurotransmitters have been investigated in migraine. Rather consistently, changes in 5-hydroxytryptamine levels and metabolism were found during and between migraine attacks (Sicuteri et al., 1961; Curran et al.,1965; Ferrari et al., 1989). But the nature of these changes in the different publications was by no means the same, so that an enormous controversy exists on this subject as well. This will also be further discussed below.

The migraine aura

That cerebral vasoconstriction and ensuing ischaemia has been held responsible for the aura phase, has been discussed above. Indeed, the use of intracarotid injection or inhalation of ^{133}Xe to measure cerebral blood flow has made it possible to demonstrate a decrease in cerebral blood flow during the migraine aura phase (Olesen et al., 1981; Lauritzen et al., 1983a; Lauritzen & Olesen, 1984). Such an area of decreased brain blood flow usually started in the occipital lobes and moved anteriorly with a velocity of 2-3 $mm.min^{-1}$. Lashley (1941), himself a migraine sufferer, calculated from the spread of his own visual aura the spread of the neurologic depression over the occipital cerebral surface and found the same velocity. Equally other sensory auras, like paraesthesias, and motor auras have a tendency to spread. A problem with the ischaemic theory is, that the areas of reduced brain flow did not observe boundaries of the different cerebral vascular beds and that the magnitude of the flow decrease did not seem to be sufficient to explain the neurological symptoms (Olesen et al., 1981; Lauritzen et al., 1983a). These authors, therefore, proposed a primarily neurological disturbance as the origin of the aura phase, with secondary changes in brain blood flow. This disturbance seemed to be equal or similar to the "spreading depression" which had been described by Leão (1944). This phenomenon can be induced by mechanical or chemical stimulation of the exposed cerebral cortex in experimental animals. This induces a slowly spreading (1-3 $mm.min^{-1}$) area of depression of neuronal activity and oligemia, with a similar speed as the spreading oligemia, found during the aura phase of migraine (Olesen et al., 1981; Lauritzen et al.,

1983a). Furthermore, spreading depression was shown to cause a similar spreading oligaemia (Lauritzen et al., 1982).

However, in re-evaluating the experiments of Olesen et al. (1981), Skyhøj Olsen et al. (1984) claimed that under the influence of Compton Scatter the reduction in cerebral blood flow values found by this group during the migraine aura probably have been underestimated, and that in fact ischaemia was likely to have occurred. Furthermore, it has not been possible to induce spreading depression in man during brain surgery (Piper et al., 1991).

Although the migraine aura is very likely to have its origin in a disturbance of cerebral function, as deducted from the half-sidedness of the symptoms, it is yet uncertain whether the aura symptoms are directly caused by this cerebral process or by the ensuing oligaemia or ischaemia. Indeed, stimulation of certain brain stem nuclei, like the dorsal raphe nucleus and the locus coeruleus nucleus influence both intracranial and extracranial vasculature (Goadsby & Lance, 1988).

The migraine headache

Vascular changes

A vascular theory on the headache phase of migraine was strengthened by the experiments of Graham & Wolff (1938) described above. In accordance with this, Ray and Wolff (1940) demonstrated by stimulating different intracranial structures in patients, undergoing neurosurgical operations under local anaesthesia, that production of pain was limited to some cranial blood vessels, especially the larger extracerebral arteries, and the dura mater. A vascular aetiology of the migraine headache was also adopted by Heyck (1969), who demonstrated an increased oxygen saturation in the jugular venous blood on the headache side and its subsequent reduction by dihydroergotamine. He proposed that dilatation of arteriovenous anastomoses and a resulting increase in non-nutrient blood flow, from which no oxygen is extracted, could be responsible for these findings, although an increased blood flow without an increased oxygen utilization can also explain these findings. This hypothesis has not been further evaluated in migraine patients.

Subsequently, the vascular concept has been questioned. Whereas in some patients the migraine headache has a pulsating character, this does not apply to the majority of patients. The use of ^{133}Xe-techniques to measure cerebral blood flow, which has been described above in conjunction with the aura phase, demonstrated a preserved cerebral blood flow in migraine without aura (Lauritzen & Olesen, 1984), while in migraine attacks with aura the decrease in cerebral blood flow in the aura phase even persisted into

the headache phase (Olesen et al., 1981; Lauritzen et al., 1983a; Lauritzen & Olesen, 1984). After this period of low cerebral flow, hyperaemia occurred, without any temporal relationship to the headache (Andersen et al., 1988). It is, therefore, quite certain that the headache phase of migraine cannot be explained by changes in *cerebral* perfusion.

Another possibility that has to be considered is that the migraine headache is accompanied by dilatation of larger intracranial arteries that do not regulate vascular resistance, rather than dilatation of blood vessels regulating cerebral perfusion. An index of arterial width can be obtained by measuring blood flow velocity in the artery by the transcranial Doppler-method. If blood flow in the artery remains constant, blood flow velocity is inversely related to vascular diameter. However, such estimations of vascular diameter have led to conflicting results. Thie et al. (1990) reported dilatation of the common and internal carotid and the anterior, middle and posterior cerebral arteries during attacks of common migraine without a clear lateralization or correlation with the side of the headache. Dilatation of the middle meningeal artery during the migraine headache was confirmed by Friberg et al. (1991), although their patients suffered both from common and classic migraine and the side of flow velocity increase correlated with the headache side. On the other hand, Zwetsloot et al. (1991) reported unchanged blood flow velocities in the major cerebral arteries, except for a bilateral dilatation of the common carotid artery. A reason for these discrepancies could be a methodological problem, since the blood flow velocity values measured with the Doppler technique depend much on the position and direction of the Doppler probe.

After the experiments of Graham & Wolff (1938), who noticed increased pulsations of the temporal artery during a migraine attack, relatively little research has been devoted to possible changes in extracranial blood flow or vascular diameter during the attack. Two groups tried to repeat the experiments of Graham & Wolff (1938). Drummond & Lance (1983) found increased pulsations of the frontal branch of the temporal artery only in one in three patients. Furthermore, it was not possible to relieve the migraine headache by vascular compression in half (Blau & Dexter, 1981) or one-third of the patients (Drummond & Lance, 1983). In a more recent study the diameter of the superficial temporal arteries was assessed by an ultrasound technique and compared to the diameter of the radial arteries during the migraine headache phase (Iversen et al., 1990). The superficial temporal artery was demonstrated to be wider on the headache side than on the other side. Furthermore, the temporal artery on the non-headache side and both radial arteries were narrower than between attacks. They suggested a local vasodilator response in the affected temporal artery, counteracting a generalized vasoconstriction (Iversen et al., 1990). In the only study measuring extracranial blood

flow during the migraine headache phase with ^{133}Xe, an increase was observed (Sakai & Meyer, 1978), although the reliability of the extracranial blood flow values measured with ^{133}Xe is questionable due to interference with intracranial radiation.

In conclusion, in spite of an unaltered cerebral blood flow, there are good claims for dilatation of extracerebral arteries in migraine subjects during the attack, at least in a large proportion of patients. Whether intracranial arteries are equally involved awaits more reliable methods of determining intracranial vascular diameters.

Neural changes

In the meantime, a neurogenic theory on the pathogenesis of migraine was strengthened by two findings. One was the demonstration of increased levels of CGRP (calcitonin gene-related peptide), a sensory peptide derived from trigeminal nerve fibres, in the jugular venous blood of migraine patients during the attack (Goadsby et al., 1990). This reflects involvement of perivascular trigeminal nerves in the migraine syndrome, although whether as a cause or as a result of the pain is not certain. Nonetheless, the release of sensory peptides can be experimentally induced by stimulation of the trigeminal ganglion (Buzzi et al., 1991) or sensory afferents in the superior sagittal sinus (Zagami et al., 1990). Such a neurogenic mechanism, involving release of sensory peptides from the trigeminal nerve links the production of pain with involvement of the vasculature, since CGRP produces vasodilatation (Brain et al., 1985). The second important discovery favouring a neurogenic etiology is that stimulation of different brain stem regions appears to have marked influences on cranial haemodynamics, giving a mechanism by which a discrete, primarily cerebral disorder might influence the cranial vasculature at one side of the head only (Goadsby & Lance, 1988; Lance et al., 1989). 5-Hydroxytryptamine may be involved in this cerebral control of the cranial circulation, since the 5-hydroxytryptamine-containing dorsal and median raphe nuclei innervate cerebral and pial blood vessels (Edvinsson et al., 1983a) and stimulation of these regions increases both cerebral and extracranial blood flow (Goadsby et al., 1985).

In conclusion, although the pathogenesis of the migraine headache remains far from elucidated, a combination of neurogenic and vascular mechanisms seems to have a role in the pathogenesis of the migraine headache.

Changes in metabolism of 5-hydroxytryptamine

A pathogenetic factor of migraine involving the neurotransmitter 5-hydroxytryptamine was proposed in the sixties, when increased urinary excretion of 5-hydroxyindoleacetic acid (Sicuteri et al., 1961), the major metabolite of 5-hydroxytryptamine, and decreased plasma levels of 5-hydroxytryptamine were found

during the migraine attack (Anthony et al., 1969). Furthermore, reserpine, a substance which causes depletion of 5-hydroxytryptamine (but also of noradrenaline and dopamine), was shown capable of inducing a migraine attack (Curzon et al., 1969a). This led to speculations that a decrease in the plasma level of 5-hydroxytryptamine might set off the migraine attack in susceptible persons. This idea was strengthened by the previous finding that infusion of 5-hydroxytryptamine alleviated the migraine headache in spite of a wide range of side effects (Kimball et al., 1960). Whether this was caused by a suppletion of 5-hydroxytryptamine or a pharmacological effect of supranormal levels of this neurotransmitter has been disputed since. A decrease in the platelet content of 5-hydroxytryptamine during attacks of migraine without aura has been confirmed in a later study (Ferrari et al., 1989), although the free plasma levels were raised in this study. On the other hand, an increased conversion of 5-hydroxytryptamine into 5-hydroxyindoleacetic acid could not be confirmed in a substantial proportion of patients in later studies: unchanged (Curzon et al., 1969b) or even decreased (Ferrari et al., 1989) levels of this metabolite were reported.

Although a decrease in the platelet-rich plasma level of 5-hydroxytryptamine seems to occur during an attack of migraine with aura, it remains uncertain if this is an etiologic factor of the migraine attack, or just a reflection of the gastrointestinal motility changes. It should be remembered that 90 % of the body 5-hydroxytryptamine is located in the chromaffin cells in the gut (Anthony, 1986). Furthermore, it remains to be determined why only in patients having migraine without aura and not in those having migraine with aura a change in the level of 5-hydroxytryptamine is observed.

Chapter 3

Pharmacological Properties of the Antimigraine Drugs

Introduction

For the treatment of migraine the physician may choose from two therapeutic approaches: prophylaxis or treatment of the attacks. Prophylaxis may be indicated in the case of frequent attacks or when the attacks cannot adequately be relieved by acute treatment. Drugs which are currently used for this purpose are (for references, see Saxena & Den Boer, 1991): some beta-adrenoceptor antagonists (e.g. propranolol), calcium channel blockers (e.g. flunarizine) or 5-HT_2-receptor antagonists (e.g. pizotifen, methysergide). Despite the fact that some of these drugs have been claimed to act via 5-hydroxytryptamine-related mechanisms, little is known about their mechanisms of action. These drugs will not be further discussed, since this thesis deals only with the attack-related treatments.

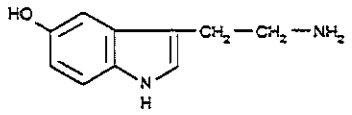
In some patients, the migraine attacks may be sufficiently treated by non-steroidal anti-inflammatory drugs, like aspirin, indomethacin or naproxen sodium. Other patients may, however, require more specific antimigraine drugs, like sumatriptan or an ergot alkaloid. Some pharmacology of the ergot alkaloids and sumatriptan will be presently discussed.

Ergotamine

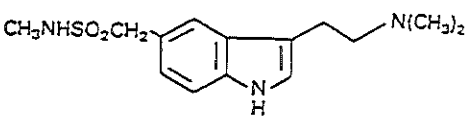
Ergotamine is a member of the group of the ergot derivatives. These have originally been derived from the fungus Claviceps purpurea, which grows on rye. In the past, the eating of such infected rye has caused epidemics of "Saint Anthony's Fire", characterized by intense vasoconstriction and gangrene of the extremities. This disease could be cured by paying a visit to the Shrine of St. Anthony, apparently in a region where such infected rye did not occur. The ergot alkaloids contain a tetracyclic ergolene-ring structure. In the case of ergotamine this ring structure is linked to an amine, forming a peptide bond (Figure 1). Ergotamine, like most ergot alkaloids, has a complex mode of action. The effects can be mediated by an interaction with 5-HT, dopamine and noradrenaline receptors (Müller-Schweinitzer & Weidmann, 1978), for all of which ergotamine has a high affinity (Table 1). Both agonist and antagonist actions at these

Figure 1. The chemical structure of 5-hydroxytryptamine and three antimigraine drugs.

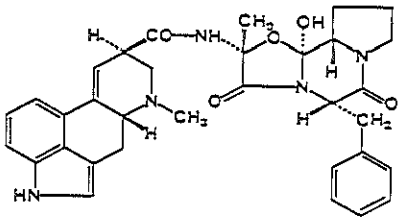
5-hydroxytryptamine



sumatriptan



ergotamine



dihydroergotamine

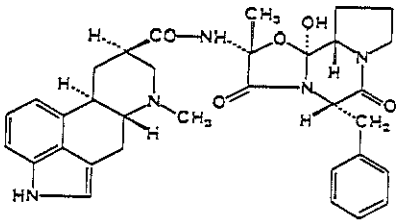


Table 1 Affinities (pK_i) of ergotamine, dihydroergotamine and methiothepin for different binding sites.

	Ergotamine	Dihydroergotamine	Sumatriptan
5-HT_{1A}	8.3[a]; 8.4[b]	8.6[a]; 8.9[bc]	6.1[c]
5-HT_{1B}	8.7[ab]	8.4[a]; 9.1[b]	6.4[c]
5-HT_{1C}	7.3[ab]	7.3[a]; 7.4[c]; 7.5[b]	4.1[c]
5-HT_{1D}	7.6[b]; 7.8[a]	7.7[bc]; 7.9[a]	7.5[c]
5-HT_2	7.7[b]; 7.9[d]	7.1[c]; 8.6[b]	<5[c]
α_1	8.0[d]	8.2[c]	<5[c]
α_2	8.2[d]	8.5[c]	<5[c]
D_2	8.5[d]	7.0[c]	<5[c]

Data from: [a], Hoyer et al., 1989; [b], Hoyer, 1989; [c], McCarthy & Peroutka, 1989; [d], Leysen & Gommeren, 1984; [e], Schoeffter & Hoyer, 1989b.

receptor types have been described (Müller-Schweinitzer & Weidmann, 1978). And even after a complete pharmacologic blockade of these receptors, some of the actions of ergotamine persist, so that an additional interaction with still unclassified receptors remains likely (Saxena et al., 1983; Bom et al., 1989a; MacLennan & Martin, 1990a). This point will be further discussed in chapter 8 of this thesis, where an extension of the receptor studies of the ergot alkaloids ergotamine and dihydroergotamine will be presented.

The first report of a migraine patient successfully treated with a fluid extract of ergot was published at the end of the last century (Thomson, 1894). It was, however, only in the twenties of this century that ergotamine was regularly established as an antimigraine agent. That the effect of ergotamine in aborting the migraine headache could involve constriction of dilated blood vessels was for the first time scientifically investigated by Graham and Wolff (1938). They used tambours, applied to the temporal skin, to measure pulsations of the superficial temporal arteries in their patients during a migraine attack. They reported a decrease in pulsatility after administration of ergotamine. This triggered a great number of publications on the vascular actions of ergotamine. The diversity of vascular effects of ergotamine have been shown to match the pharmacological complexity of this agent. Both vasoconstrictor and vasodilator effects of ergotamine have been described (Lennox, 1938; Saxena & De Vlaam-Schluter, 1974). The vasodilator effects predominate when the existing blood pressure and sympathetic tone are high (Lennox, 1938; Aellig & Berde, 1969). The mechanism of this vasodilator effect is not known. A cerebral mechanism has been postulated for this effect (Clark et al., 1978). Although a presynaptic inhibition of noradrenaline release, mediated by stimulation of presynaptic α_2-adrenoceptors could theoretically be involved, ergotamine has usually been observed to increase noradrenaline overflow (Müller-Schweinitzer & Weidmann, 1978).

On the other hand, in the case of a normal arterial blood pressure, intravenous injection of ergotamine usually causes vasoconstriction. Two phases in this vasoconstriction can be observed. A short-lasting increase in vascular resistance in arterioles and small arteries results in a short-lasting rise in mean arterial blood pressure (Tfelt-Hansen et al., 1983). Such an effect on resistance blood vessels is too brief to explain the long-lasting therapeutic effect of ergotamine. The second vasoconstrictor phase of ergotamine consists in a long lasting constriction of larger arteries, which do not regulate vascular resistance (Tfelt-Hansen et al., 1983). Such a prolonged constriction of arteries can also be observed in organ-bath studies in isolated blood vessels, where it may be impossible to wash ergotamine out from the tissue. In spite of a relative selectivity for cranial arteries, most arteries in the body are susceptible to such an effect

(Müller-Schweinitzer & Weidmann, 1978; Tfelt-Hansen et al., 1983). Furthermore, ergotamine has a venoconstrictor property (Aellig, 1976). This property enables its use as prophylaxis against venous thrombosis, although dihydroergotamine is preferred for this indication.

After the study of Graham & Wolff (1938) there was little doubt that the mechanism of action of ergotamine in migraine involved constriction of cranial arteries. Some more recent studies with the aim of confirming the experiment by Graham & Wolff (1938) have been less successful in demonstrating involvement of the vasculature in migraine, as discussed in chapter 2 of this thesis. Nonetheless, a vascular involvement was observed even in these studies in more than half the patients (Drummond & Lance, 1983). Therefore, a therapeutic action of ergotamine on dilated arteries remains a possibility. However, even constriction of cranial arteries which are sensitized but not dilated might be involved in the action of ergotamine.

The constrictor effects of ergotamine extend further to the arteriovenous anastomoses in the cranial circulation (Saxena et al., 1983; Bom et al, 1989a). Based on the finding of increased oxygen saturations in the jugular venous blood, a dilatation of arteriovenous anastomoses has been implicated in migraine (Heyck, 1969). This was, however, a small study and other factors could be responsible for an increased oxygen saturation (Heyck, 1981). Therefore, the contribution of constriction of arteriovenous anastomoses to the antimigraine effect of ergotamine remains to be ascertained. A second point which remains to be elucidated is the location of the arteriovenous anastomoses that are constricted by ergotamine. Chapters 4, 9 and 10 of this thesis deal further with this point.

Recent studies at Harvard University have disclosed another, neurovascular effect of ergotamine. The drug was found to inhibit the 'neurogenic inflammation' in the rat dura mater, due to stimulation of the trigeminal ganglion or intravenous injection of capsaicin (Markowitz et al., 1987, 1988; Saito et al., 1988). As discussed above, such a neurogenic 'inflammatory' reaction of the dura mater is one of the current pathogenetic theories on the migraine headache (Buzzi et al., 1991). Since ergotamine and dihydroergotamine, but not angiotensin II or phenylephrine, inhibited neurogenic and capsaicin-induced oedema, the authors concluded that the effect of ergotamine is independent of vasoconstriction and may be a result of direct inhibition of trigeminal afferent function (Saito et al., 1988). However, these results were not backed up by simultaneous measurement of dural (or carotid) blood flow or arterial blood pressure and, therefore, the effectiveness of angiotensin II and phenylephrine to induce dural vasoconstriction or even systemic vasoconstriction is uncertain. Furthermore, the dose of ergotamine used to block the protein extravasation (100 $\mu g.kg^{-1}$) is high compared to the

usual clinical dose to abort the migraine attack (less than 10 $\mu g.kg^{-1}$). A study on the vascular effects of ergotamine in the dura mater will be presented in Chapter 9 of this thesis.

An action of ergotamine in the central nervous system has also been postulated, because the drug inhibited the discharge of neurons in the lateral cervical nucleus of the cat in response to stimulation of the superior sinus sagittalis (Lance, 1988). However, a vascular effect of ergotamine in the sinus sagittalis to inhibit neural function has not been excluded, and it is not likely that ergotamine penetrates the blood-brain barrier to a great extent (Eckert et al., 1978; Hovdal et al., 1982). Therefore, an action of ergotamine at the level of the blood vessel is still likely.

In conclusion, both vascular and neural effects of ergotamine have been proposed in the antimigraine effect of this drug. Since during the migraine headache a disturbance has been observed in both cranial blood vessels and perivascular trigeminal afferents, a combination of these effects of ergotamine may contribute to the relief of the headache.

Dihydroergotamine

The structure and basic pharmacologic features of dihydroergotamine resemble those of ergotamine, notwithstanding small quantitative differences in receptor affinity (Figure 1, Table 1). However, when compared with ergotamine, dihydroergotamine has a stronger α-adrenoceptor antagonist property (Müller-Schweinitzer & Weidmann, 1978) and behaves as a milder vasoconstrictor (Clark et al., 1978). When given intravenously it is less potent in raising the arterial blood pressure than ergotamine (Clark et al., 1978). Moreover, the drug is more effective in constricting capacitance vessels than resistance vessels (Rall & Schleifer, 1985). This is the rationale for the use of the drug during surgery for the prevention of venous thrombosis and embolism (Sagar et al., 1976). In spite of this, the drug shares many effects with ergotamine, like a vasoconstrictor effect on arteries (Müller-Schweinitzer & Weidmann, 1978) and arteriovenous anastomoses (Spierings & Saxena, 1980a) and inhibition of neurogenic extravasation in rat dura mater (Markowitz et al., 1988; Saito et al., 1988). The drug equally reduces the release of CGRP, which accompanies this neurogenic extravasation (Buzzi et al., 1991). Therefore, a similar mechanism as for ergotamine is very likely in the antimigraine action of the drug.

Although the vasoconstrictive effects of dihydroergotamine may be mediated by α_2 adrenoceptors (Roquebert & Grenié, 1986) or 5-HT receptors (Müller-Schweinitzer & Rosenthaler, 1987), no pharmacologic characterization of the receptor involved in the effect of dihydroergotamine on arteriovenous anastomoses has yet been reported. Chapter 8 of this thesis will deal with this point.

Sumatriptan

Sumatriptan is a recently developed antimigraine drug, which is effective in aborting the attack. Unlike the ergot alkaloids, it is a tryptamine derivative, obtained by chemically modifying the 5-hydroxytryptamine molecule (Figure 1). Compared with the ergot alkaloids, the drug has a high pharmacological selectivity. The agonist profile of the drug is limited to 5-HT_1-like receptors (Humphrey et al., 1988; Peroutka & McCarthy, 1989; Table 1).

Despite this pharmacologic selectivity, both vascular and neural effects of sumatriptan have been reported, which are quite similar to those of the ergot alkaloids. As with ergotamine, the vascular effects are predominantly constrictor (Humphrey et al., 1988; Connor et al., 1989a), but dilatation may be observed under certain circumstances (Schoeffter & Hoyer, 1989a). In contrast to ergotamine, arterial blood pressure is not much changed by sumatriptan and the toe-arm systolic gradient, a measure of constriction of peripheral arteries, is also not affected (Nielsen & Tfelt-Hansen, 1989). In spite of this lack of effect on systemic haemodynamics, sumatriptan causes contraction of many isolated arteries: among these are basilar arteries, both human (Parsons et al., 1989) and animal (Connor et al., 1989a). Equally, the vascular bed of the isolated human dura mater, which is one of the possible sites of the migraine headache, is constricted by sumatriptan (Humphrey et al., 1991a). *In vivo*, sumatriptan selectively increases carotid vascular resistance in the dog and the cat (Feniuk et al., 1989a; Perren et al., 1989); in the latter species the increase in carotid resistance has been shown to be confined to its arteriovenous anastomotic fraction (Perren et al., 1989). Evidence for a constrictor effect on cranial arteries of migraine patients during the attack is also present (Friberg et al., 1991; Caekebeke et al., 1991). Therefore, a vascular mechanism in the abortion of the migraine attack by sumatriptan is quite likely, whether the cranial arteries were dilated during the attack or not. Chapters 6 and 7 of this thesis will discuss the receptor mechanism of the constrictor effect of sumatriptan on arteriovenous anastomoses and the dilator effect on cutaneous arterioles, especially if one of the present known 5-HT_1

receptor subtypes is involved in these effects. The localization of arteriovenous anastomoses susceptible to sumatriptan will be discussed in chapter 9.

Sumatriptan has been reported to inhibit the extravasation of plasma from dural vessels following trigeminal ganglion stimulation or capsaicin injection in the rat (Buzzi & Moskowitz, 1990). A reflection of this process is the normalization of CGRP levels in the jugular venous blood by sumatriptan, which can be observed both in migraine patients during the attack (Goadsby & Edvinsson, 1991) and in experimental animals during trigeminal ganglion stimulation (Buzzi et al., 1991). Like for ergotamine, it is not known whether this effect is a result of vasoconstriction leading to the relief of pain or reflects a direct inhibitory action on trigeminal nerve afferents. Such a direct action of sumatriptan on sensory nerve fibres could, however, not be established in isolated arteries (Waldron et al., 1992; Butler et al., 1992). Chapter 9 of this thesis will further discuss the vascular effects of sumatriptan in the dura mater.

Sumatriptan is more effective than the ergot alkaloids in relieving the associated symptoms of the migraine attack, like the nausea, vomiting and photophobia. Whether the relief of these associated symptoms is caused by the relief of the headache or reflects another, possibly central, effect of the drug remains an interesting point of debate. Furthermore, the ergot alkaloids by themselves induce nausea and vomiting (Loew et al., 1978), probably by stimulating D_2 receptors in the chemoreceptor trigger zone, which is located outside the blood brain barrier (Wislocki & Leduc, 1952).

In conclusion, like for the ergot alkaloids two possible therapeutic mechanisms have been proposed for sumatriptan, one vascular and the other neurogenic. It remains to be determined which mechanism predominates but again a combination of the latter on the trigeminal-vascular interface is possible.

Chapter 4

Anatomical, Physiological and Pharmacological Aspects of the Cranial Circulation: Cerebrum, Arteriovenous Anastomoses and Dura Mater

Introduction

This thesis is based on the effect of pharmacologic interventions, predominantly antimigraine drugs, on the cranial vasculature in the pig. In this chapter aspects of the anatomy and physiological regulation of the cerebral and the extracerebral circulation are discussed. Since the porcine cranial circulation is used as a model for the human cranial circulation, some differences between the two species are mentioned. One major difference is concerned with the relative contributions of the carotid arteries and vertebral arteries to the brain perfusion. Since the dura mater will be discussed in this thesis, the arterial supply to this tissue has been included. A major pharmacological difference is the absence of α-adrenoceptors on porcine in contrast to human cerebral arteries (Bevan, 1991).

Craniovascular anatomy

This paragraph has been based on two articles (Ghoshal & Nanda, 1975; Dahl & Edvinsson, 1988), to which the reader is referred for further information.

The cranial arterial supply is derived both in man and in the pig from two carotid and two vertebral arteries. Both carotid arteries bifurcate into an external carotid and an internal carotid artery. Whereas this bifurcation in man occurs in the neck, the bifurcation in the pig is located on a higher level, behind the lower jaw. While the external carotid artery is important in both species and supplies almost all of the extracranial and part of the intracranial tissues, the internal carotid artery is much larger in man than in the pig.

Brain

The anatomy of the human and porcine brain vasculature is presented in figures 1 and 2, respectively.

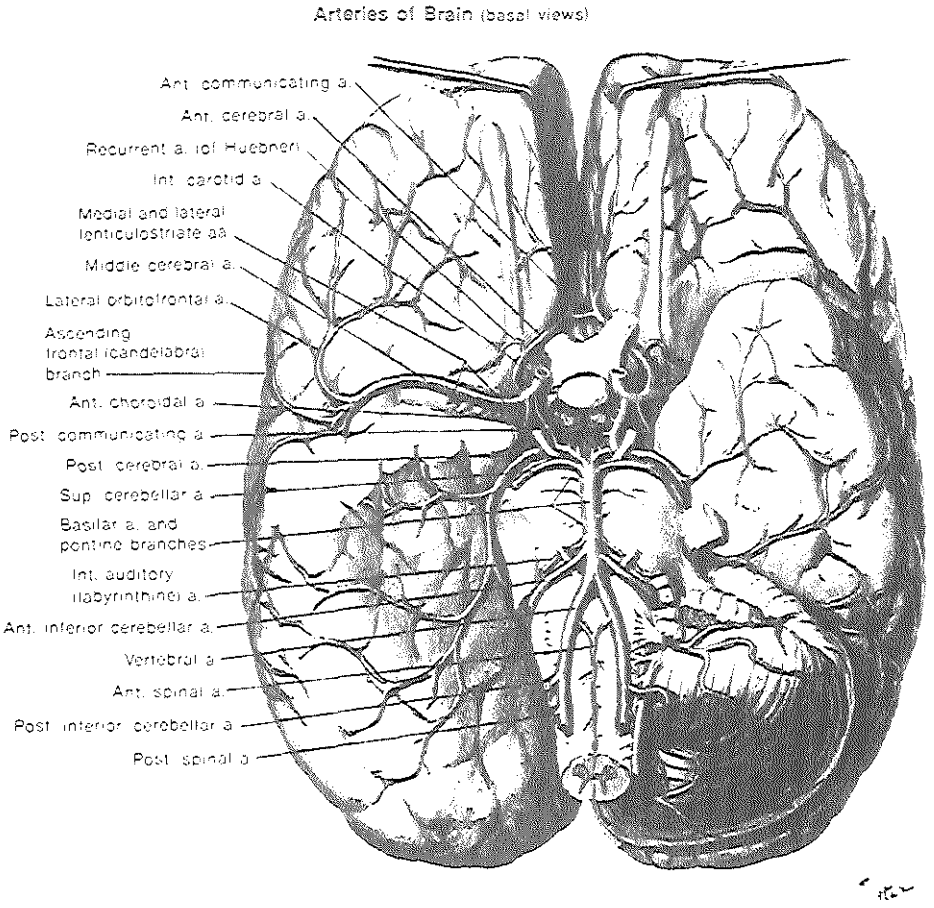


Figure 1 The anatomy of the cerebral arterial vasculature in man. Reproduced with permission from Dahl & Edvinsson, 1988.

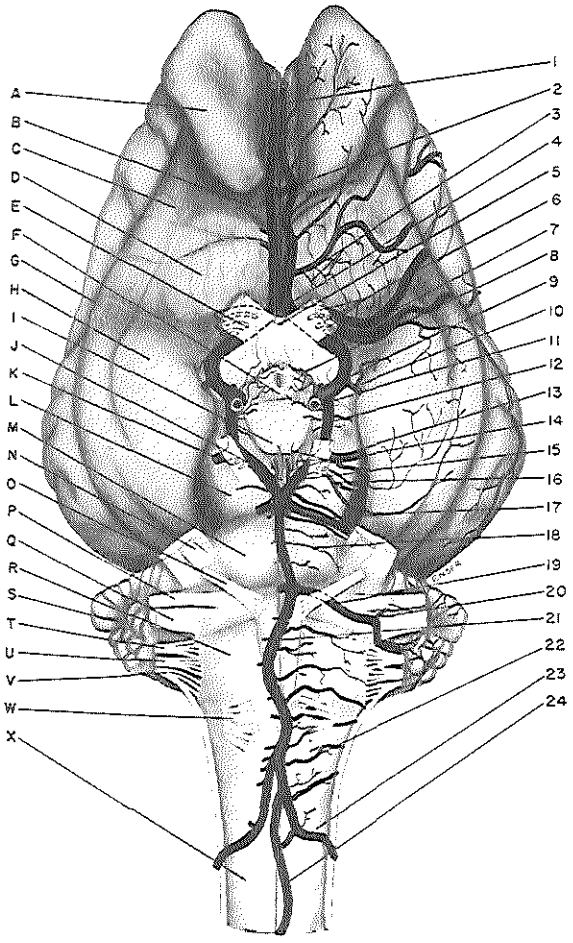


Figure 2 The anatomy of the cerebral arterial vasculature in the pig. Reproduced with permission from Ghoshal & Nanda, 1975. 1. Internal ethmoidal a.; 2. Common (median) a. of corpus callosum; 3. central (medial striate) branches; 4. Rostral cerebral a.; 5. Rostral communicating a.; 6. Middle cerebral a.; 7. Internal carotid a.; 8. Rostral (superior) hypophysial aa.; 9. Internal ophthalmic a.; 10. Rostral choroid a.; 11. Internal carotid a.; 12. Caudal communicating a. (proximal part); 13. Caudomedial (dorsomedial) branches; 14. Caudal cerebral a.; 15. Mesencephalic a.; 16. Branch to rostral mesencephalic tectum; 17. Rostral cerebellar a.; 18. Branch to pons; 19. Caudal cerebellar a.; 20. Middle cerebellar a. and labyrithine a.; 21. Basilar a.; 22. Medullary branch; 23. Vertebral a.; 24. Ventral spinal a.; A. Olfactory bulb; B. Medial olfactory tract; C. Lateral olfactory tract; D. Olfactory tubercle; E. Optic n.; F. Tuber cinereum; G. Lateral rhinal sulcus; H. Piriform lobe; I. Mamillary body; J. Caudal perforate substance; K. Oculomotor n.; L. Cerebral crus; M. Pons; N. Trigeminal n.; O. Abducent n.; P. Facial n.; Q. Vestibulocochlear n.; R. Medulla oblongata; S. Cerebellum; T. Glossopharyngeal n.; U. Vagus n.; V. Accessory n.; W. Hypoglossal n.; X. Spinal cord.

In man most of the brain (80%) derives its blood from the internal carotid arteries, whereas in pig most of the cerebral blood flow is derived from the external carotid arteries and the vertebral arteries. The vertebral arteries join in both species to form the basilar artery, which is wider in pigs than in man. In the human cerebral circulation the number and size of arterial anastomoses are limited. The internal carotid arteries form the anterior and middle cerebral arteries and perfuse the anterior two-thirds of the brain and the basilar artery forms the two posterior cerebral arteries which perfuse the occipital part of the brain. Anastomoses between these vessels occur in the Circle of Willis at the base of the brain, but these are usually badly developed and are not sufficient to prevent cerebral infarction if one of the arteries is obliterated.

In the porcine cerebral circulation a different situation exists. The Circle of Willis is well developed with large caudal communicating arteries between the internal carotid and basilar arteries and multiple anterior communicating arteries between the internal carotid arteries. Furthermore, the pig, like most other domestic animals but in contrast to man, possesses extensive rostral epidural retia mirabile. These are arterial plexuses at the base of the brain between the internal carotid arteries and at the side of the hypophysis. These plexuses derive their arterial input for a large part from branches of the external carotid artery, like the maxillary arteries and the middle and rostral meningeal arteries and for a smaller part from the internal carotid artery. Both retia mirabile are connected to each other by vascular anastomoses. The outflow from these two plexuses is by the internal carotid arteries, which at this point have enormously gained in size. Because of these extensive ramifications and plexuses every part of the porcine brain may be perfused by any of the cranial arteries and infarctions cannot be induced by occlusion of a single artery.

From the Circle of Willis branch off the six major cerebral arteries, two anterior, two middle and two posterior cerebral arteries. These are localized in the pia mater on the brain surface and branches from these perforate the brain tissue to perfuse the brain. The pial arteries behave differently from other arteries in the body in that they contribute for a large part to the vascular resistance in the brain. Furthermore, typical arterioles are not found in the brain circulation (Stromberg & Fox, 1972). Instead, precapillary arterioles have a thin muscular layer, consisting of only one or two layers of smooth muscle cells (McCulloch & Edvinsson, 1984).

The consequences of the differences in arterial supply of the brain are limited in the present kind of studies. The only exception is an underestimation of brain flow, since the contribution from the vertebral arteries has not been taken into account. The pharmacologic effects of the drugs on brain blood flow and resistance are determined in the smaller pial and cerebral arteries, where a similar anatomic situation exists as in man.

The brain arterioles, capillaries and venules and, to a lesser extent, the pial arteries, arterioles, and venules possess special features, termed the blood-brain barrier (Knudsen et al., 1988). Anatomically, the endothelial cells are joined together with tight junctions, only allowing lipid soluble substances to pass (Reese & Karnovsky, 1967; Westergaard & Bightman, 1973). Furthermore, pinocytotic vesicles are scarce and endothelial enzymes are present to degrade or modify ingested substances, like monoamine oxidase or tyrosine hydroxylase (Hardebo & Owman, 1980). This could be one reason why only perivascular application and not intravenous injection of some vasoactive substances influences the brain circulation (Connor et al., 1992). Different pathological circumstances may disrupt the blood-brain barrier, like sudden severe hypertension (Westergaard & Brøndsted, 1974) or cerebrovascular ischaemia (Siesjö & Ljundggren, 1973). Whether disruption of the blood-brain barrier occurs during migraine is still a matter of debate.

Dura mater

The dura mater is a well vascularized tissue, both in man (Kerber & Newton, 1973) and in the pig. In man the arterial supply of the dura mater is largely derived from the external carotid arteries, predominantly via the middle meningeal arteries, but branches from the internal carotid and vertebral arteries also contribute to dural perfusion. In the pig a similar situation exists, except that the external and internal carotid systems are connected to each other by the rostral epidural rete mirabile, from which branches supply the dura mater. In contrast to the cerebral microcirculation, capillaries and venules in the dura mater contain fenestrated endothelium, implying the absence of a blood-brain barrier (Andres et al., 1987; Knudsen et al., 1988). Rather, the layer of arachnoid cells bordering the dura mater is tied together with tight junctions (Knudsen et al., 1988). Therefore, the dura mater seems to be located outside the blood-brain barrier. A further feature of the dura mater is the occurrence of many arteriovenous anastomoses both in man (Rowbotham & Little, 1965; Kerber & Newton, 1973) and in the pig (this thesis).

Extracranial tissues

In man and in the pig the extracranial tissues are exclusively supplied by branches of the external carotid artery. They do not possess the specific features of the cerebral circulation and are comparable to peripheral vascular beds. Especially the cutaneous circulation of the pig is enriched with arteriovenous anastomoses (Saxena & Verdouw, 1985a). It is likely that they contribute to the temperature regulation, like the arteriovenous anastomoses in the rat tail (Richardson et al., 1991) or human hand (Raman & Vanhuyse, 1983).

Craniovascular physiology and pharmacology

Cerebral arteries and arterioles

The cerebral circulation presents some unique features. These are for a part related to the strict regulation of cerebral blood flow according to local demand. There is a strict relationship between local oxygen and glucose consumption and blood flow (Raichle et al., 1976; Kuschinsky et al., 1981). The major mechanism by which the brain regulates its perfusion appears to be metabolic. Neural mechanisms seem to play only a secondary role.

The metabolic regulation seems to be mediated by extracellular potassium- and hydrogen-ions: a short lasting hypoxia leads to an increase in the extracellular levels of these ions, again leading to vasodilatation (Kuschinsky et al., 1972). The metabolic requirements and the cerebral blood flow may be uncoupled during cerebral ischaemia (Nemoto et al., 1975) and during the migraine aura (Lauritzen et al., 1983b).

The innervation of the cerebral vascular bed differs from that in other body regions in that the cerebral arterioles are scarcely innervated. Most of the studies concerning the innervation and neurotransmitters of the cerebral blood vessels have been performed in rats, rabbits, cats or monkeys and evidence in man or pigs is scarce. The basilar artery, the cerebral arteries of the Circle of Willis and those running in the pia mater possess dense perivascular nerve plexuses innervated. Generally, the innervation becomes less dense when the vessels become smaller and very few nerve fibres accompany the intraparenchymal arterioles. Both vasodilator and vasoconstrictor neurotransmitters are found within these nerves (Edvinsson et al., 1988a; Brayden & Bevan, 1988; Lee et al., 1988). Constrictor neurotransmitters are noradrenaline (Nielsen & Owman, 1967; Lee et al., 1976), adenosine triphosphate (Muramatsu et al., 1981), neuropeptide Y (Edvinsson et al., 1983b) and 5-hydroxytryptamine (Chan-Palay, 1976; Chédotal & Hamel, 1990; Bonvento et al., 1991), while dilator neurotransmitters in these vessels comprise acetylcholine (Duckles, 1981), vasoactive intestinal polypeptide (Larsson et al., 1976), substance P (Edvinsson et al., 1981) and calcitonin gene-related peptide (Matsuyama et al., 1985; Uddman et al., 1985). For all these neurotransmitters receptors are found on the cerebral arteries, but the receptor density seems to decline when the blood vessels become smaller.

Constrictor neurotransmitters

Noradrenaline is localized in perivascular sympathetic axons, which have been demonstrated on pial arteries of the cat (Nielsen & Owman, 1967; Kuschinsky & Wahl, 1975) and on the rabbit basilar artery (Lee et al., 1976). It has been argued that all of these stem from the superior cervical ganglion (Edvinsson et al., 1972), although the brainstem locus coeruleus nuclei may provide also part of the noradrenaline neurons (Raichle et al., 1975; Bonvento et al., 1991). The effect of the sympathetic nervous system on the cerebral perfusion is uncertain. Conflictive results have been reported: no effect was seen in the cat (Alm & Bill, 1973) or dog (Heistad et al., 1977), but, on the other hand, profound decreases in cerebral blood flow were observed in the baboon (James et al., 1969), goat (Lluch et al., 1975), dog (D'Alecy & Feigel, 1972) and cats (Kuschinsky & Wahl, 1975). Species and methodological differences are likely to be involved. Similar differences have been found in the reactivity of cerebral blood vessels to noradrenaline. In a study on isolated porcine cerebral arteries, noradrenaline or transmural nerve stimulation failed to constrict these vessels; instead, relaxation was observed which was mediated by $ß_1$-adrenoceptors (Winquist et al., 1982). Likewise, noradrenaline is a very poor constrictor of the rabbit basilar artery (Lee et al., 1976). On the other hand, human cerebral arteries are susceptible to contraction with noradrenaline (Edvinsson et al., 1988a). This may reflect a difference in α- and ß-adrenoceptor density or coupling between the two species.

In the sympathetic axons noradrenaline seems to be co-stored with neuropeptide Y (Edvinsson et al., 1983b) and ATP (Burnstock et al., 1984). An additional role for neuropeptide Y in sympathetically induced contraction has been proposed, because such contractions, and especially the latter phase, cannot be completely inhibited by α-adrenergic antagonists (Lee et al., 1976). Furthermore, neuropeptide Y is a strong vasoconstrictor in feline cerebral arteries (Edvinsson et al., 1983b). The function of the sympathetic innervation of cerebral arteries is uncertain, although it may have a role in the protection of the cerebral circulation against hypertension (Kuschinsky, 1988).

Another neurotransmitter which can be demonstrated by immunocytochemistry in cerebral blood vessels is 5-hydroxytryptamine (Chan-Palay, 1976; Reinhard et al., 1979). There is a continuing debate on the origin of the neurons where the amine is stored. It has been proposed that all of the perivascular 5-hydroxytryptamine is contained in sympathetic neurones, where it has been taken up as a result of an immersion fixation procedure, in other words an artifact (Saito & Lee, 1987; Jackowski et al., 1989). This followed the observation of absent 5-hydroxytryptamine immunoreactivity after perfusion of the animal with Krebs buffer before sacrifice (Saito & Lee, 1987), or the pretreatment of the animal with 6-hydroxydopamine (Jackowski et al., 1989). On the other hand, there

is evidence in favour of genuinely serotonergic neurons originating in the dorsal raphe nuclei: 5-hydroxytryptamine immunoreactivity in rat intracerebral blood vessels disappeared after lesioning the dorsal raphe nuclei (Reinhard et al., 1979) and tryptophan-hydroxylase was found in neurons innervating some pial arteries and arterioles (Chédotal & Hamel, 1990). However, recently it has been demonstrated that such differences reflect different origins of 5-hydroxytryptaminergic nerves of large and small pial arteries, since cervical sympathectomy affected mainly the levels of the amine in the larger pial arteries and destruction of the intracerebral serotonergic pathways mainly affected the smaller pial vessels (Bonvento et al., 1991). Therefore, whether a serotonergic innervation of cerebral blood vessels exists, and in which type of blood vessel, remains to be established. It is, on the other hand, generally accepted that 5-hydroxytryptamine, when applied in vitro or perivascularly, is a potent constrictor of the larger cerebral arteries (Edvinsson et al., 1984; Hamel et al., 1989), including the human basilar artery (Forster & Whalley, 1982).

Two different 5-hydroxytryptamine receptors may be involved in these constrictor responses, depending on the species investigated: 5-HT_1 or 5-HT_2 receptors (see Bom & Saxena, 1991). In the pig basilar artery only a 5-HT_1 receptor contribution to the contractile response has been found (Van Charldorp & Wouters, 1990), while in human basilar artery both these receptors are involved (Forster & Whalley, 1982; Parsons et al., 1989). Whereas the effect of 5-hydroxytryptamine on larger cerebral arteries is predominantly constrictor, smaller cerebral arteries and arterioles tend to be dilated by the compound (Harper & McKenzie, 1977a). Although a physiological role of 5-hydroxytryptaminergic nerves in the cerebral circulation may be suspected, no definite function has yet been ascribed to this amine. Another source of 5-hydroxytryptamine may the blood platelets, but in the presence of an intact blood-brain barrier intravascular 5-hydroxytryptamine is devoid of activity (Harper & MacKenzie, 1977b). The amine may, however, be responsible for the vascular spasm accompanying subarachnoidal haemorrhage (Gaw et al., 1990). Furthermore, a role in migraine is suspected on the basis of a disturbed metabolism of 5-hydroxytryptamine in this disorder and the therapeutic efficacy of drugs related to this amine (Anthony et al., 1969; Saxena & Ferrari, 1989).

Dilator neurotransmitters

Two different neural systems have been reported to supply dilator neurotransmitters to the cerebral vascular bed. A parasympathetic, cholinergic system innervates the cerebral arteries running in the pia mater of many species, including man, although data on the pig are lacking (Lee et al., 1978; Florence & Bevan, 1979; Saito et

al., 1985). This system arises from the sphenopalatine and otic ganglia and uses acetylcholine and vasoactive intestinal polypeptide as neurotransmitters (Hara et al., 1985; Saito et al., 1985). This system is involved in the vasodilatation observed after experimental stimulation of the facial nerve, the superior sagittal sinus (lying in the dura mater) or other intracranial structures (Goadsby et al., 1984). This vasodilatation is resistant to atropine (Duckles, 1986), presumably because of the dilator effects of the co-released vasoactive intestinal polypeptide (Lee et al., 1984; Zagami et al., 1990).

Secondly, the cerebral blood vessels that run in the pia mater are innervated by afferents from the trigeminal nerve (Uddman et al., 1985). These afferents contain the sensory peptides, substance P, calcitonin gene-related peptide and neurokinin A (Uddman et al., 1985; Edvinsson et al., 1988b). All three peptides are vasodilators of which calcitonin gene-related peptide is the most potent, while substance P and neurokinin A increase vascular permeability and promote edema of the vascular wall (Moskowitz et al., 1990). Both the parasympathetic and the trigeminal systems have been implied in the pathogenesis of migraine, as described in Chapter 2 of this thesis. The physiological role of the parasympathetic vasodilator system is still unknown, although it has been proposed to be involved in coupling cerebral blood flow to metabolic requirements (Fahrenkrug, 1982).

Extracerebral blood vessels

Generally, extracerebral blood vessels have been less studied than the cerebral vessels described above. Since this thesis deals with pharmacologic effects on arteriovenous anastomoses, their regulation will be described. This paragraph will equally deal with some aspects of the circulation in the dura mater, because this tissue is thought to be involved in migraine.

Arteriovenous anastomoses

Arteriovenous anastomoses can be divided into those located on body surfaces and those in deep tissues. The superficial arteriovenous anastomoses play a role in the regulation of body temperature and in man and in the pig are located in the skin (Hales & Molyneux, 1988; this thesis), although in some other species, which predominantly lose heat through the tongue, they are most abundant in this tissue (Pleschka, 1984). The temperature regulation is complex and has been extensively investigated by the group of Hales (for a review, see Hales & Molyneux, 1988). Deep arteriovenous anastomoses are found in both the human (Rowbotham & Little, 1965; Kerber & Newton, 1973) and the

porcine (this thesis) dura mater, but their function is unknown and their regulation and pharmacology has remained uninvestigated. A role for the dura mater in the migraine attack has been proposed and antimigraine drugs may profoundly influence arteriovenous anastomotic blood flow. Therefore, the effect of antimigraine drugs on dural arteriovenous anastomoses has been investigated in Chapter 9 of this thesis.

The regulation of arteriovenous anastomoses has exclusively been investigated in the skin and tongue of different species. These are generally under a tight constrictor tone which can be reduced by a warm environment (but less than 42 °C) or heating of the spinal cord, but in general not by local heating (Hales & Molyneux, 1988). The sympathetic nature of this tone has been demonstrated by measuring electrical activity in sympathetic nerves during different thermal circumstances. They possess a dense sympathetic innervation which extends into the muscular layer of the media, whereas in most other blood vessels these nerves generally do not penetrate deeper than the adventitia (Hales & Molyneux, 1988). Furthermore, arteriovenous anastomoses in the sheep can be dilated by phentolamine and constricted by noradrenaline (Hales et al., 1982). This is in contrast to arteriovenous anastomoses in the head of the pig, which are rather insensitive to noradrenaline or sympathetic nerve stimulation (Verdouw et al., 1984a). The reason for this insensitivity is unknown, especially since in the conscious pig arteriovenous anastomoses are equally under a constrictor tone (Van Woerkens et al., 1990). There is a possibility of a role for neuropeptide Y which is present in the walls of arteriovenous anastomoses and functions as a co-transmitter of noradrenaline (Molyneux & Haller, 1988). 5-Hydroxytryptamine, which is commonly found in the wall of different cranial arteries and may also be co-stored with noradrenaline in sympathetic terminals, may also contribute to the tone of arteriovenous anastomoses. Furthermore, 5-hydroxytryptamine is a very powerful constrictor of arteriovenous anastomoses, a response that is mediated by a combination of 5-HT_1 and 5-HT_2 receptors (Saxena & Verdouw, 1982). The role of noradrenaline and 5-hydroxytryptamine is further investigated in chapter 11 of this thesis.

Arteriovenous anastomoses are not exclusively innervated by constrictor nerve fibres. Dilator cholinergic nerves have been demonstrated on some arteriovenous anastomoses, like the duck's foot (Molyneux & Harmon, 1982) and sheep tongue (Molyneux & Haller, 1988). In the skin, the evidence in favour of a cholinergic innervation is at best equivocal (Hales & Molyneux, 1988). Furthermore, there is a claim in favour of a dopaminergic, dilator innervation of the duck's foot and dog hindpaw (Bell & Lang, 1979; Bell & Rome, 1984). These systems may have a role in the active vasodilatation which has been observed in arteriovenous anastomoses containing tissues after adrenergic blockade (Hillman et al., 1982). Lastly, arteriovenous anastomoses are innervated by sensory fibres derived from the trigeminal nerve which contain the

neuropeptides substance P and calcitonin gene-related peptide (Molyneux & Haller, 1988). Especially calcitonin gene-related peptide is a potent dilator of arteriovenous anastomoses (Mogg et al., in press).

In conclusion, besides well known noradrenergic mechanisms controlling the diameter of superficial arteriovenous anastomoses, other neurotransmitters and systems are present, the function of which has not been elucidated.

Dura mater

As stated above, the dura mater is a well vascularized tissue. It contains both in human beings and pigs arteriovenous anastomoses, besides a capillary network (Kerber & Newton, 1973; this thesis), although in dogs functional arteriovenous anastomoses could not be demonstrated (Faraci et al., 1989). Little research has been devoted to the regulation and pharmacology of the circulation of the dura mater. Unlike the capillaries and venules of the brain and the pia mater, no blood-brain barrier is present on these vessels of the dura mater and the endothelium contains fenestrations (Knudsen et al., 1988)

The human dura mater is a very pain-sensitive structure and receives an innervation from the trigeminal nerve (Ray & Wolff, 1940). Furthermore, perivascular sympathetic nerves from the superior cervical ganglion (containing noradrenaline and neuropeptide Y) and a modest parasympathetic innervation from the sphenopalatine and otic ganglia (containing acetylcholine and, possibly, vasoactive intestinal polypeptide) have been demonstrated in this tissue (Uddman et al., 1989). The effects of the sympathetic and the parasympathetic systems on the dural circulation are not known, although it might be postulated from their effects on other vascular beds that they subserve a vasoconstrictor, respectively a vasodilator action. The trigeminal afferents innervating the dura mater contain substance P and calcitonin gene-related peptide (Keller & Marfurt, 1991). Besides a sensory role of these fibres, they have also been shown to have an efferent function. Substance P, when injected intravenously in dogs, causes an increase in dural blood flow (Faraci et al., 1989). Electrical stimulation of the trigeminal ganglion or the administration of capsaicin (which causes sensory afferents to release peptides) causes oedema and protein extravasation of the dura mater (Markowitz et al., 1987). This process, its pharmacological modification and possible role in migraine have been discussed in chapter 3. Lastly, a modest serotonergic innervation was observed (Keller & Marfurt, 1991). Conflictive results have been obtained with respect to the effect of 5-hydroxytryptamine on dural blood flow. In anaesthetized dogs the amine caused an

increase in dural blood flow (Faraci et al., 1989), whereas in the human isolated dura mater vasoconstriction was observed, which was mimicked by the selective 5-HT_1 agonists 5-carboxamidotryptamine and sumatriptan (Humphrey et al., 1991a). Postmortem changes or the effect of denervation on the dura mater may be responsible for this discrepancy. In chapter 9 of this thesis the effects of antimigraine drugs, including sumatriptan, on dural haemodynamics are presented.

Chapter 5
Aims of the Thesis

1. To characterize the receptor(s) involved in the constrictor effect of sumatriptan on the arteriovenous anastomoses and the dilator effect on arterioles in the porcine carotid circulation by the use of suitable antagonists (chapters 6 and 7).

2. To characterize the receptor(s) involved in the constrictor effect of ergotamine and dihydroergotamine on the arteriovenous anastomoses in the porcine carotid circulation (chapter 8).

3. To localize in the carotid circulation the arteriovenous anastomoses that are constricted by the three antimigraine drugs (chapter 9).

4. To compare the effects of ergotamine and sumatriptan on haemodynamics and the distribution of cardiac output in the pig (chapter 10).

5. To determine the receptor and neurotransmitter involved in the tonic constriction of arteriovenous anastomoses in conscious pigs by using a type of anaesthesia mimicking haemodynamically the conscious state (chapter 11).

Chapter 6

Role of 5-HT_1-Like Receptors in the Reduction of Porcine Cranial Arteriovenous Anastomotic Shunting by Sumatriptan

Summary

The new tryptamine derivative sumatriptan (GR43175) is effective in the treatment of migraine. Since several antimigraine agents reduce cranial arteriovenous anastomotic blood flow in the anaesthetized pig, we have investigated the carotid haemodynamic effects of sumatriptan.

Sumatriptan (10, 30, 100 and 300 $\mu g\ kg^{-1}$, i.v.) reduced total common carotid blood flow, exclusively by affecting its arteriovenous anastomotic fraction; the capillary fraction even increased with the highest doses. These reductions in the carotid arteriovenous anastomotic ("shunt") blood flow were mediated by a 5-HT_1-like receptor, as methiothepin, but not ketanserin, antagonized the responses to sumatriptan. Sumatriptan increased the difference in oxygen saturation between arterial and jugular venous blood, which is likely to be a consequence of the reduction of the carotid shunt blood flow.

The selective reduction in arteriovenous anastomotic blood flow produced by sumatriptan may reflect its antimigraine action, thought to involve vasoconstriction of those cranial vessels, be they "shunt" vessels or not, which are distended and inflamed during a migraine attack.

Introduction

Sumatriptan, a new tryptamine derivative, has a high affinity for 5-HT_{1D} (K_i=17 nM) and 5-HT_{1B} (K_i=27 nM) binding sites, an appreciable affinity for 5-HT_{1A} (K_i=100 nM) binding sites and minimal affinity for other 5-hydroxytryptamine (5-HT), adrenaline, dopamine, muscarine or benzodiazepine binding sites (Peroutka & McCarthy, 1989). Furthermore, sumatriptan seems to discern between two types of 5-HT_{1D} binding sites (Sumner & Humphrey, 1989). Functionally, sumatriptan causes contraction of different isolated blood vessels (Humphrey et al., 1989a, 1990a; Saxena & Ferrari, 1989), like the

dog saphenous vein (Humphrey et al., 1988) and middle cerebral artery (Humphrey et al., 1989a) and the dog, monkey and human basilar artery (Connor et al., 1989a; Parsons et al., 1989), by acting on a 5-HT_1-like receptor. In the anaesthetized dog and cat, sumatriptan increases the carotid arterial resistance (Feniuk et al., 1989a; Perren et al., 1989; MacLennan & Martin, 1990b). In the dog this decrease is mediated by a 5-HT_1-like receptor (Feniuk et al., 1989). Regional blood flow measurements with radioactive microspheres in the cat have shown that the increase in the carotid resistance is confined to its arteriovenous anastomotic fraction (Perren et al., 1989; MacLennan & Martin, 1990b); the receptor mechanism of this effect is not known.

Sumatriptan is effective in the treatment of the acute migraine attack and can abolish both the headache and the associated symptoms of migraine (Doenicke et al., 1988; Perrin et al., 1989). The selective reduction of cranial arteriovenous anastomotic blood flow in several anaesthetized species, amongst these the domestic pig, is a property shared with many antimigraine agents, including ergotamine (Johnston & Saxena, 1978; Spierings & Saxena, 1980a; Saxena et al., 1983; Bom et al., 1989a), dihydroergotamine (Spierings & Saxena, 1980a), methysergide (Saxena & Verdouw, 1984) and isometheptene (Spierings & Saxena, 1980b). In addition, 5-HT (Saxena & Verdouw, 1982; Saxena et al., 1986) and other drugs with affinity for 5-HT receptors — 5-carboxamidotryptamine (Saxena & Verdouw, 1985b), BEA 1654 (Verdouw et al., 1985), 8-OH-DPAT (Bom et al., 1989b), RU 24969 (Bom et al., 1989c) and indorenate (Villalón et al., 1990) — also constrict carotid arteriovenous anastomoses by acting on 5-HT_1-like receptors.

Since sumatriptan possesses both antimigraine properties and 5-HT_1-like agonist activity, we set out to study its effect on porcine carotid blood flow and its distribution, with special emphasis on the arteriovenous anastomotic fraction, with the radioactive microsphere method (Saxena et al., 1980). By using the 5-HT receptor antagonists methiothepin (5-HT_1 and 5-HT_2) and ketanserin (5-HT_2), we also tried to establish if indeed sumatriptan acted via a 5-HT_1-like receptor, since, despite a high binding affinity to 5-HT_1-binding sites, the vasoconstrictor action of the antimigraine drug ergotamine on arteriovenous anastomoses seems to be mediated by undefined receptors, distinct from 5-HT_1-like, 5-HT_2 or adrenoceptors (Bom et al., 1989a). A part of this study has been presented to the British Pharmacological Society (Den Boer et al., 1990).

Methods

General

After an overnight fast 33 domestic pigs (Yorkshire x Landrace; 16-22 kg) were anaesthetized with 120 mg azaperone i.m. and 150 mg metomidate i.v. (both from Janssen Pharmaceutica, Beerse, Belgium), intubated and connected to a respirator (Bear 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; pO_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of pentobarbitone sodium (Sanofi, Paris, France) at 20 $mg.kg^{-1}.h^{-1}$ for the first hour and thereafter 12 $mg.kg^{-1}.h^{-1}$).

Catheters were placed in the inferior vena cava via a femoral vein for the administration of drugs and in the aortic arch via a femoral artery, connected to a Statham pressure transducer (P23 Dc, Hato Rey, Puerto Rico) for the measurement of arterial blood pressure and the withdrawal of arterial blood for determining blood gases (ABL-2, Radiometer, Copenhagen, Denmark). Mean arterial blood pressure (MAP) was calculated from the systolic (SAP) and diastolic (DAP) arterial pressures: MAP = (SAP + 2 x DAP) / 3. The common carotid arteries were dissected free and the cervical vagosympathetic trunks were cut. Blood flow was measured in one of the common carotid arteries with a flow probe (internal diameter: 2.5 or 3 mm) connected to a sine-wave electromagnetic flow meter (Transflow 600-system, Skalar, Delft, The Netherlands). Heart rate was measured with a tachograph triggered from the blood pressure or the flow signal, depending on their shape. A 0.5 mm (external diameter) needle, connected to a polyethylene tubing was inserted into the common carotid artery against the direction of the blood flow for the administration of radioactive microspheres. At the same side the jugular vein was cannulated in order to obtain venous blood samples for determining blood gases.

During the experiment body temperature was kept at about 37 °C and the animal was continuously infused with 100 $ml.h^{-1}$ saline to compensate for fluid losses.

Distribution of common carotid blood flow

The distribution of common carotid blood flow was determined with 15 $\pm$ 1 (S.D.) μm diameter microspheres labelled with either ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Company, Dreieich, West Germany). For each measurement a suspension of about 200,000 microspheres, labelled with one of the isotopes, was mixed and injected into the carotid artery against the direction of the blood flow to ensure uniform mixing.

At the end of the experiment the animals were killed and the heart, kidneys, lungs and the different cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 5-10 min in a γ-scintillation counter (Packard, Minaxi Autogamma 5000) using suitable windows for discriminating the different isotopes.

The ratio between the radioactivity in a particular tissue and the total radioactivity was calculated with a set of specially developed computer programs (Saxena et al., 1980). By multiplying this ratio with the total carotid blood flow value at the time of the injection, blood flow to the tissues (nutrient blood flow) was determined. No radioactivity could be detected in the heart or the kidneys, so all microspheres reaching the venous side by arteriovenous anastomoses were trapped in the lungs. Therefore, the amount of radioactivity in the lungs was used as an index for the arteriovenous anastomotic part of the common carotid blood flow (see Johnston & Saxena, 1978; Saxena & Verdouw, 1982). The respective conductances were determined by dividing blood flow by mean arterial blood pressure.

Experimental protocol

After a stabilization period of one hour the animals were divided into five groups. The first group (n=6) received 10 ml boluses of saline at the times the other animals received the respective doses of sumatriptan; in this group the stability of the preparation was evaluated. The four other groups received sumatriptan in cumulative doses of 10, 30, 100 and 300 $\mu g.kg^{-1}$, administered every 15 min, after pretreatment with either saline (n=8), ketanserin (0.5 $mg.kg^{-1}$,n=6), methiothepin (1 $mg.kg^{-1}$, n=7) or methiothepin (3 $mg.kg^{-1}$, n=6). All pretreatments were given i.v. over 30 min. After the pretreatment and after each dose of sumatriptan, measurements of heart rate, mean blood pressure, carotid blood flow and its distribution and arterial and jugular venous blood gases were made.

Data presentation and statistical analysis

All data have been expressed as means $\pm$ SEM. The significance of the differences between the variables within one group was evaluated with Duncan's new multiple range test once an analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). Between groups the respective changes at the same dose of sumatriptan were evaluated with a Student's t-test. Statistical significance was accepted at $P<0.05$ (two-tailed).

Drugs

Apart from the anaesthetics the drugs used in this study were: sumatriptan (GR43175; gift: Glaxo Research Laboratories, Ware, UK), ketanserin tartrate (gift: Dr J.M. Van Nueten, Janssen Pharmaceutica, Beerse, Belgium), methiothepin maleate (gift: Hoffman La Roche B.V., Mijdrecht, The Netherlands) and heparin sodium (Thromboliquine, Organon Teknika B.V., Boxtel, The Netherlands) to prevent clotting of the catheters. Sumatriptan was dissolved in physiological saline. Ketanserin tartrate and methiothepin maleate were dissolved in 5 ml propylene glycol 20% in distilled water and subsequently diluted with 45 ml physiological saline. All doses refer to the respective salts.

Results

Stability of the preparation during saline treatment

The effects of four consecutive bolus injections of saline after saline pretreatment are presented in Table 1. No significant changes occurred during the experiment in total common carotid blood flow and its distribution in arteriovenous anastomotic and nutrient blood flow. Heart rate also remained constant, but there was a small decrease in mean arterial blood pressure (-5 ± 2%).

Table 1 Stability of the preparation during the experimental period where 5 i.v. bolus injections of physiological saline were given (n=6).

	Time of injection after the start of the experiment (min)				
	0	15	30	45	60
HR	87±6	86±6	86±6	86±6	84±6
MAP	78±7	77±8	77±8	76±8	75±8*
Car F	198±18	190±19	194±17	187±14	186±13
AVA F	151±22	147±22	153±23	148±20	140±17
Nut F	46±7	43±7	40±7	38±7*	46±7

HR, Heart rate (beats.min^{-1}); MAP, mean arterial pressure (mmHg); Car, total carotid; AVA, arteriovenous anastomotic; Nut, nutrient; F, flow (ml.min^{-1}). All values have been presented as means ± SEM.
*, $P<0.05$ vs baseline.

Table 2 Effects of intravenous bolus injections of sumatriptan (10, 30, 100 and 300 $\mu g.kg^{-1}$) on systemic haemodynamic variables after pretreatment with saline, ketanserin (0.5 mg.kg-1), methiothepin (1 $mg.kg^{-1}$) or methiothepin (3 $mg.kg^{-1}$).

Pretreatment	Baseline	Sumatriptan ($\mu g.kg^{-1}$)			
		10	30	100	300
Heart rate (beats.min^{-1})					
Saline	93±2	90±2*	88±2*	85±3*	82±3*
Ketanserin	88±7	86±6	83±5	82±4	84±5
Methiothepin 1	91±4	90±5	87±5	82±4*	78±4*
Methiothepin 3	84±2	83±2	82±2*	80±3*	78±3*+
Mean arterial blood pressure (mmHg)					
Saline	85±4	86±4	85±4	83±6	76±8
Ketanserin	75±7	75±6	76±5	74±4	74±3
Methiothepin 1	89±7	86±5	82±5	82±6	83±6
Methiothepin 3	89±5	88±7	86±6	85±6	83±6*

All values have been presented as means ± SEM. *, $P<0.05$ vs baseline.
+, $P<0.05$ vs the corresponding dose in saline pretreated animals.

Changes in the systemic haemodynamics by sumatriptan

The effects on heart rate and blood pressure of i.v. infusions of sumatriptan after pretreatments with either physiological saline, ketanserin (0.5 $mg.kg^{-1}$) or methiothepin (1 or 3 $mg.kg^{-1}$) are summarized in Table 2. None of the pretreatments resulted in a significant difference in mean arterial blood pressure, compared to the saline pretreated group, though after ketanserin blood pressure was slightly decreased. In the group pretreated with the higher dose of methiothepin, the initial heart rate was significantly lower than in the saline pretreated group.

Sumatriptan induced a small dose-dependent decrease in heart rate, which was not present after ketanserin. Also after methiothepin 3 $mg.kg^{-1}$ the bradycardia after the highest dose of sumatriptan was blunted, although it must be noted that the baseline (pre-sumatriptan) heart rate in this group was lower.

There was a small (in most cases statistically not significant) decrease in mean blood pressure during the experiments in all groups except in the ketanserin pretreated group, in which the baseline blood pressure was already lower.

Changes in the carotid haemodynamics

The effects of i.v. injections of sumatriptan on the total carotid blood flow and its distribution into arteriovenous anastomotic and arteriolar (nutrient) blood flow are shown in Figure 1 (absolute values) and Figure 2 (% changes from baseline). In all groups about 80% of the carotid blood flow was initially diverted through arteriovenous anastomoses. In the saline-pretreated animals sumatriptan caused a dose-dependent decrease in arteriovenous anastomotic blood flow. At the highest dose of sumatriptan (300 $\mu g.kg^{-1}$ or 440 $\mu g.kg^{-1}$ cumulative dose) arteriovenous anastomotic blood flow had decreased by 66 $\pm$ 10%. Total carotid blood flow had decreased only by 39 $\pm$ 9%, because nutrient (tissue) blood flow had increased by 36 $\pm$ 18%.

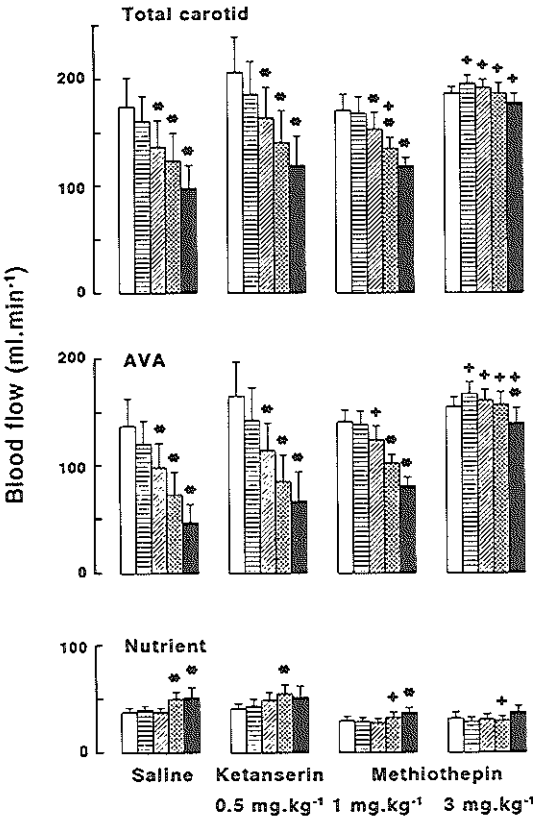


Figure 1 Effect of sumatriptan i.v. on the total carotid blood flow and its arteriovenous anastomotic (AVA) and nutrient fractions in pigs, pretreated i.v. with either saline (n=8), ketanserin 0.5 $mg.kg^{-1}$ (n=6), methiothepin 1 $mg.kg^{-1}$ (n=7) or methiothepin 3 $mg.kg^{-1}$ (n=6). From left to right the bars signify: Before sumatriptan (after pretreatment) and sumatriptan 10, 30, 100 and 300 $\mu g.kg^{-1}$. All values have been presented as means $\pm$ SEM. *, $P<0.05$ vs baseline; +, $P<0.05$ vs the corresponding dose of sumatriptan in the saline pretreated animals.

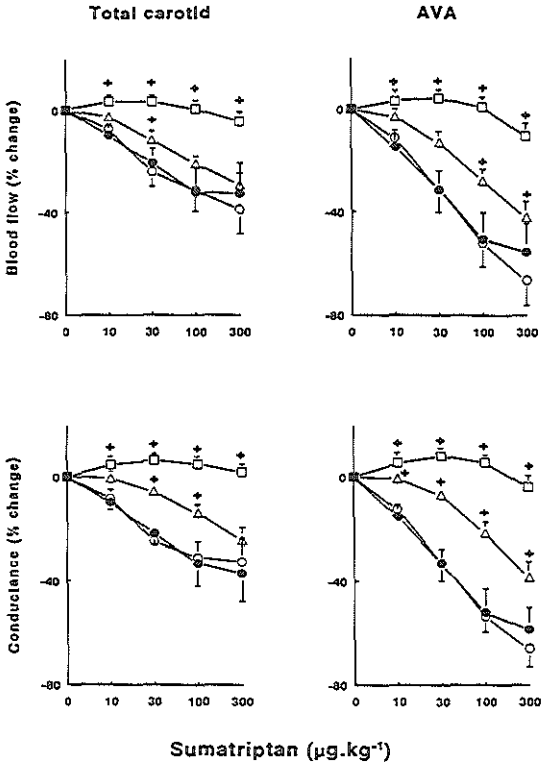


Figure 2 Percent change from baseline by sumatriptan i.v. in the total carotid and arteriovenous anastomotic blood flow and conductance in pigs, pretreated i.v. with either saline (closed circles, n=8), ketanserin 0.5 mg.kg^{-1} (open circles, n=6), methiothepin 1 mg.kg^{-1} (triangles, n=7) or methiothepin 3 mg.kg^{-1} (squares, n=6). All values have been presented as means ± SEM. +, $P<0.05$ vs the corresponding dose of sumatriptan in the saline pretreated animals.

In the animals pretreated with ketanserin (0.5 mg.kg^{-1}) none of these responses was modified, either on the arteriovenous anastomoses or on the arteriolar fraction. However, pretreatment with methiothepin at 1 mg.kg^{-1} attenuated and at 3 mg.kg^{-1} almost completely blocked the effects of sumatriptan on the arteriovenous anastomoses. Methiothepin also attenuated the effect of sumatriptan on nutrient blood flow, especially after 100 μg.kg^{-1} of sumatriptan. Since arterial blood pressure was not much changed during the experiments, the pattern of changes in the vascular conductances by sumatriptan was similar to that in the blood flows. Ketanserin did not modify, but methiothepin antagonized the sumatriptan-induced increases in both total carotid and arteriovenous anastomotic conductance.

The effects of sumatriptan on the distribution of arteriolar, nutrient blood flow to the different tissues are shown in figure 3. Sumatriptan increased the arteriolar blood flow to the ears (up to 188 ± 78%), head skin (up to 148 ± 47%), head bones (up to 47 ± 27%) and head fat (up to 148 ± 74%), whereas the arteriolar blood flow to the muscles, eyes, brain and salivary glands remained unchanged. The sumatriptan-induced increase in arteriolar blood flow to the above mentioned tissues was unchanged by ketanserin. Methiothepin, however, attenuated this response to 30 and 100 $\mu g.kg^{-1}$ of sumatriptan.

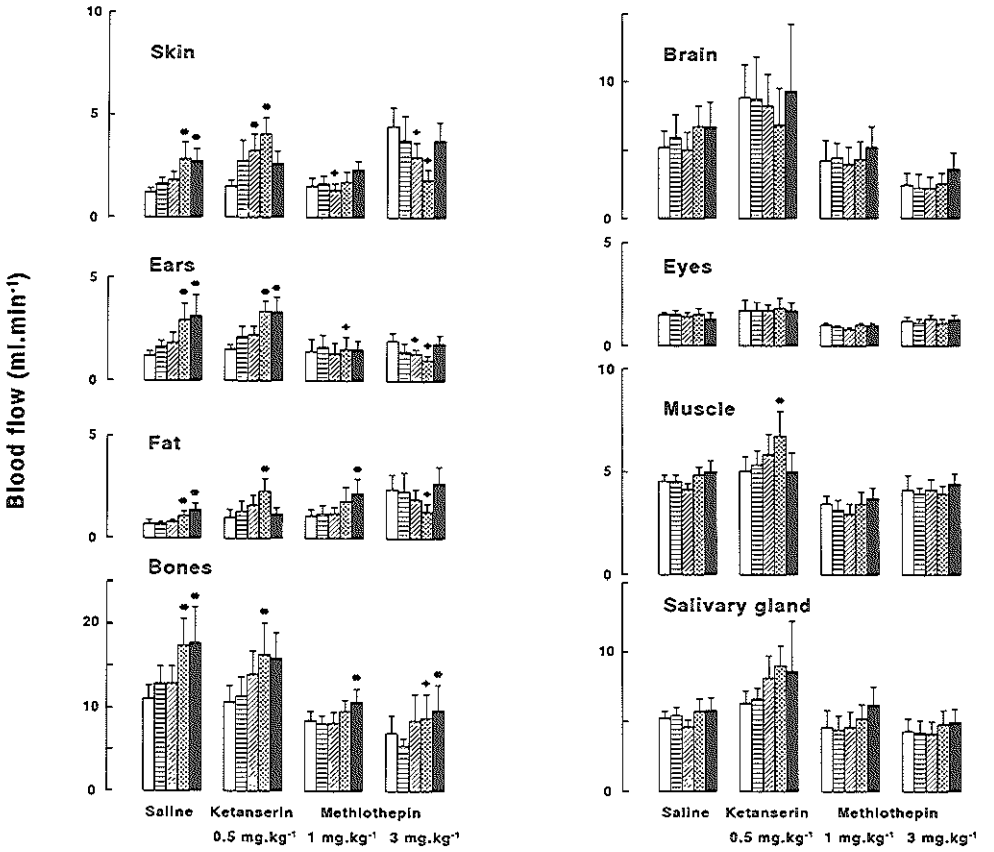


Figure 3 Effect of sumatriptan i.v. on the regional carotid blood flow distributed to different cranial tissues in pigs, pretreated i.v. with either saline (n=8), ketanserin 0.5 $mg.kg^{-1}$ (n=6), methiothepin 1 $mg.kg^{-1}$ (n=7) or methiothepin 3 $mg.kg^{-1}$ (n=6). From left to right the bars signify: Before sumatriptan (after pretreatment) and sumatriptan 10, 30, 100 and 300 $\mu g\ kg^{-1}$. All values have been presented as means ± SEM. *, $P<0.05$ vs baseline; +, $P<0.05$ vs the corresponding dose of sumatriptan in the saline pretreated animals.

Changes in the blood gases

Sumatriptan caused an increase in the difference in oxygen saturation between arterial and jugular venous blood (Table 3). There was a clear tendency towards attenuation of this effect in the methiothepin pretreated groups. Though the oxygen saturation changes by sumatriptan in the methiothepin- and saline-treated groups did not differ significantly ($P>0.05$), the values after the two highest doses of sumatriptan in the animals treated with methiothepin (3 $mg.kg^{-1}$), in contrast to those in the saline-treated animals, were not significantly different from the baseline values (Table 3).

Table 3 Effects of intravenous bolus injections of sumatriptan (10, 30, 100 and 300 $\mu g.kg^{-1}$) on the arteriovenous difference in oxygen saturation (%) after pretreatment with saline, ketanserin (0.5 mg.kg-1), methiothepin (1 $mg.kg^{-1}$) or methiothepin (3 $mg.kg^{-1}$).

Pretreatment	Baseline	Sumatriptan ($\mu g.kg^{-1}$)			
		10	30	100	300
Saline	9.4±4.2	9.9±4.1	13.2±6.3	13.7±5.6*	15.3±5.2*
Ketanserin	7.6±2.5	7.7±2.4	8.4±2.6	9.8±2.5*	11.0±3.6*
Methiothepin 1	5.6±1.4	6.4±1.1	6.6±1.2	7.8±1.5*	7.6±1.0*
Methiothepin 3	5.3±1.2	4.3±1.2	6.0±2.1	7.1±2.8	7.3±2.1

All values have been presented as means ± SEM. *, $P<0.05$ vs baseline.
+, $P<0.05$ vs the corresponding dose in saline pretreated animals.

Discussion

Systemic haemodynamic changes after sumatriptan

After each dose of sumatriptan a small decrease in heart rate was observed. This is likely to be an effect of the drug, as in the saline treated group no significant changes in heart rate were observed. Furthermore, in anaesthetized dogs almost identical falls in heart rate occurred after sumatriptan administration (Feniuk et al., 1989a). The mechanism of this decrease in heart rate is unknown. After ketanserin pretreatment the sumatriptan-induced decreases in heart rate were slightly blunted. This is unlikely to have been caused by the 5-HT_2 or α_1-adrenoceptor antagonist action of ketanserin, since sumatriptan has minimal affinity for these receptors (Peroutka & McCarthy, 1989). Likewise, after 3 mg kg^{-1} of methiothepin the highest dose of sumatriptan caused significantly less heart rate reduction. In this case the baseline heart rate differed from

the other groups. It should, however, be noted that these changes were rather small, so that their significance is doubtful. In clinical studies with sumatriptan no changes in heart rate have been reported (Perren et al., 1989).

Unlike the antimigraine drug ergotamine which usually increases blood pressure by increasing total peripheral resistance (see Saxena & De Vlaam-Schluter, 1974; Johnston & Saxena, 1978), sumatriptan did not increase arterial blood pressure. This lack of a hypertensive effect of sumatriptan can be explained by its more selective vasoconstrictor action on cranial blood vessels. In anaesthetized dogs sumatriptan had no influence on total peripheral resistance at doses which induced a marked increase in carotid resistance (Feniuk et al., 1989a).

Carotid haemodynamics

Sumatriptan elicited a dose-dependent reduction in common carotid blood flow, which was directly related to a decrease in its arteriovenous anastomotic fraction, as found in the cat (Perren et al., 1989; MacLennan & Martin, 1990b). The decrease in the arteriovenous anastomotic blood flow was also reflected in the decrease in the oxygen saturation in the jugular venous blood. Pretreatment with ketanserin (0.5 $mg.kg^{-1}$) did not change the effects of sumatriptan. This dose of ketanserin is adequate to block 5-HT_2 receptor mediated responses (Saxena & Lawang, 1985; Schalekamp & Wenting, 1990). In contrast to ketanserin, methiothepin antagonized the sumatriptan-induced reductions in both total carotid and arteriovenous anastomotic blood flows. Therefore, sumatriptan appears to reduce arteriovenous anastomotic blood flow via stimulation of a 5-HT_1-like receptor. In this sense sumatriptan behaves in a similar way as 8-OH-DPAT (Bom et al., 1989b), RU 24969 (Bom et al., 1989c) and indorenate (Villalón et al., 1990). Whereas 1 $mg.kg^{-1}$ of methiothepin markedly antagonized the reduction in arteriovenous anastomotic blood flow by the above-mentioned agonists, in the present experiments the antagonist potency of this dose of methiothepin was less marked and for a full inhibition of the response of sumatriptan a dose of 3 $mg.kg^{-1}$ of methiothepin was needed. A possible reason of the difference may be the greater selectivity of sumatriptan which does not appear to affect the carotid circulation via a 5-HT_2-receptor mechanism, since ketanserin left the responses unchanged. In contrast, with 5-HT, RU 24969 and indorenate a small ketanserin-sensitive 5-HT_2 receptor component is also noticed in the reduction of arteriovenous anastomotic blood flow (see Saxena & Verdouw, 1984; Verdouw et al., 1984b; Bom et al., 1989c; Villalón et al., 1990). RU 24969 is also known to activate 5-HT_2 receptors (Feniuk & Humphrey, 1989). It may be that the slightly greater antagonist activity of methiothepin against these agonists reflects this additional 5-HT_2

receptor component; indeed, methiothepin is a more potent 5-HT_2 receptor than a 5-HT_1-like receptor antagonist (Bradley et al., 1986).

Sumatriptan caused a modest increase in the arteriolar fraction of the carotid blood flow to the ears, head skin, head bones and fat. This is possibly a consequence of a decrease in arteriovenous anastomotic flow in these tissues and the resulting diversion of blood flow. However, a vasodilator action, either direct or via inhibition of neurogenically mediated vasoconstrictor tone, may be involved, since indorenate did not cause an increase in the nutrient fraction of the carotid blood flow, despite similarly decreasing arteriovenous anastomotic blood flow (Villalón et al., 1990). Moreover, sumatriptan can elicit endothelium-dependent relaxation in the pig coronary artery (Schoeffter & Hoyer, 1989a) and it can also inhibit sympathetically mediated constriction and cyclic-AMP accumulation in the dog saphenous vein (Humphrey et al., 1988; Sumner & Humphrey, 1990). Since in our experiments the cranial vascular bed was sympathetically denervated, such an inhibition of sympathetic tone seems unlikely. In any case, if such a dilator action of sumatriptan is present in the cephalic vascular beds, it is very weak compared to 5-HT (Saxena & Verdouw, 1982) or 5-carboxamidotryptamine (Saxena & Verdouw, 1985b), both of which induce massive vasodilation and redness in the skin. The attenuation of the vasodilator effect by methiothepin is compatible with both a direct and an indirect effect of sumatriptan.

Nature of the 5-HT_1-like receptors involved in the reduction of cephalic arteriovenous anastomotic blood flow

In view of the heterogeneity of 5-HT_1-like receptors (Bradley et al., 1986; Humphrey & Feniuk, 1988; Saxena & Villalón, 1990) one may ask which of the several subtypes of the 5-HT_1-like receptor mediates the reduction in arteriovenous anastomotic blood flow by sumatriptan in the pig? Sumatriptan has a high affinity for 5-HT_{1D} and 5-HT_{1B} binding sites and a reasonably high affinity for 5-HT_{1A} sites (Peroutka & McCarthy, 1989; Schoeffter & Hoyer, 1989b). Because the other putative 5-HT_{1A} receptor agonists BEA 1654 (Verdouw et al., 1985), 8-OH-DPAT (Bom et al., 1989b) and indorenate (Villalón et al., 1990), also reduce arteriovenous shunting it is tempting to suggest that 5-HT_{1A} receptors are involved. However, the selective 5-HT_{1A} receptor agonist ipsapirone (Peroutka, 1986) was inactive in the pig carotid circulation (Bom et al., 1988) and the reduction in arteriovenous anastomotic blood flow by RU 24969, a 5-HT_{1A} and 5-HT_{1B} receptor agonist, is not amenable to blockade by (±)-pindolol, an antagonist at both these receptors (Hoyer, 1988). Therefore, neither 5-HT_{1A} nor 5-HT_{1B} receptors seem to be involved. The involvement of 5-HT_{1C} receptors is also unlikely because of a

very high potency of 5-carboxamidotryptamine in the present experimental model (Saxena & Verdouw, 1985b).

Compared to the above 5-HT_1-like receptor subtypes, the 5-HT_{1D} receptor would seem a more likely candidate, since sumatriptan, ergotamine, dihydroergotamine, methysergide, RU 24969, 8-OH DPAT and indorenate all bind with a reasonable affinity to the 5-HT_{1D} receptor (see Hoyer, 1989). On the other hand, the sumatriptan-induced contraction of the dog saphenous vein (Humphrey et al., 1988) and the indorenate-induced reduction in the porcine carotid arteriovenous anastomotic blood flow (Villalón et al., 1990) are both resistant to metergoline, which has a high affinity for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} receptors (Hoyer, 1988) and can antagonize a potentially 5-HT_{1D} receptor-mediated effect, the endothelium-dependent relaxation of the pig coronary artery by 5-HT (Schoeffter & Hoyer, 1990). Though in another putative functional model for 5-HT_{1D} receptors, inhibition of adenylate cyclase production in calf substantia nigra, metergoline behaved almost as a full agonist (Schoeffter et al., 1988), no agonist effect of metergoline was observed in the cranial arteriovenous anastomoses (Villalón et al., 1990). Taken together, the evidence to-date indicates that the 5-HT_1-like receptor mediating the reduction of arteriovenous anastomotic blood flow in the pig does not fully correspond to any of the subtypes characterized so far.

Do sumatriptan and ergotamine constrict porcine arteriovenous anastomoses by acting on different receptors?

We have earlier reported that the constriction of arteriovenous anastomoses by ergotamine, being resistant to blockade by phentolamine (0.5 $mg.kg^{-1}$), pizotifen (0.5 $mg.kg^{-1}$), ketanserin (0.5 $mg.kg^{-1}$) and methiothepin (1.0 $mg.kg^{-1}$), is not mediated by either α-adrenoceptors or 5-HT_2 and 5-HT_1-like receptors (Saxena et al., 1983; Bom et al., 1989a). However, 1 $mg.kg^{-1}$ of methiothepin did not prove much more effective against sumatriptan in the present study than it was against ergotamine earlier (Bom et al., 1989a). Therefore, in view of this new data with sumatriptan, the lack of involvement of the 5-HT_1-like receptor (on which sumatriptan acts) in the ergotamine-induced constriction of the shunt vessels remains open until the use of 3 $mg.kg^{-1}$ dose of methiothepin against ergotamine in this model.

Is the constriction of arteriovenous anastomoses likely to contribute to the antimigraine activity of sumatriptan?

In a study in 7 migraine patients during an attack, Heyck (1969) found the jugular venous oxygen saturation to be elevated on the affected side; treatment with dihydroergotamine normalized the oxygen saturation on the affected side. Heyck suggested that one

explanation for this phenomenon could be an abnormal dilation of arteriovenous anastomoses during the migraine attack, increasing the amount of shunted blood from which no oxygen can be extracted (see also Saxena & Ferrari, 1989; Saxena, 1990). Arteriovenous anastomoses do exist in human cranial, non-cerebral tissues such as the dura mater and skin (Rowbotham & Little, 1965; Kerber & Newton, 1973), but it is fair to point out that they may not convey the same volume of blood as in anaesthetized domestic animals and that there is no direct information on their behaviour during a migraine attack. Nevertheless, in view of the possible involvement of arteriovenous anastomoses in migraine and the fact that the reduction of arteriovenous anastomotic blood flow in our animal experimental model is a feature sumatriptan shares with several other antimigraine agents like ergotamine (Johnston & Saxena, 1978; Spierings & Saxena, 1980a; Saxena et al., 1983; Bom et al., 1989a), dihydroergotamine (Spierings & Saxena, 1980a), methysergide (Saxena & Verdouw, 1984) and isometheptene (Spierings & Saxena, 1980b), it seems reasonable to suggest that the constriction of arteriovenous anastomoses may play a part in the mechanism of therapeutic action of sumatriptan and other antimigraine drugs.

It has recently been shown that ergotamine, dihydroergotamine, methysergide and sumatriptan inhibit the extravasation of plasma proteins from dural blood vessels following stimulation of the trigeminal ganglion and that this action could be responsible for their antimigraine action (Markowitz et al., 1988; Saito et al., 1988; Buzzi & Moskowitz, 1990). It should, however, be noted that, except for sumatriptan, the doses of these antimigraine drugs needed to inhibit protein extravasation were higher than the doses used in patients against migraine attacks and also higher than the doses needed to cause a substantial decrease in arteriovenous anastomotic blood flow. Furthermore, it cannot be ruled out that the reduction in plasma extravasation by these drugs is secondary to the constriction of the leaking vessels within the carotid vascular bed. The lack of inhibition of neurogenic and capsaicin-induced plasma extravasation by angiotensin II and phenylephrine (see Markowitz et al., 1988; Saito et al., 1988) may not signify independence from the vasoconstrictor effect of the antimigraine drugs, since these results were not backed up by simultaneous measurement of dural (or carotid) blood flow or even arterial blood pressure. It is uncertain if the bolus injections of phenylephrine, as used by Markowitz et al. (1988), have a sustained vasoconstrictor effect or if phenyleprine or angiotensin II actually increase resistance in that dural vascular bed responsible for protein extravasation.

Chapter 7

5-HT_1-Like Receptor Mediated Changes in Porcine Carotid Haemodynamics: Are 5-HT_{1D} Receptors Involved?

Summary

5-Hydroxytryptamine (5-HT) reduces porcine arteriovenous shunting in the carotid vascular bed by stimulation of both 5-HT_1-like and 5-HT_2 receptors and increases capillary flow to some tissues, like the skin and ears, by different 5-HT_1-like receptors. In view of the heterogeneous nature of the 5-HT_1-like receptors and the relative selectivity for the 5-HT_{1D} binding sites of sumatriptan, which also reduces porcine arteriovenous shunting and slightly increases capillary blood flow towards skin and ears by 5-HT_1-like receptors, we have attempted to determine whether one or both of these carotid 5-HT_1-like receptors belong to the 5-HT_{1D} subtype. Pentobarbitone anaesthetized pigs, subjected to bilateral cervical vagosympathectomy, received either 5-HT (2 $\mu g.kg^{-1}.min^{-1}$) in the carotid artery or cumulative i.v. doses of sumatriptan (10, 30, 100 and 300 $\mu g.kg^{-1}$). Their effect on the total carotid blood flow and its distribution into capillary and arteriovenous anastomotic parts was determined with radioactive microspheres. The effect of metergoline (1 $mg.kg^{-1}$), a substance with a very high affinity for the 5-HT_{1D} receptor as well as for the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_2 receptors, was studied on the responses to 5-HT and sumatriptan.

Both 5-HT and sumatriptan reduced carotid arteriovenous anastomotic blood flow. 5-HT and, to a lesser extent, sumatriptan also increased capillary blood flow towards some tissues. Metergoline by itself did not affect the distribution of porcine carotid blood flow. It attenuated the constrictor response, but increased the vasodilator response to 5-HT, in a manner similar to the 5-HT_2 receptor antagonists cyproheptadine, ketanserin and WAL 1307 in our former experiments. These effects seem, therefore, to be related to the blockade of 5-HT_2 receptors by metergoline. On the other hand, metergoline had no significant effect against the responses to sumatriptan.

It is concluded that neither the constrictor nor the dilator carotid 5-HT_1-like receptors seem to be related to the known 5-HT_1 binding subtypes, including the 5-HT_{1D} subtype.

Introduction

Both 5-hydroxytryptamine (5-HT) and sumatriptan decrease arteriovenous shunting of carotid blood flow in anaesthetized animals, including the domestic pig. This decrease in arteriovenous shunting by 5-HT is mediated by both 5-HT_1-like and 5-HT_2 receptors, since this effect is abolished by methiothepin (Saxena et al., 1986) and reduced by cyproheptadine (Saxena & Verdouw, 1982) and ketanserin (Verdouw et al., 1984b). In the case of sumatriptan, constriction of arteriovenous anastomoses (Perren et al., 1989; Den Boer et al., 1991a) is mediated exclusively via 5-HT_1-like receptors, since methiothepin, but not ketanserin, acts as an antagonist (Den Boer et al., 1991a). Furthermore, 5-HT and, to a lesser extent, sumatriptan increase capillary flow towards some tissues, like ears and skin; this effect is mediated by 5-HT_1-like receptors (Saxena et al., 1986; Den Boer et al., 1991a). These dilator 5-HT_1-like receptors appear to be different from the constrictor type, since indorenate, a 5-HT_1-like receptor agonist which also decreases arteriovenous shunting, does not increase skin or ear blood flow (Villalón et al., 1990).

Within the 5-HT_1 receptor family sumatriptan has a relative selectivity for the 5-HT_{1D} receptors (Peroutka & McCarthy, 1989; Schoeffter & Hoyer, 1989b), which have been defined in the brain of various species, including the domestic pig (Waeber et al., 1988). The 5-HT_{1D} receptor has received much interest lately because several actions of 5-HT and/or sumatriptan, for example, endothelium-dependent coronary vasodilatation in the pig (Schoeffter & Hoyer, 1989a, 1990), inhibition of noradrenaline release in the human saphenous vein (Molderings et al., 1990), constriction of the human pial arteries (Hamel & Bouchard, 1991) and contralateral rotation after unilateral activation of the substantia nigra in the guinea-pig (Higgins et al., 1991), have been ascribed to this or a similar receptor. Equally, the presynaptic serotonin autoreceptor in the porcine brain cortex resembles the 5-HT_{1D} receptor (Schlicker et al., 1989). On the other hand, vascular 5-HT_1-like receptors mediating 5-HT- and sumatriptan-induced constrictions of the dog saphenous vein or flow reductions in the carotid arterial bed could not be identified as being of the 5-HT_{1D} subtype (Perren et al., 1991). These responses were unaffected by metergoline, a compound with high affinity for 5-HT_2 and all 5-HT_1 binding subtypes (Hoyer, 1989). On similar grounds, we have reported that the 5-HT_1-like receptor mediating the reduction in porcine carotid arteriovenous anastomotic blood flow by indorenate is not related to any of the subtypes of 5-HT binding sites, including the 5-HT_{1D} (Villalón et al., 1990).

In the present experiments, we have further explored the nature of the constrictor and dilator 5-HT_1-like receptors in the pig carotid circulation, especially with the aim of

verifying if one or both of these receptors belong to the 5-HT_{1D} subtype. Therefore, we studied the carotid vascular effects of the autogenous ligand 5-HT, and the putative 5-HT_{1D} receptor agonist sumatriptan in the absence or the presence of metergoline (1 $mg.kg^{-1}$). Of all the substances that have currently been tested, metergoline possesses the highest affinity for the 5-HT_{1D} receptors (Waeber et al., 1988).

Methods

General

After an overnight fast 20 domestic pigs (Yorkshire x Landrace; 16-22 kg) were anaesthetized with azaperone (120 mg, i.m.) and metomidate (150 mg, i.v.), intubated and connected to a respirator (Bear 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; Po_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of pentobarbitone sodium (Sanofi, Paris, France) at 20 $mg.kg^{-1}.h^{-1}$ for the first hour and thereafter 12 $mg.kg^{-1}.h^{-1}$).

Catheters were placed in the inferior vena cava via a femoral vein for the administration of drugs and in the aortic arch via a femoral artery, connected to a Statham pressure transducer (P23 Dc, Hato Rey, Puerto Rico) for the measurement of arterial blood pressure and the withdrawal of arterial blood for determining blood gases (ABL-2, Radiometer, Copenhagen, Denmark). Mean arterial blood pressure (MAP) was calculated from the systolic (SAP) and diastolic (DAP) arterial pressures:
MAP = (SAP + 2 x DAP) / 3. The common carotid arteries were dissected free and the cervical vagosympathetic trunks were cut. Blood flow was measured in one of the common carotid arteries with a flow probe (internal diameter: 2.5 or 3 mm) connected to a sine-wave electromagnetic flow meter (Transflow 600-system, Skalar, Delft, The Netherlands). Heart rate was measured with a tachograph triggered from the blood pressure or the flow signal, depending on their shape. A 0.5 mm (external diameter) needle, connected to a polyethylene tubing was inserted into the common carotid artery against the direction of the blood flow for the administration of radioactive microspheres. In the animals that were given 5-HT a second needle was inserted into the same carotid artery for the infusion of 5-HT. At the same side the jugular vein was cannulated in order to obtain venous blood samples for determining blood gases.

During the experiment body temperature was kept at about 37 °C and the animals were continuously infused with 100 $ml.h^{-1}$ saline to compensate for fluid losses.

Distribution of common carotid blood flow

The distribution of common carotid blood flow was determined with 15±1 (S.D.) μm diameter microspheres labelled with either ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Company, Dreieich, Germany). For each measurement a suspension of about 200,000 microspheres, labelled with one of the, randomly assigned, isotopes, was mixed and injected into the carotid artery against the direction of the blood flow to ensure uniform mixing. At the end of the experiment the animals were killed and the heart, kidneys, lungs and the different cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 5-10 min in a gamma-scintillation counter (Packard, Minaxi Autogamma 5000) using suitable windows for discriminating the different isotopes.

The ratio between the radioactivity in a particular tissue and the total radioactivity was calculated with a set of specially developed computer programs (Saxena et al., 1980). By multiplying this ratio with the total carotid blood flow value at the time of the injection, blood flow to the tissues (capillary blood flow) was determined. No radioactivity could be detected in the heart or the kidneys, so all microspheres reaching the venous side by arteriovenous anastomoses were trapped in the lungs. Therefore, the amount of radioactivity in the lungs was used as an index for the arteriovenous anastomotic part of the common carotid blood flow (see Johnston & Saxena, 1978; Saxena & Verdouw, 1984).

Experimental protocols

5-HT experiments. The experiments were started after a stabilization period of about 1 h. After measuring heart rate, mean arterial blood pressure, carotid blood flow and its distribution and arterial and jugular venous blood gases at baseline, an intracarotid infusion of 5-HT (2 $\mu g.kg^{-1}.min^{-1}$), lasting about 15 min, was started. All parameters were reassessed towards the end of this infusion and, again, 30 min after terminating the infusion (recovery). Then, metergoline (1 $mg.kg^{-1}$) was administered i.v. over a period of 30 min. All parameters were again assessed before and during a second 5-HT infusion (same protocol as above).

Sumatriptan experiments. After a stabilization period of about 1 h, the animals were divided into two groups. The first group received cumulative i.v. doses of sumatriptan (10, 30, 100 and 300 $\mu g.kg^{-1}$) every 20 min after saline pretreatment (n=8). The second group received the same doses of sumatriptan after pretreatment with metergoline (1 $mg.kg^{-1}$), administered over a 30 min period (n=7). Just before and 15 min after each dose of sumatriptan measurements of heart rate, mean blood pressure, carotid blood flow and its distribution and arterial and jugular venous blood gases were made.

Data presentation and statistical analysis

All data have been expressed as means ± SEM. The significance of the differences between the variables within one group was evaluated with Duncan's new multiple range test, once an analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel and Torrie, 1980). Between the two sumatriptan groups the respective changes at the same dose of sumatriptan were evaluated with a Student's t-test. Statistical significance was accepted at $P<0.05$ (two-tailed).

Drugs

Apart from the anaesthetics, azaperone and metomidate (both from Janssen Pharmaceutica, Beerse, Belgium), the drugs used in this study were: 5-HT creatinine sulphate (Sigma, St. Louis, MO, USA), sumatriptan (Glaxo group research, Ware, UK), metergoline (Farmitalia, Milan, Italy) and heparin sodium (Thromboliquine, Organon Teknika B.V., Boxtel, The Netherlands) to prevent clotting of the catheters. 5-HT and sumatriptan were dissolved in physiological saline. Metergoline was dissolved in 5 ml ascorbic acid (10%) in distilled water and subsequently diluted with 45 ml physiological saline. The dose of 5-HT refers to the free base.

Results

Effects of 5-HT

Systemic haemodynamics

Intracarotid infusion of 5-HT (2 $\mu g.kg^{-1}.min^{-1}$) caused no change in either heart rate or mean arterial blood pressure. Similarly, i.v. administration of metergoline (1 $mg.kg^{-1}$) failed to elicit any significant change in these variables (Table 1).

Carotid haemodynamics

Intracarotid infusion of 5-HT (2 $\mu g.kg^{-1}.min^{-1}$) caused a decrease in the total carotid flow by 58±8% (Figure 1). This was caused predominantly by decreasing the arteriovenous anastomotic fraction by 95±1%, since the overall capillary fraction was not significantly changed (Figure 1). An increase in the arterial-jugular venous oxygen saturation difference of 59±19% (Table 1) is in keeping with this effect on the arteriovenous anastomoses.

Table 1 Values of heart rate (HR; beats.min^{-1}), mean arterial blood pressure (MAP; mmHg) and difference in arterial and jugular venous oxygen saturation (DAVO; %) at baseline, during intracarotid infusion of 2 μg.kg^{-1}.min^{-1} of 5-HT, after 30 min. of recovery, after metergoline (1 mg.kg^{-1}) and during a second infusion of 2 μg.kg^{-1}.min^{-1} 5-HT.

	Baseline	5-HT	Recovery	Metergoline	5-HT
HR	102±5	106±5	100±4	96±5	101±5
MAP	87±3	89±4	85±4	83±4	80±4
DAVO	7.3±0.5	11.7±1.6a	7.9±0.8	9.6±1.0	7.7±0.8

All values have been presented as means ± SEM. a, $P<0.05$ vs baseline

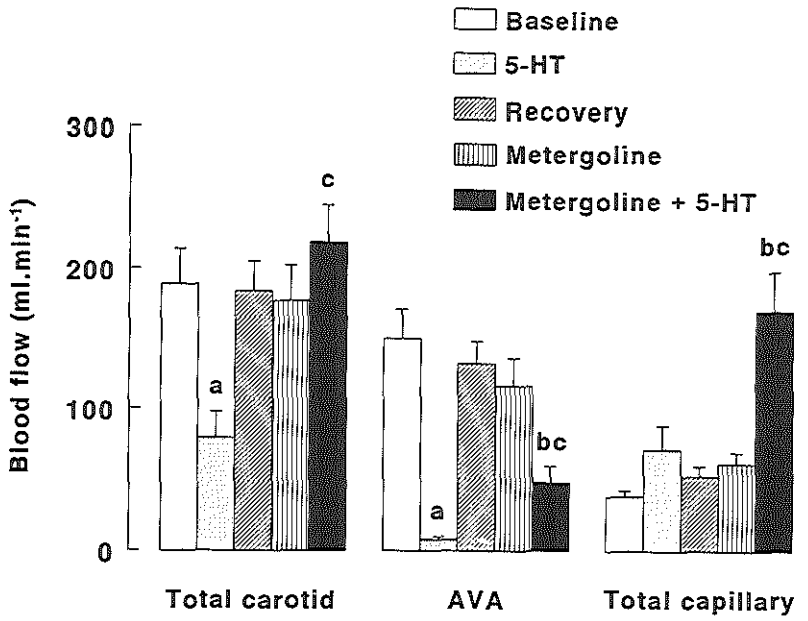


Figure 1. Effect of 5-HT (2 μg.kg^{-1}.min^{-1}, i.a.) on the total carotid, arteriovenous anastomotic (AVA) and nutrient blood flow in anaesthetized pigs (n=6) initially and after metergoline (1 mg.kg^{-1}, i.v.). Data are expressed as means ± SEM. a, $P<0.05$ vs. baseline. b, $P<0.05$ vs state after metergoline. c, $P<0.05$ vs first response to 5-HT.

Despite the fact that the overall carotid capillary flow did not change significantly, there was an increase in capillary flow towards the skin (by 711±233%), ears (by 491±202%), muscle (by 103±35%) and bones (by 62±27%; Figure 2). On the other hand, the amount of carotid blood distributed to the brain was severely decreased by

5-HT (by -98±1%). Thirty minutes after discontinuation of the 5-HT infusion, all variables had returned towards baseline values; there was, however, a tendency for bone blood flow to remain elevated (Figure 2).

Intravenous injection of metergoline (1 mg.kg^{-1}) had by itself no effects on carotid haemodynamics. It attenuated, however, the decrease in arteriovenous anastomotic blood flow following intracarotid infusion of 5-HT (Figure 1). The increase in the arterial-jugular venous oxygen saturation difference was equally abolished by metergoline (Table 1). On the other hand, the 5-HT-induced increase in blood flow towards skin, ears, muscle and bones was greatly enhanced (Figure 2), to such an extent, that the total capillary flow was equally increased (Figure 1). The sharp decrease in brain blood flow by 5-HT was prevented by metergoline (Figure 2).

Since mean arterial blood pressure remained unaltered, the changes in the vascular resistances (data not shown) essentially paralleled those in blood flows.

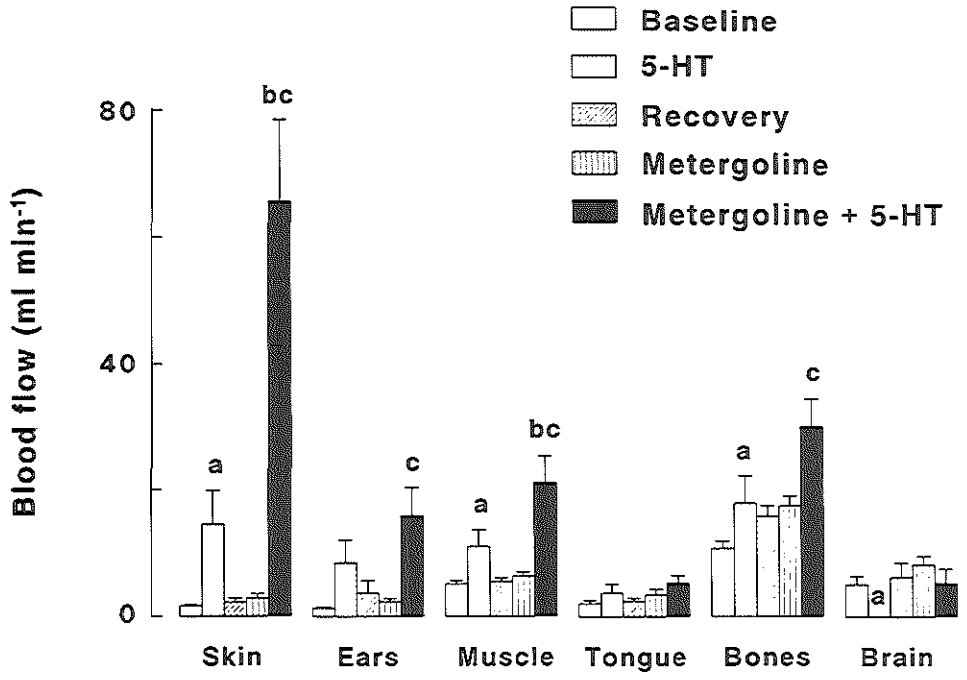


Figure 2. Effect of 5-HT (2 μg.kg^{-1}.min^{-1}, i.a.) on blood flow to different cranial tissues in anaesthetized pigs (n=6) initially and after metergoline (1 mg.kg^{-1}, i.v.). Data are expressed as means ± SEM. a, $P<0.05$ vs baseline. b, $P<0.05$ vs state after metergoline. c, $P<0.05$ vs first response to 5-HT.

Effects of sumatriptan

Systemic haemodynamics

Bolus injections of sumatriptan (10-300 $\mu g.kg^{-1}$, i.v.) did not affect mean arterial blood pressure, except for a slight increase with the two highest doses in the metergoline-treated group. The drug slightly decreased heart rate in both saline- and metergoline-treated groups (Table 2).

Table 2 Changes in heart rate (beats.min^{-1}), mean arterial blood pressure (mmHg) and difference in arterial and jugular venous oxygen saturation (%) caused by cumulative doses of sumatriptan after saline (n=8) or metergoline (1 mg.kg^{-1}; n=7).

	Sumatriptan ($\mu g.kg^{-1}$)				
	0	10	30	100	300
Heart rate					
Saline	92±2	89±2a	87±2a	84±3a	81±2a
Metergoline	89±3	86±3a	85±4a	84±4a	82±4a
Mean arterial blood pressure					
Saline	88±4	90±4	90±5	89±6	84±8
Metergoline	91±5	90±5	91±4	96±5a	96±6a
Arteriovenous difference in oxygen saturation					
Saline	5.5±1.5	5.9±1.9	7.1±2.1	8.3±2.3a	10.4±2.5a
Metergoline	5.9±1.7	6.2±1.6	6.4±1.5	6.9±1.5	7.3±1.6

All values have been presented as means ± SEM; a, $P<0.05$ vs baseline.

Carotid haemodynamics

Sumatriptan caused dose-dependent reductions in the total carotid blood flow (Figure 3). The decrease at the dose of 300 $\mu g.kg^{-1}$ was 31±7%. This was caused exclusively by a decrease in its arteriovenous anastomotic part (by up to 61±9%), since the capillary blood flow was increased by up to 49±16% (Figure 3). An increase in the arterial-jugular venous oxygen saturation difference (Table 2) is in keeping with this effect on the arteriovenous anastomoses. As shown in Figure 4, an increase in flow was observed in the skin (by 182±41%), ears (by 209±75%) and bones (by 64±24%).

Though there was a tendency to attenuate the effect on blood gases, metergoline did not significantly change the responses to sumatriptan (Figures 3 and 4; Table 2). The changes in arteriovenous anastomotic and capillary blood flows with the highest dose of sumatriptan in the metergoline-treated group were, respectively, -50±5% and 67±12%.

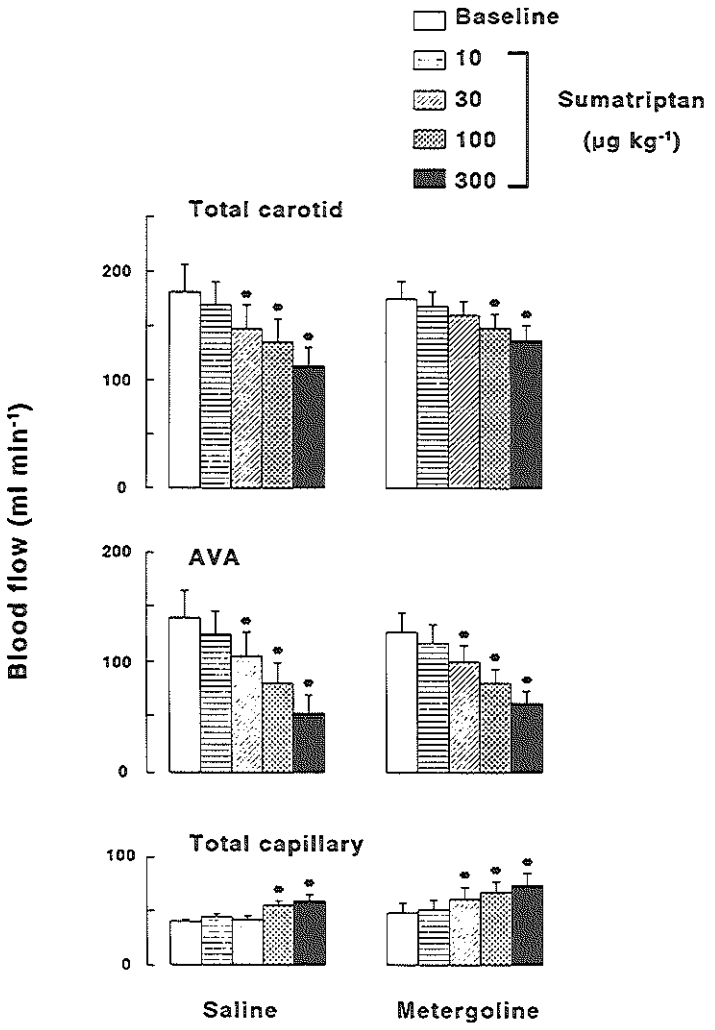


Figure 3. Effect of i.v. sumatriptan on the total carotid, arteriovenous anastomotic (AVA) and nutrient blood flow after pretreatment with saline (left panel) or metergoline (1 mg.kg^{-1}, i.v., right panel). Data are expressed as means ± SEM. *, $P<0.05$ vs baseline. No significant differences were found between the saline and metergoline pretreated animals.

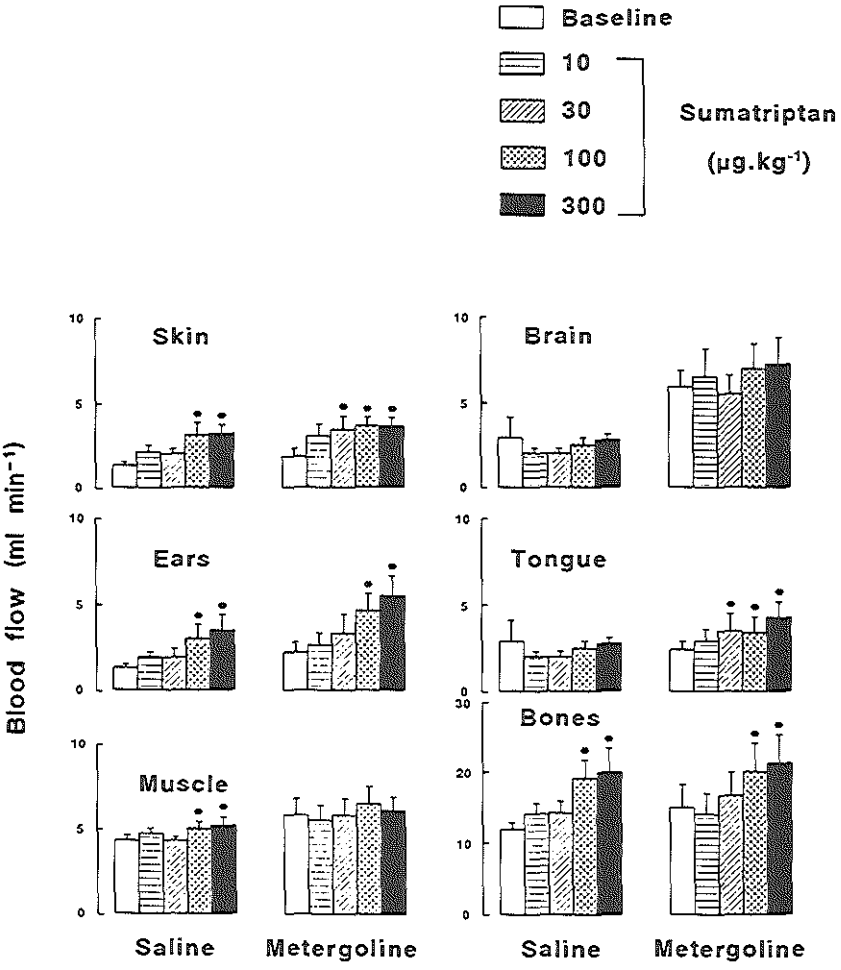


Figure 4. Effect of i.v. sumatriptan on blood flow to different cranial tissues after pretreatment with saline (left panel) or metergoline (1 mg.kg^{-1}, i.v., right panel). Data are expressed as means ± SEM. *, $P<0.05$ vs baseline. No significant differences were found between the saline and metergoline pretreated animals.

Discussion

Vascular constriction and blood flow reduction by 5-HT can be mediated by 5-HT_1-like or 5-HT_2 receptors, depending on the species and blood vessel studied (see Müller-Schweinitzer, 1986; Saxena & Villalón, 1990). It has been demonstrated before that both 5-HT_1-like and 5-HT_2 receptors are involved in the reduction of arteriovenous anastomotic flow in the carotid circulation by 5-HT, since this effect is partly inhibited by the 5-HT_2 receptor antagonists, cyproheptadine, WAL 1307 (3-amino-6-methyl-1,13b-dihydro-di-benz(b,f)imidazo(1,5-d)-(1,4)-oxazepin hydrochloride-hemihydrate) and ketanserin (Saxena & Verdouw, 1982; Verdouw et al., 1984b) and abolished by methiothepin (Saxena et al., 1986). Sumatriptan, which has negligible affinity for 5-HT_2 receptors (Humphrey et al., 1988; Peroutka & McCarthy, 1989), increases vascular resistance in the canine carotid vascular bed and reduces carotid arteriovenous anastomotic flow in pigs exclusively by 5-HT_1-like receptors; methiothepin, but not ketanserin, antagonizes this effect (Feniuk et al., 1989a; Den Boer et al., 1991a). In the present experiments, 5-HT reduced arteriovenous shunting much more effectively than sumatriptan. This might be due to an additional agonistic effect at 5-HT_2 receptors, although differences in dose or intrinsic activity at the 5-HT_1-like receptor may also be involved. The other 5-HT_1-like receptor agonists - 5-CT (Saxena & Verdouw, 1985b), 8-OH-DPAT (Bom et al., 1989b), RU 24969 (Bom et al., 1989c) and indorenate (Villalón et al., 1990) — reduce porcine carotid arteriovenous anastomotic blood flow via constrictor 5-HT_1-like receptors as well. Apart from the carotid arteriovenous anastomoses, constrictor 5-HT_1-like receptors have also been demonstrated on other blood vessels, like the dog (Humphrey et al., 1988) and rabbit (Martin & MacLennan, 1990; Van Heuven-Nolsen et al., 1990) saphenous vein and the human, canine and primate basilar artery (Connor et al., 1989a; Parsons et al., 1989).

A different 5-HT_1-like receptor seems to be involved in the increase in capillary blood flow towards, for example, the skin and ears by 5-HT. Apart from 5-HT, 5-CT is a full agonist at this receptor (Saxena & Verdouw, 1985b). Sumatriptan and the other 5-HT_1-like agonists 8-OH-DPAT and RU 24969 are much less potent vasodilators than 5-HT and indorenate is none at all (Bom et al., 1989b, c; Villalón et al., 1990).

There is now further evidence that 5-HT_1-like receptors are heterogeneous; in different species 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D} and, possibly, 5-HT_{1E} receptors have been discerned (see Hoyer, 1989; Göthert & Schlicker, 1990; Saxena & Villalón, 1990) and some of them have even been cloned (Lübbert et al., 1987; Hamblin & Metcalf, 1991). Despite this multiplicity of the 5-HT_1 receptor subtypes, some 5-HT_1-like receptors, particularly those on extra- and intracranial blood vessels, do not seem to be

related to the above subtypes (Connor et al., 1989a; Saxena & Ferrari, 1989). For example, the '5-HT_{1A} receptor-selective' agonist indorenate (Villalón et al., 1990) constricts porcine carotid arteriovenous anastomoses by a 5-HT_1-like receptor, which is blocked by methiothepin, but not by metergoline, yet both compounds have high affinity for all 5-HT_1 binding sites (Hoyer, 1989). The reduction in arteriovenous anastomotic blood flow by the '5-HT_{1A} and 5-HT_{1B} receptor-selective' agonist RU 24969 (Bom et al., 1989c) was resistant to pindolol despite its high affinity for 5-HT_{1A} and 5-HT_{1B} binding sites (Hoyer, 1989). The present investigation strengthens this notion, since we found that the reduction in carotid arteriovenous shunting by sumatriptan, a compound with a high affinity for the 5-HT_{1D} and a somewhat lower affinity for the 5-HT_{1A} binding sites (Peroutka & McCarthy, 1989; Schoeffter & Hoyer, 1989b, 1990), was not antagonized by metergoline (which also lacked agonist activity), despite being susceptible to blockade by methiothepin (Den Boer et al., 1991a). Neither the sumatriptan-induced increase in the oxygen saturation difference between arterial and jugular venous blood was significantly changed by metergoline, although there was a tendency to blunting of this effect. Although metergoline reduced the constrictor response to 5-HT on arteriovenous anastomoses and prevented the 5-HT-induced rise in the arteriovenous oxygen saturation difference, this was accompanied by a marked enhancement of the increase in the capillary blood flow by 5-HT (see Figures 1 and 2). Using the same model, we have observed effects similar to those of metergoline with the selective 5-HT_2 receptor antagonists cyproheptadine, ketanserin and WAL 1307 (Saxena & Verdouw, 1982; Verdouw et al., 1984b). This is in contrast to the non-selective 5-HT_1-like and 5-HT_2 receptor antagonist methiothepin, which effectively blocks both the vasoconstrictor and vasodilator responses to 5-HT in the carotid circulation of the pig (Saxena et al., 1986). Therefore, the attenuation of the 5-HT-induced reduction in arteriovenous anastomotic shunting by metergoline can best be ascribed to antagonism at 5-HT_2 receptors rather than at 5-HT_1-like receptors. Similarly, the enhancement by metergoline of the vasodilatation caused by 5-HT in the skin and ear capillary bed seems to be due to 5-HT_2 receptor antagonism by metergoline, which also left the dilator 5-HT_1-like receptors intact.

The striking differences between metergoline and methiothepin in their effects on the carotid vascular responses to sumatriptan and 5-HT suggest that the 5-HT_1-like receptors mediating vasoconstriction or even vasodilatation cannot be equated with the known 5-HT_1 binding site subtypes, including the 5-HT_{1D}. Equally, the 5-HT_1-like receptor mediating an increase in resistance within the canine carotid circulation as well as constriction of the isolated saphenous vein from the same species does not correspond to the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, or 5-HT_{1D} receptor, because only methiothepin, but not metergoline or some other more selective 5-HT_{1A} or 5-HT_{1D} receptor antagonists

(spiperone, spiroxatrine, pindolol, rauwolscine and yohimbine), showed any antagonism (Perren et al., 1991).

Several other vascular effects of 5-HT and sumatriptan have been claimed to be mediated by 5-HT_{1D} or similar receptors: inhibition of 5-HT release from cortical serotonergic nerves (Schlicker et al., 1989), contraction of human isolated pial arteries (Hamel & Bouchard, 1991), endothelium-dependent relaxation of the pig coronary artery (Schoeffter & Hoyer, 1989a, 1990) and inhibition of noradrenaline release from the sympathetic nerves in the human saphenous vein (Molderings et al., 1990). Though, as in the present experiments, Molderings et al. (1990) failed to detect any agonist effect of metergoline, its antagonist effect against 5-HT-induced inhibition of noradrenaline release in the human saphenous vein was not studied. In the experiments on human isolated pial arteries (Hamel & Bouchard, 1991) both methiothepin and metergoline (having a pK_i of, respectively, 6.8 and 7.9 at human 5-HT_{1D} sites; Waeber et al., 1988) inhibited the constrictor response to 5-HT. Whereas methiothepin had a clear dose-dependent effect, metergoline was completely inactive in concentrations of 10 and 100 nM and, suddenly, showed a rather strong antagonistic property at 1 μM. Therefore, the activities of these drugs were inversely correlated with their affinities at the 5-HT_{1D} receptor. Furthermore, since metergoline clearly showed non-competitive antagonism, it is difficult to distinguish between a receptor-mediated and a non-specific effect of metergoline. The relaxation of the pig coronary artery to 5-HT was inhibited by different antagonists of the cerebral 5-HT_{1D} receptor, like metergoline, yohimbine, rauwolscine and corynanthine (Schoeffter & Hoyer, 1990). In these experiments matching of the antagonist potencies with their affinities at 5-HT_{1D} binding sites yielded a good correlation. Again a problem is the low antagonist potency of metergoline, despite a very high affinity of this compound for the 5-HT_{1D} receptor. Also in the study by Schlicker et al. (1989), metergoline was less potent in antagonizing the 5-HT autoreceptor than methiothepin, which does not correlate with their respective affinities. In the experiments on sumatriptan-induced relaxation of pig coronary arteries no 5-HT_{1D} receptor antagonist was used to confirm the receptor mechanism (Schoeffter & Hoyer, 1989a).

Lastly, it may be pointed out that in this study 5-HT caused a sharp decrease in the part of the carotid artery blood flow supplied to the brain. This is in contrast with our earlier studies in the pig, where intracarotid infusion of the same dose of 5-HT did not cause any change in the carotid arterial contribution to brain blood flow (Saxena & Verdouw, 1982; Verdouw et al., 1984b). The reason for this difference is unclear. It is, however, not certain if brain ischaemia occurred, since in pigs the circle of Willis is well developed and compensatory blood flow from other cerebral arteries is possible. Pretreatment with metergoline prevented this fall in brain blood supply from the carotid artery, which may be due to antagonism at 5-HT_2 receptors.

Chapter 8

Carotid Vascular Effects of Ergotamine and Dihydroergotamine in the Pig: No Exclusive Mediation via 5-HT_1-like Receptors

Summary

Though it is well known that the antimigraine drugs ergotamine and dihydroergotamine reduce carotid arteriovenous anastomotic shunting, it is uncertain whether a 5-HT_1-like receptor is responsible for this effect. Using a high dose of methiothepin (3 mg kg^{-1}), which completely blocks the carotid vascular effects of sumatriptan, we have attempted to study the role of 5-HT_1-like receptors in the carotid vascular effects of ergotamine as well as dihydroergotamine in anaesthetized pigs.

Both ergotamine and dihydroergotamine increased arterial blood pressure and decreased heart rate. The ergot alkaloids reduced dose-dependently total carotid blood flow and conductance as a result of a selective decrease in the arteriovenous anastomotic fraction. The nutrient fraction increased, particulary to bones, tongue and salivary glands with ergotamine and to ears, head skin, bones and salivary glands with dihydroergotamine. In contrast, dural vascular conductance tended to decrease.

Methiothepin (3 mg kg^{-1}) partially antagonized the decrease in total carotid and arteriovenous anastomotic blood flow and conductance by the ergot alkaloids; the ED_{30} for ergotamine and dihydroergotamine (agonist dose eliciting a 30% decrease in arteriovenous anastomotic conductance) was raised by 3.1 and 5.2 fold, respectively.

These results indicate that the effects of ergotamine and dihydroergotamine are partly mediated by methiothepin-sensitive receptors, which may probably belong to either 5-HT_1-like or α_2-adrenergic category. An important part of the effect of the ergot alkaloids, however, is left after methiothepin and this could be mediated by other, perhaps novel, receptors.

Introduction

The pharmacology of the antimigraine drugs ergotamine and dihydroergotamine is complex. Apart from being capable of inhibiting the sympathetic nervous system and blunting cardiovascular reflexes (Saxena & De Vlaam-Schluter, 1974; Clark et al., 1978; Saxena & Cairo-Rawlins, 1979), the ergot alkaloids possess a direct vasoconstrictor property on veins and larger arteries. Depending on the species, the blood vessel and the experimental procedure used, stimulation of both adrenoceptors (α_1 and α_2) and 5-hydroxytryptamine (5-HT_1-like and 5-HT_2) receptors have been implicated (Berde & Stürmer, 1978; Müller-Schweinitzer & Weidmann, 1978; Müller-Schweinitzer & Rosenthaler, 1987; Glusa & Markwardt, 1988; Müller et al., 1988). A reduction of carotid arteriovenous anastomotic blood flow by ergotamine is a property shared by other antimigraine drugs such as dihydroergotamine, isometheptene, methysergide and sumatriptan (Johnston & Saxena, 1978; Spierings & Saxena, 1980a,b; Saxena & Verdouw, 1984; Perren et al., 1989; Saxena et al., 1989; Den Boer et al., 1991a; Saxena & Den Boer, 1991). In different species this effect of ergotamine was only partially affected by pretreatment with phentolamine (0.5 $mg.kg^{-1}$) and methiothepin (1 $mg.kg^{-1}$) and not at all by pizotifen (0.5 $mg\ kg^{-1}$) or ketanserin (0.5 $mg\ kg^{-1}$) (Saxena et al., 1983; Bom et al., 1989a). These findings apparently excluded a major role of 5-HT_1-like, 5-HT_2 and D_2 receptors as well as α_1- and α_2-adrenoceptors.

Recent investigations have revealed that: (i) ergotamine and dihydroergotamine, besides their well known ability to bind to α_1- and α_2-adrenoceptors and D_2 receptors, show high affinities for 5-HT_1 and 5-HT_2 binding sites (Table 1), (ii) the reduction of arteriovenous anastomotic blood flow by sumatriptan, a selective 5-HT_1-like receptor agonist (Humphrey et al., 1988) with antimigraine action is only partially inhibited by 1 $mg\ kg^{-1}$ of methiothepin (Den Boer et al., 1991a), and (iii) for a full inhibition of the effect of sumatriptan, a dose of 3 $mg.kg^{-1}$ is required (Den Boer et al., 1991a). Thus, the adequacy of the dose of methiothepin (1 $mg.kg^{-1}$) used in the ergotamine experiments (Bom et al., 1989a) to antagonize 5-HT_1-like receptors is questionable. Therefore, the present experiments were devoted to studying the effect of 3 $mg.kg^{-1}$ methiothepin on the reduction in cranial arteriovenous anastomotic shunting by ergotamine in the pig. In addition, dihydroergotamine was included in this study, since no data are available on the receptors involved in the carotid vascular action of this ergot derivative.

Table 1 Affinities (pK_i) of ergotamine, dihydroergotamine and methiothepin for different binding sites.

	Ergotamine	Dihydroergotamine	Methiothepin
5-HT_{1A}	8.3[a]; 8.4[b]	8.6[a]; 8.9[bc]	7.1[b]
5-HT_{1B}	8.7[ab]	8.4[a]; 9.1[b]	7.3[b]
5-HT_{1C}	7.3[ab]	7.3[a]; 7.4[c]; 7.5[b]	7.6[b]
5-HT_{1D}	7.6[b]; 7.8[a]	7.7[bc]; 7.9[a]	7.3[b]
5-HT_2	7.7[b]; 7.9[d]	7.1[c]; 8.6[b]	8.7[c]; 8.8[b]
α_1	8.0[d]	8.2[c]	9.3[e]; 9.3[f]
α_2	8.2[d]	8.5[c]	7.3[e]; 7.3[f]
D_2	8.5[d]	7.0[c]	8.4[f]

Data from: [a], Hoyer et al., 1989; [b], Hoyer, 1989; [c], McCarthy & Peroutka, 1989; [d], Leysen & Gommeren, 1984; [e], Leysen et al., 1981; [f], Leysen, 1985.

Methods

General

After an overnight fast 24 domestic pigs (Yorkshire x Landrace; 16-22 kg) were anaesthetized with azaperone (120 mg, i.m.) and metomidate (150 mg, i.v.), intubated and connected to a respirator (Bear 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; pO_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of pentobarbitone sodium (Sanofi, Paris, France) at 20 $mg.kg^{-1}.h^{-1}$ for the first hour and thereafter 12 $mg.kg^{-1}.h^{-1}$).

Catheters were placed in the inferior vena cava via a femoral vein for the administration of drugs and in the aortic arch via a femoral artery, connected to a Statham pressure transducer (P23 Dc, Hato Rey, Puerto Rico) for the measurement of arterial blood pressure and the withdrawal of arterial blood for determining blood gases (ABL-2, Radiometer, Copenhagen, Denmark). Mean arterial blood pressure (MAP) was calculated from the systolic (SAP) and diastolic (DAP) arterial pressures:
MAP = (SAP + 2 x DAP) / 3. The common carotid arteries were dissected free and the cervical vagosympathetic trunks were cut. Blood flow was measured in one of the common carotid arteries with a flow probe (internal diameter: 2.5 or 3 mm) connected to a sine-wave electromagnetic flow meter (Transflow 600-system, Skalar, Delft, The Netherlands). Heart rate was measured with a tachograph triggered from the blood

pressure or the flow signal, depending on their shape. A 0.5 mm (external diameter) needle, connected to a polyethylene tubing was inserted into the common carotid artery against the direction of the blood flow for the administration of radioactive microspheres. At the same side the jugular vein was cannulated in order to obtain venous blood samples for determining blood gases.

During the experiment body temperature was kept at about 37 °C and the animal was continuously infused with 100 $ml.h^{-1}$ saline to compensate for fluid losses.

Distribution of common carotid blood flow

The distribution of common carotid blood flow was determined with 15 ± 1 (S.D.) μm diameter microspheres labelled with either ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Company, Dreieich, West Germany). For each measurement a suspension of about 200,000 microspheres, labelled with one of the isotopes, was mixed and injected into the carotid artery against the direction of the blood flow to ensure uniform mixing. At the end of the experiment the animals were killed and the heart, kidneys, lungs and the different cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 5-10 min in a gamma-scintillation counter (Packard, Minaxi Autogamma 5000) using suitable windows for discriminating the different isotopes.

The ratio between the radioactivity in a particular tissue and the total radioactivity was calculated with a set of specially developed computer programs (Saxena et al., 1980). By multiplying this ratio with the total carotid blood flow value at the time of the injection, blood flow to the tissues (nutrient blood flow) was determined. No radioactivity could be detected in the heart or the kidneys, so all microspheres reaching the venous side by arteriovenous anastomoses were trapped in the lungs. Therefore, the amount of radioactivity in the lungs was used as an index for the arteriovenous anastomotic part of the common carotid blood flow (see Johnston & Saxena, 1978; Saxena & Verdouw, 1984). The respective conductances were determined by dividing blood flow by mean arterial blood pressure.

Experimental protocol

After a stabilization period of about one hour, the animals were divided into four groups. The first group received cumulative bolus injections of ergotamine (2.5, 5, 10 and 20 $\mu g.kg^{-1}$), every 25-30 min after saline pretreatment. The second group received the same doses of ergotamine, but after pretreatment with methiothepin (3 $mg.kg^{-1}$). The third and fourth groups received cumulatively dihydroergotamine (3, 10, 30 and 100 $\mu g.kg^{-1}$), every 25-30 min after pretreatment with, respectively, saline and methiothepin (3 $mg.kg^{-1}$). All pretreatments were given i.v. over a 30 min period. Just before and

after about 15-20 min each dose of the ergot alkaloids, measurements of heart rate, mean blood pressure, carotid blood flow and its distribution and arterial and jugular venous blood gases were made.

Data presentation and statistical analysis

All data have been expressed as means ± SEM. The significance of the differences between the variables within one group was evaluated with Duncan's new multiple range test, once an analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). Between groups the respective changes at the same dose of ergotamine or dihydroergotamine were evaluated with a Student's t-test. Statistical significance was accepted at $P < 0.05$ (two-tailed).

Drugs

Apart from the anaesthetics, azaperone and metomidate (both from Janssen Pharmaceutica, Beerse, Belgium), the drugs used in this study were: ergotamine tartrate (Wander-Pharma, Uden, The Netherlands), dihydroergotamine mesylate (Wander-Pharma, Uden, The Netherlands), methiothepin maleate (gift: Hoffman La Roche B.V., Mijdrecht, The Netherlands) and heparin sodium (Thromboliquine, Organon Teknika B.V., Boxtel, The Netherlands) to prevent clotting of the catheters. Methiothepin maleate was dissolved in 5 ml propylene glycol 20% in distilled water and subsequently diluted with 45 ml physiological saline. All doses refer to the respective salts.

Results

Changes in the systemic haemodynamics by the ergot alkaloids

The systemic haemodynamic effects of the ergot alkaloids are shown in Table 2. Ergotamine caused a dose-dependent decrease in heart rate up to -12±3% at the maximum dose. Dihydroergotamine also reduced heart rate until the 30 $\mu g.kg^{-1}$ dose (-6±3%) but after the highest dose heart rate had returned to baseline. Though the responses were rather small, methiothepin seemed to attenuate those caused by ergotamine.

Mean arterial blood pressure was dose-dependently increased by 29±2% and 40±7% at the highest dose of ergotamine and dihydroergotamine, respectively. Methiothepin attenuated the increases in blood pressure by the two compounds; it should, however, be noted that in both methiothepin pretreated groups initial arterial blood pressure was higher (Table 2).

Table 2 Effects of intravenous bolus injections of ergotamine and dihydroergotamine on systemic haemodynamic variables and arteriovenous difference in oxygen saturation after pretreatment with saline or methiothepin (3 mg kg^{-1}).

	Ergotamine ($\mu g\ kg^{-1}$)					Dihydroergotamine ($\mu g\ kg^{-1}$)				
	0	2.5	5	10	20	0	3	10	30	100
Heart rate (beats min^{-1})										
Saline	88±3	84±3	81±4*	79±4*	77±4*	90±2	87±1	85±1*	84±2*	89±2
Methiothepin	87±4	86±4	85±4	84±3	81±3*	86±3	84±3	83±3	84±3	87±3
Mean arterial blood pressure (mmHg)										
Saline	85±6	97±7	99±6*	103±7*	109±7*	90±2	104±3*	110±4*	118±5*	126±5*
Methiothepin	95±4	97±6+	99±7	102±8+	104±8+	96±6	98±7+	101±6+	108±7*+	112±7*+
Arteriovenous difference in oxygen saturation (%)										
Saline	5±1	7±1	8±1	9±2	10±2	6±1	8±1	9±2	10±2*	11±2*
Methiothepin	7±2	8±2	10±2	12±3*	13±3*	4±1	5±1	5±1	7±1*	8±1*

All values have been presented as means±s.e.m. *, $P<0.05$ vs. baseline.
+, $P<0.05$ vs. the corresponding dose in saline pretreated animals.

Changes in the arteriovenous oxygen saturation difference

Dihydroergotamine caused an increase in the difference in oxygen saturation between arterial and jugular venous blood (Table 2). Ergotamine tended to equally increase arteriovenous oxygen saturation difference, although this did not reach statistical significance. The responses to dihydroergotamine remained unaffected and those to ergotamine were even slightly accentuated in animals pretreated with methiothepin.

Changes in the carotid haemodynamics

In the four groups between 71 and 80% of the carotid blood flow passed through arteriovenous anastomoses. In the saline-pretreated animals, both ergotamine and dihydroergotamine caused dose-dependent decreases in arteriovenous anastomotic blood flow (Figures 1 and 2).

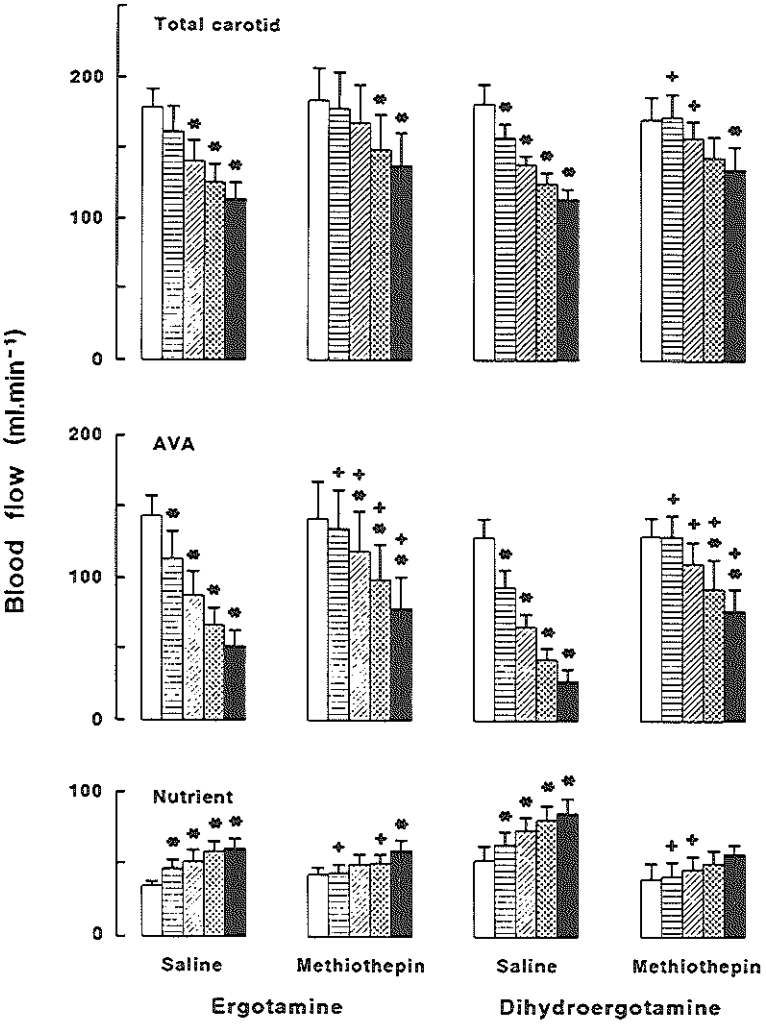


Figure 1 Effects of ergotamine and dihydroergotamine on total carotid blood flow and its arteriovenous anastomotic (AVA) and nutrient fractions in anaesthetized pigs pretreated with saline (n=6 each) or methiothepin (3 mg.kg^{-1}; n=6 each). The five columns in each panel represent, from left to right, the following doses: Ergotamine: 0 (baseline), 2.5, 5, 10, and 20 μg.kg^{-1}; Dihydroergotamine: 0 (baseline), 3, 10, 30, and 100 μg.kg^{-1}. *, $P < 0.05$ vs baseline; +, $P < 0.05$ vs the corresponding dose in the saline pretreated animals.

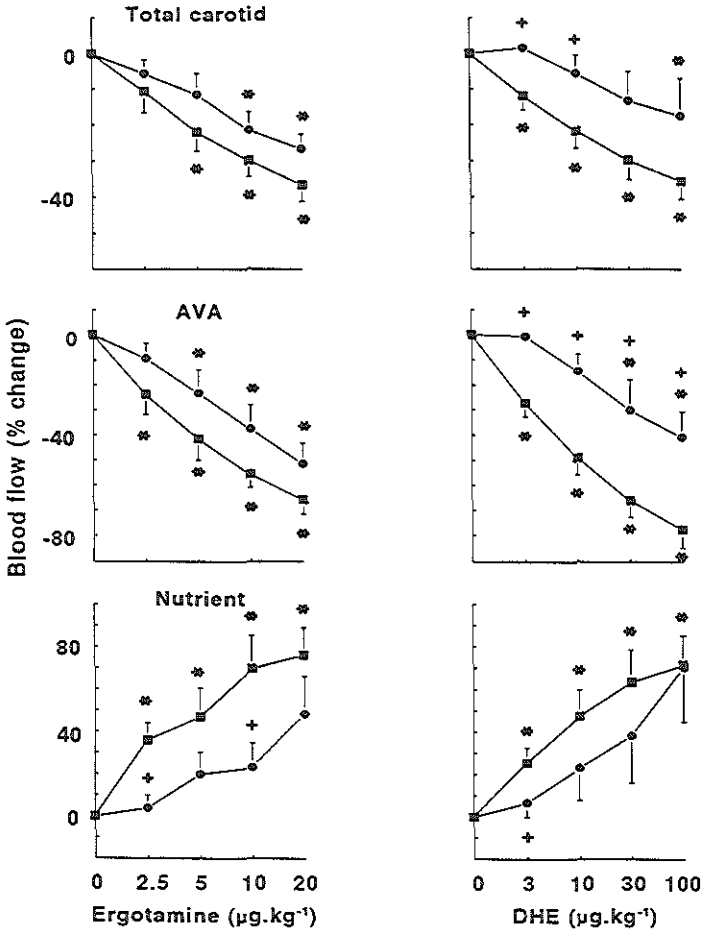


Figure 2 Percent changes in total carotid blood flow and its arteriovenous anastomotic (AVA) and nutrient fractions induced by ergotamine and dihydroergotamine in anaesthetized pigs pretreated with saline (squares; n=6 and 6, respectively) or methiothepin (3 mg.kg^{-1}, circles; n=6 and 6, respectively). *, $P < 0.05$ vs baseline; +, $P < 0.05$ vs the corresponding dose in the saline pretreated animals.

At the highest dose of ergotamine (20 μg.kg^{-1}, cumulatively 37.5 μg.kg^{-1}), arteriovenous anastomotic blood flow had decreased by 66±6%, but total carotid blood flow only by 37±5% because nutrient (tissue) blood flow had increased by 76±13%. The highest dose of dihydroergotamine (100 μg.kg^{-1}, 143 μg.kg^{-1} cumulatively) decreased arteriovenous anastomotic blood flow by 78±7% and total carotid flow by 36±5%, while nutrient flow increased by 72±14%.

Because both ergotamine and dihydroergotamine increased arterial blood pressure (Table 2), they caused even larger decreases in total carotid and arteriovenous anastomotic vascular conductances; the increases at the highest doses were: 51±3% and 74±4%, respectively, for ergotamine and 53±5% and 84±6%, respectively, for dihydroergotamine (Figures 3 and 4).

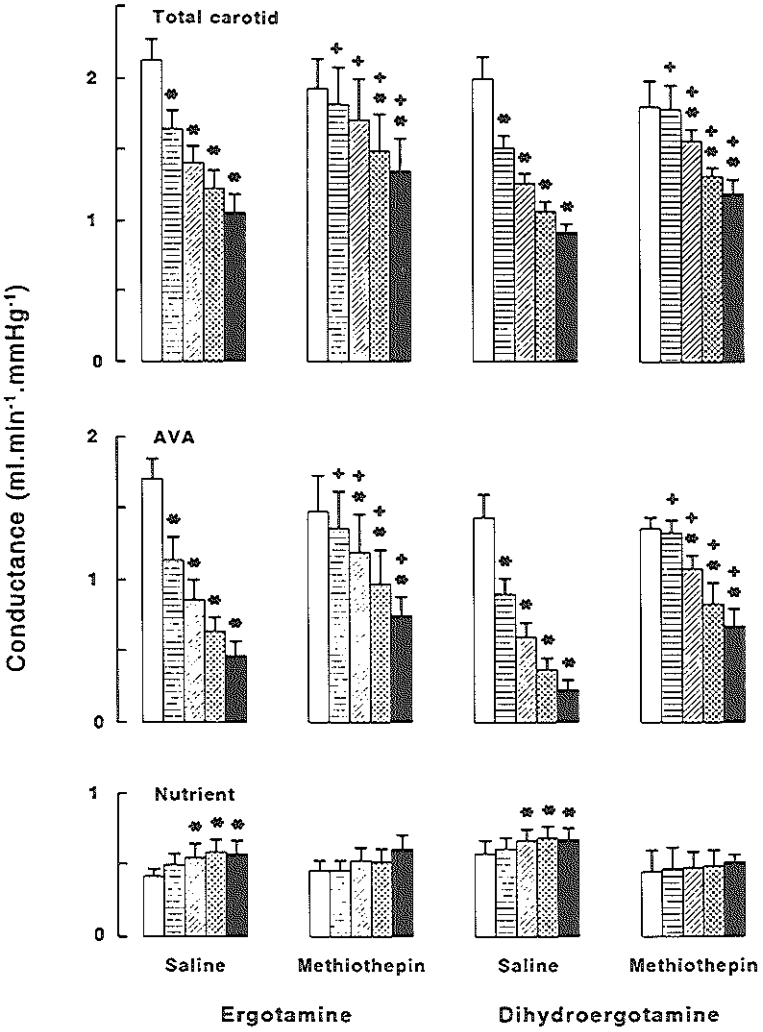


Figure 3 Effects of ergotamine and dihydroergotamine on total carotid vascular conductance and its arteriovenous anastomotic (AVA) and nutrient fractions in anaesthetized pigs pretreated with saline (n=6 each) or methiothepin (3 mg.kg^{-1}; n=6 each). The five columns in each panel represent, from left to right, the following doses: Ergotamine: 0 (baseline), 2.5, 5, 10, and 20 μg.kg^{-1}; Dihydroergotamine: 0 (baseline), 3, 10, 30, and 100 μg.kg^{-1}. *, $P < 0.05$ vs baseline; +, $P < 0.05$ vs the corresponding dose in the saline pretreated animals.

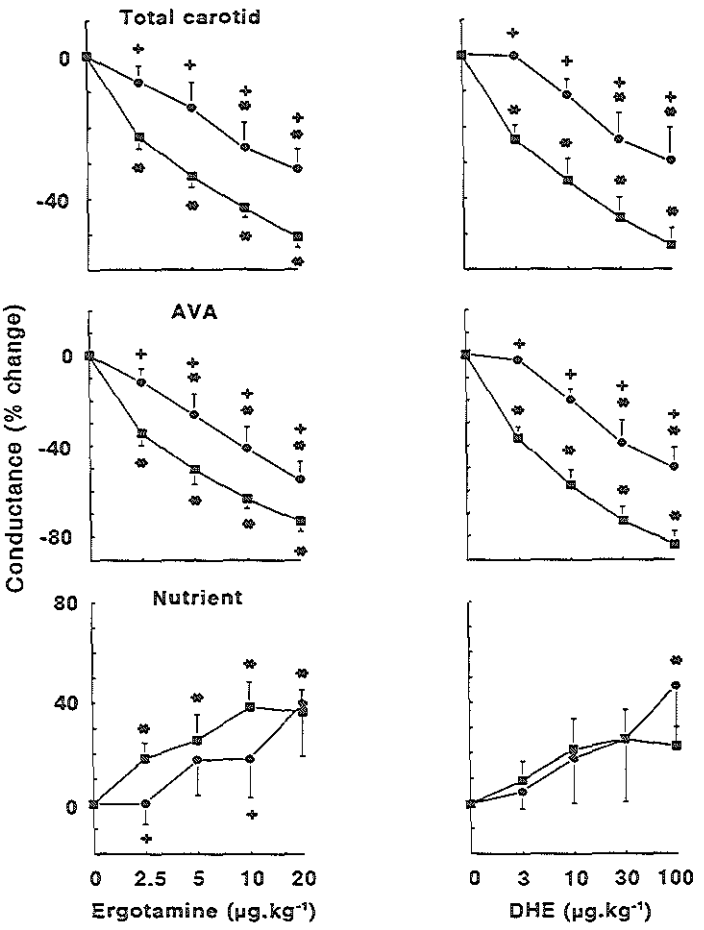


Figure 4 Percent changes in total carotid vascular conductance and its arteriovenous anastomotic (AVA) and nutrient fractions induced by ergotamine and dihydroergotamine in anaesthetized pigs pretreated with saline (squares; n=6 and 6, respectively) or methiothepin (3 mg.kg^{-1}, circles; n=6 and 6, respectively). *, P< 0.05 vs baseline; +, P< 0.05 vs the corresponding dose in the saline pretreated animals.

Pretreatment with methiothepin (3 mg.kg^{-1}) attenuated the effects of both ergotamine and dihydroergotamine on arteriovenous anastomotic blood flow and conductance; there was a 3.1 and 5.2 fold shift in the ED_{30} (dose eliciting a 30% decrease in arteriovenous anastomotic conductance) of ergotamine and dihydroergotamine, respectively.

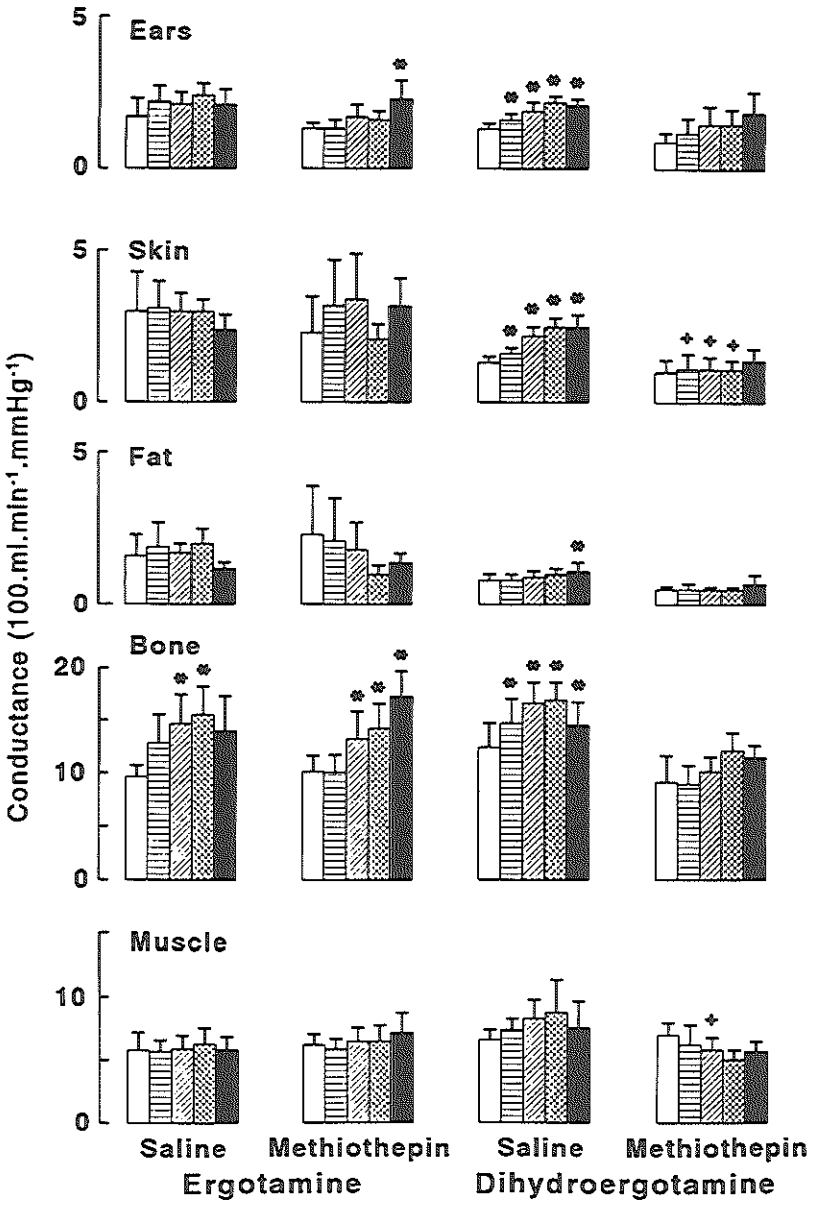


Figure 5 Effects of ergotamine and dihydroergotamine on vascular conductance in different cranial tissues in anaesthetized pigs pretreated with saline (n=6 each) or methiothepin (3 mg.kg^{-1}; n=6 each). The five columns in each panel represent, from left to right, the following doses: Ergotamine: 0 (baseline), 2.5, 5, 10, and 20 μg kg^{-1}; Dihydroergotamine: 0 (baseline), 3, 10, 30, and 100 μg.kg^{-1}. *, $P < 0.05$ vs baseline; +, $P < 0.05$ vs the corresponding dose in the saline pretreated animals.

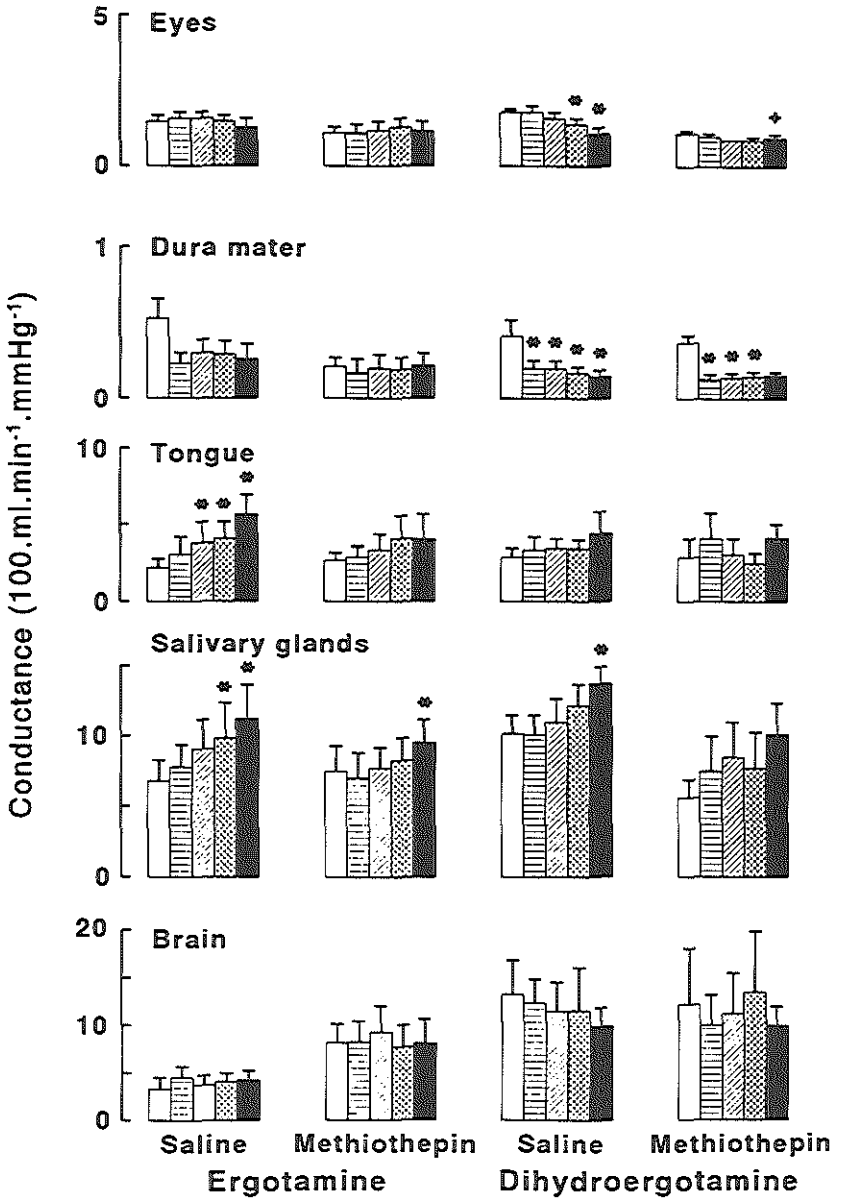


Figure 6 Effects of ergotamine and dihydroergotamine on vascular conductance in different cranial tissues in anaesthetized pigs pretreated with saline (n=6 each) or methiothepin (3 mg.kg^{-1}; n=6 each). The five columns in each panel represent, from left to right, the following doses: Ergotamine: 0 (baseline), 2.5, 5, 10, and 20 μg kg^{-1}; Dihydroergotamine: 0 (baseline), 3, 10, 30, and 100 μg.kg^{-1}. *, $P < 0.05$ vs baseline; +, $P < 0.05$ vs the corresponding dose in the saline pretreated animals.

The effects of ergotamine and dihydroergotamine on the nutrient conductance to the different tissues are shown in figure 5 and 6. Ergotamine increased the arteriolar conductance in the bones, tongue and salivary glands, whereas the arteriolar conductance in the other tissues remained unchanged. Methiothepin (3 $mg.kg^{-1}$) had no significant effect on these changes in nutrient conductance. Dihydroergotamine slightly increased the arteriolar conductance in the ears, head skin, bones and salivary glands. Methiothepin (3 $mg.kg^{-1}$) attenuated most of these effects (figures 5 & 6).

Figure 5 and 6 also show that, despite an increase in total nutrient conductance as well as conductance in several tissues, the conductance in the dura mater tended to decrease with both ergot alkaloids. However, this vascular effect reached statistical significance only in the case of dihydroergotamine. Methiothepin did not have a clear effect on the responses of the dural vessels.

Discussion

Systemic haemodynamics

Ergotamine reduced dose-dependently heart rate. Such a bradycardiac action has already been shown in different species, like dogs and man (Clark et al., 1978), cats (Saxena & Cairo-Rawlins, 1979) and pigs (Schamhardt et al., 1979; Bom et al., 1989a). In cats, an agonist action at presynaptic dopamine receptors on sympathetic cardioaccelerator neurons has been implicated (Saxena & Cairo-Rawlins, 1979), but in rats stimulation of presynaptic α_2-adrenoceptors may be involved (Roquebert & Grenié, 1986). The mechanism involved in pigs is, however, unknown at present. Dihydroergotamine (3-30 $\mu g.kg^{-1}$) also reduced heart rate in a dose-dependent way, probably by the same mechanism as ergotamine; in man a moderate decrease in heart rate has been observed after 10-15 $\mu g.kg^{-1}$ of dihydroergotamine (Harris et al., 1963; Nordenfeldt & Mellander, 1972). Since the highest dose of dihydroergotamine (100 $\mu g.kg^{-1}$) produced less bradycardia than the lower doses in the present experiments, the drug may have a partial agonist activity on the receptor involved.

The pressor response to the ergot alkaloids is generally believed to be a peripheral action, depending much on the pre-existing sympathetic tone (Clark et al., 1978). In the case of a high sympathetic tone, the sympatholytic action of the ergots predominates and no or less marked pressor responses are found, as has been the case in some earlier studies (Saxena & De Vlaam-Schluter, 1974; Bom et al., 1989a). In the present investigation, arterial blood pressure was increased by both ergotamine and dihydroergotamine. In the rat the ergot alkaloids-induced pressor response seems to be

mediated partly by α_2-adrenoceptors and partly by 5-HT receptors, but probably not by α_1-adrenoceptors (Roquebert & Grenié, 1986; Müller et al., 1988). Although the receptor mechanism in the pig is unknown, both the α-adrenoceptor and the 5-HT receptor antagonist potency of methiothepin may be responsible for the attenuation of the pressor responses to the two ergot alkaloids (see Table 1).

Carotid haemodynamics

Both ergotamine and dihydroergotamine caused dose-dependent decreases in carotid blood flow and vascular conductance, exclusively by affecting the arteriovenous anastomotic fraction. The effects of the drugs on the arteriovenous oxygen saturation difference is in keeping with an effect on the arteriovenous anastomoses. Heyck (1969) observed a decreased arterio-jugular venous oxygen saturation difference during migraine attacks and administration of dihydroergotamine raised this difference, as in our experiments. It is interesting to note that, despite an increase in vascular conductance in several cranial extracerebral tissues and the nutrient conductance as a whole, dural vascular conductance decreased with the ergots. There was no effect on the cerebral component of the carotid blood flow in our experiments, which is in keeping with the observations in both animals and man that total cerebral blood flow is little affected by ergotamine and dihydroergotamine (Hatchinski et al., 1978; Johnston & Saxena, 1978; Andersen et al., 1987).

Pretreatment with 3 $mg.kg^{-1}$ methiothepin partly antagonized the effects of the two ergot alkaloids. This antagonism was slightly more marked in the case of dihydroergotamine than ergotamine; the increase in ED_{30} was, respectively, 5.2 and 3.1 fold. Since methiothepin 1 $mg.kg^{-1}$ had caused a 3.2 fold increase in ED_{30} of ergotamine in the experiments of Bom et al. (1989a), the higher dose of methiothepin did not seem to have any further effect. This is in contrast with sumatriptan where methiothepin dose-dependently antagonized the carotid vascular effects and with the higher dose (3 $mg.kg^{-1}$) a complete blockade was observed (Den Boer et al., 1991a). At high concentrations, methiothepin is an antagonist at 5-HT_1-like and 5-HT_2 receptors (Table 1; Bradley et al., 1986) as well as at α_1- and α_2-adrenoceptors and D_2 receptors (Table 1). Since ergotamine and dihydroergotamine also have reasonable affinities for these receptors (Table 1), theoretically any of these could mediate the methiothepin-sensitive component of the effect of the ergot alkaloids. Involvement of α_1, 5-HT_2 or D_2 receptors seems less likely, since blockade of 5-HT_2 receptors by pizotifen in dogs and of 5-HT_2 and α_1 receptors by ketanserin in cats left the responses to ergotamine unchanged (Saxena et al., 1983; Bom et al., 1989a) and the presence of functional D_2 receptors on blood vessels is at present uncertain (De Keyser et al., 1988). Since phentolamine slightly

inhibited the carotid vasoconstrictor responses to ergotamine in dogs (Saxena & De Vlaam-Schluter, 1974; Saxena et al., 1983), the attenuation observed with methiothepin in the present experiments could be due to blockade of postsynaptic α_2-adrenoceptors. Nevertheless, we cannot rule out the involvement of 5-HT_1-like receptors, especially since phentolamine also has a slight antagonist action at 5-HT receptors (Parsons et al., 1989; Hoffman & Lefkowitz, 1990). Ergotamine and dihydroergotamine as well as sumatriptan share a high affinity for the 5-HT_{1D} subtype of the 5-HT_1-like receptors (Table 1) and all three inhibit forskolin-stimulated adenylate cyclase activity in calf substantia nigra (Hoyer & Schoeffter, 1991), a putative 5-HT_{1D} receptor mediated effect. However, a possible role of this receptor in the carotid vasoconstrictor responses to these drugs has still to be elucidated.

The present experiments show that, despite the use of a high dose (3 $mg.kg^{-1}$) of methiothepin, a substantial part of the arteriovenous anastomotic flow reduction by the ergot alkaloids was not amenable to blockade by methiothepin. This is in marked contrast to the antimigraine drug sumatriptan (Den Boer et al., 1991a) and other drugs, such as 5-HT (Saxena et al., 1986), 8-OH DPAT (Bom et al., 1989b), RU 24969 (Bom et al., 1989c) and indorenate (Villalón et al., 1990), all of which reduce arteriovenous anastomotic blood flow by acting on a 5-HT_1-like receptor which is quite susceptible to blockade by methiothepin (Saxena & Ferrari, 1989; Den Boer et al., 1991a). Therefore, the carotid vasoconstrictor effects of the ergot alkaloids must be in part mediated by a novel mechanism involving 'ergot receptors' that remain to be characterized. Such receptors have also been postulated in the contraction of isolated rabbit saphenous vein to the non-peptide ergot alkaloids methysergide, ergometrine and methylergometrine (MacLennan & Martin, 1990a). These contractions were clearly biphasic and only the first part was susceptible to blockade by methiothepin.

Possible mechanisms in the antimigraine effect

The exact mechanism of the antimigraine activity of the ergot alkaloids and, indeed of other drugs is under debate (see Saxena & Ferrari, 1989; Buzzi & Moskowitz, 1990; Humphrey et al., 1990a; Saxena, 1990). The limited understanding of the migraine syndrome itself is in large part the reason for this. Due to the unilaterality of the symptoms, migraine is likely to be caused by a neural disturbance in the central nervous system, possibly originating in brain stem nuclei. This could lead to a dilatation of extracerebral cranial blood vessels, which has been observed, at least in some patients, during a migraine attack (Drummond & Lance, 1988; Saxena & Ferrari, 1989; Iversen et al., 1990).

Notwithstanding the mechanism, a common feature of the antimigraine drugs effective against acute migraine attacks (ergotamine, dihydroergotamine and sumatriptan) is their ability to constrict, relatively selectively, arteries and arteriovenous anastomoses in the cranial circulation, without affecting tissue blood flow (Müller-Schweinitzer & Weidmann, 1978; Saxena & Ferrari, 1989; Saxena & Den Boer, 1991). Since sumatriptan does not seem to penetrate the blood brain barrier (Dallas et al., 1989) and the evidence regarding penetration by ergotamine and dihydroergotamine is at best equivocal (in favour: Ala-Hurula et al., 1979; Goadsby & Gundlach, 1991; against: Eckert et al., 1978; Kanto et al., 1981; Hovdal et al., 1982), it would appear that the antimigraine effect of these drugs is related to a vascular effect, possibly involving a powerful vasoconstrictor activity on dilated and painful blood vessels. In addition, these drugs have been reported to inhibit extravasation of plasma in the dura mater following stimulation of the trigeminal ganglion in the rat (Markowitz et al., 1988; Saito et al., 1988; Buzzi & Moskowitz, 1990); such an effect may also contribute to the antimigraine effect of these drugs. However, the exact mechanism responsible for the inhibition of plasma extravasation is not well understood. Although an effect on receptors on sensory afferent fibres in the muscular layer of the blood vessel wall is possible, this effect could equally be secondary to vasoconstriction. Indeed, sumatriptan has been shown to have a vasoconstrictor action in human dural blood vessels (Feniuk et al., 1991). Furthermore, apart from arteries and veins, the presence of arteriovenous anastomoses in the dura mater has been anatomically demonstrated (Rowbotham & Little, 1965; Kerber & Newton, 1973).

Chapter 9

Lack of Effect of the Antimigraine Drugs, Sumatriptan, Ergotamine and Dihydroergotamine on Arteriovenous Anastomotic Shunting in the Dura Mater of the Pig

Summary

In anaesthetized animals, the antimigraine drugs, sumatriptan, ergotamine and dihydroergotamine reduce carotid arteriovenous anastomotic shunting. Within the carotid vascular bed arteriovenous anastomoses are located, amongst others, in the dura mater, which is a putative site of the pain during a migraine attack. In this investigation, we have localized and measured the arteriovenous shunting within the carotid vascular bed of the pig by using simultaneous intracarotid injections of radiolabelled microspheres of three different sizes (10, 15 and 50 μm), which provides an index of blood flow via arteriovenous anastomoses larger than approximately 14, 27 and 90 μm diameter, respectively. The effects of sumatriptan (0.3 mg kg^{-1}), ergotamine (0.02 mg kg^{-1}), dihydroergotamine (0.1 mg kg^{-1}) and saline were studied by repeating the injections of 15 and 50 μm spheres after the treatments.

There was no difference in shunting or entrapment between the 10 and 15 μm microsphere, indicating the absence of arteriovenous anastomoses with a diameter between 14 and 27 μm. Arteriovenous anastomoses with a diameter between 27 and 90 μm, as indicated by the difference in blood flow measured by 15 and 50 μm spheres, were located in the dura mater, ears, skin, fat and, to a lesser extent, in the skeletal muscles and eyes. Sumatriptan, ergotamine and dihydroergotamine reduced the overall flow in the smaller arteriovenous anastomoses (diameter between 27 and 90 μm), and even more in larger shunts (wider than 90 μm). Locally, blood flow in the smaller arteriovenous shunts was reduced in the skin and fat, but not in the dura mater, ears, eyes and muscles. It is not possible to determine in which tissues blood flow in the larger arteriovenous anastomoses was reduced. Tissue blood flow measured with 15 μm microspheres remained unchanged after the three antimigraine drugs, implying a lack of effect on capillary flow.

It is concluded that in the anaesthetized pigs the only evident effect of these antimigraine drugs on carotid haemodynamics is a decrease in blood flow in both smaller and larger arteriovenous anastomoses; the smaller arteriovenous anastomoses were affected in the skin and fat, but not in other tissues.

Introduction

The antimigraine drugs, sumatriptan, ergotamine and dihydroergotamine are vasoconstrictors. *In vitro*, different medium sized and small arteries as well as certain veins are constricted (Müller-Schweinitzer & Weidmann, 1978; Müller-Schweinitzer & Rosenthaler, 1987; Humphrey et al., 1988). Amongst the arteries, those of the cranial circulation are more sensitive to these drugs than those in other body regions (Müller-Schweinitzer & Weidmann, 1978; Humphrey et al., 1988). *In vivo*, these drugs cause an increase in resistance in the carotid vascular bed of different species; this increase seems to be localized only in arteriovenous anastomoses in this vascular bed, because capillary blood flow remains either unchanged or is even increased (see Saxena, 1978; Bom et al., 1989a; Perren et al., 1989; Den Boer et al., 1991a,b).

Arteriovenous anastomoses have been located in various cranial tissues, but it is at present unclear which of them are constricted by the antimigraine drugs. Furthermore, it is uncertain whether a constrictor action in anastomoses is involved at all in the clinical action of the antimigraine drugs. A possible contribution of dilatation of arteriovenous anastomoses to the migraine headache has been postulated by Heyck (1969), who found the jugular venous oxygen saturation to be elevated during a migraine attack. Interestingly, arteriovenous anastomoses have been anatomically located in the human dura mater, which is endowed with rich vascularization (Rowbotham & Little, 1965; Kerber & Newton, 1973). The dura mater is currently held as a possible origin of the migraine pain, involving vasodilatation and extravasation of plasma protein, induced by peptide release from the peripheral endings of the trigeminal nerve (Markowitz et al., 1988; Goadsby et al., 1990). Such vascular changes in the dura mater, when experimentally induced in rats and guinea-pigs, can be inhibited by the same antimigraine drugs (Markowitz et al., 1988; Buzzi & Moskowitz, 1990). The mechanism involved in this inhibition of protein extravasation has not yet been fully elucidated; both a presynaptic inhibition of neuropeptide release (Buzzi et al., 1991) and cranial vasoconstriction (Humphrey et al., 1990b; Saxena & Den Boer, 1991) remain as possibilities. Indeed, sumatriptan increases vascular resistance in the human isolated, perfused dura mater (Humphrey et al., 1991a). However, at present, no direct information on the effects of these antimigraine agents on dural haemodynamics *in vivo* is available. Therefore, we studied the effects of sumatriptan, ergotamine and dihydroergotamine on the carotid circulation in anaesthetized pigs, with special emphasis on the dura mater. In order to obtain direct information about the blood flow in arteriovenous anastomoses of different tissues, we injected radioactive microspheres of different sizes into the carotid artery.

Methods

General

After an overnight fast 24 domestic pigs (Yorkshire x Landrace; 16-22 kg) were anaesthetized with azaperone (120 mg, i.m.) and metomidate (150 mg, i.v.), intubated and connected to a respirator (Bear 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; Po_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of pentobarbitone sodium (Sanofi, Paris, France) at 20 $mg.kg^{-1}.h^{-1}$ for the first hour and thereafter 12 $mg.kg^{-1}.h^{-1}$).

Catheters were placed in the inferior vena cava via a femoral vein for the administration of drugs and in the aortic arch via a femoral artery, connected to a Statham pressure transducer (P23 Dc, Hato Rey, Puerto Rico) for the measurement of arterial blood pressure and the withdrawal of arterial blood for determining blood gases (ABL-2, Radiometer, Copenhagen, Denmark). Mean arterial blood pressure (MAP) was calculated from the systolic (SAP) and diastolic (DAP) arterial pressures:
MAP = (SAP + 2 x DAP) / 3. The common carotid arteries were dissected free and the cervical vagosympathetic trunks were cut. Blood flow was measured in one of the common carotid arteries with a flow probe (internal diameter: 2.5 or 3 mm) connected to a sine-wave electromagnetic flow meter (Transflow 600-system, Skalar, Delft, The Netherlands). Heart rate was measured with a tachograph triggered from the blood pressure or the flow signal, depending on their shape. A 0.6 mm (external diameter) needle, connected to a polyethylene tubing was inserted into the common carotid artery against the direction of the blood flow for the administration of radioactive microspheres. At the same side the jugular vein was cannulated in order to obtain venous blood samples for determining blood gases.

During the experiment body temperature was kept above 37 °C and the animals were continuously infused with 100 $ml.h^{-1}$ saline to compensate for fluid loss.

Radioactive microsphere method

The distribution of common carotid blood flow was determined with a mixture of 10, 15 and 50 μm microspheres at baseline and of 15 and 50 μm microspheres after drug treatment. These were labelled with either ^{141}Ce (10 μm), ^{113}Sn (50 μm), ^{103}Ru (50 μm), ^{95}Nb (15 μm) or ^{46}Sc (15 μm; NEN Company, Dreieich, Germany). The order of 15 and 50 μm spheres injected before and after the drugs was randomized. The approximate number of microspheres given per isotope was: 10 μm, 700,000; 15 μm, 300,000; 50

μm, 30,000). The microspheres were vortexed for about half a minute and then injected into the carotid artery against the direction of the blood flow to ensure uniform mixing. At the end of the experiment the animals were killed and the heart, kidneys, lungs and the different cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 5-10 min in a gamma-scintillation counter (Packard, Minaxi Autogamma 5000) using suitable windows for discriminating the different isotopes.

The ratio between the radioactivity in a particular tissue and the total radioactivity was calculated with a set of specially developed computer programs (Saxena et al., 1980). By multiplying this ratio with the total carotid blood flow value at the time of the injection, blood flow values to the lungs and tissues were determined.

By video recording of the motion of microspheres in the blood vessels in the hamster cheek pouch, Dickhoner et al. (1978) have demonstrated that microspheres with a diameter of 9, 15 and 24 μm are trapped in blood vessels with a diameter of 11.5, 27.7 and 42.7 μm, respectively. Therefore, in the present investigation blood flow values measured with the 10, 15 and 50 μm microspheres in the lungs have been assumed to represent cranial shunt flow in arteriovenous anastomoses wider than approximately 14, 28 and 90 μm, respectively. Similarly, in the cranial tissues, the difference between blood flow values measured with 10 and 15 μm and with 15 and 50 μm microspheres was assumed to represent the flow in local arteriovenous anastomoses with a diameter, respectively, between 14 and 28 μm and between 28 and 90 μm. The rationale for these assumptions is amplified at the beginning of the discussion section.

Experimental protocol

After a stabilization period of about 1 h, arterial blood pressure, heart rate, blood flow in one of the common carotid arteries, and arterial and jugular venous blood gas values were determined. Then a mixture of 10, 15 and 50 μm microspheres was injected into the carotid artery. After another stabilization period of about 15 minutes, the animals received an intravenous injection of either sumatriptan (0.3 $mg.kg^{-1}$), ergotamine (0.02 $mg.kg^{-1}$), dihydroergotamine (0.1 $mg.kg^{-1}$) or saline. These doses of the drugs have been shown to cause a 70 to 80% decrease in arteriovenous anastomotic shunt flow in our previous experiments (Den Boer *et al.*, 1991a,b). Fifteen minutes after this injection, systemic haemodynamic values were collated and blood gases and regional carotid haemodynamics (by using a mixture of 15 and 50 μm microspheres) were again determined.

Data presentation and statistical analysis

All data have been expressed as means ± SEM. The overall significance of the differences between the baseline flow values has been evaluated in the four groups together with a multivariate analysis of variance (repeated-measurements design). In the case of a significant F, individual differences were analyzed with a Student's t-test for paired data (two-tailed). The effect of the treatment was compared in every group separately with a repeated measurements analysis of variance with the factors size and treatment. Individual differences were analyzed with a Student's t-test for paired data. Statistical significance was accepted at $P < 0.05$ (two-tailed).

Drugs

Apart from the anaesthetics, azaperone and metomidate (both from Janssen Pharmaceutica, Beerse, Belgium), the drugs used in this study were: sumatriptan (Glaxo group research, Ware, UK), ergotamine tartrate (Wander-Pharma, Uden, The Netherlands), dihydroergotamine mesylate (Wander-Pharma, Uden, The Netherlands) and heparin sodium (Thromboliquine, Organon Teknika B.V., Boxtel, The Netherlands) to prevent clotting of the catheters. Sumatriptan was dissolved in physiological saline. All doses refer to the respective salts.

Results

Localization and size of arteriovenous anastomoses

In the carotid circulation 63±3 % of 10 μm microspheres, 61±3 % of 15 μm spheres and 41±3 % of 50 μm spheres were shunted to the venous side via arteriovenous anastomoses. Arteriovenous anastomotic blood flows, measured with the 10, 15 and 50 μm microspheres, were 108±11, 105±11 and 71±7 $ml.min^{-1}$, respectively, so that 3±1 $ml.min^{-1}$ passed through shunts with a diameter between 14 and 28 μm, 34±4 $ml.min^{-1}$ through shunts of 28-90 μm diameter and 71±7 $ml.min^{-1}$ through larger shunts. In the dura mater, ears, skin, fat and, to a lesser extent, skeletal muscles and eyes, the respective blood flow values measured with 15 μm microspheres were less than those measured with 50 μm microspheres, thus implying the presence of arteriovenous anastomoses with a diameter between approximately 28 and 90 μm in these tissues (Table 1; Figure 1). In the skin and the eyes there was a small, but significant, difference between entrapment of 10 and 15 μm microspheres, so that a few arteriovenous anastomoses with a diameter between approximately 15 and 30 μm appear to be present in these tissues. Since there was no difference in the blood flow measured with the

Table 1. Tissue flow values, measured with 10, 15 and 50 μm diameter radioactive microspheres.

	10 μm	15 μm	50 μm
Dura mater	0.12±0.02	0.13±0.02	0.28±0.02*
Ears	2.3±0.3	2.7±0.5	9.4±1.0*
Skin	2.1±0.1*	2.9±0.3	16.9±1.3*
Fat	2.6±0.3	2.7±0.4	11.6±1.5*
Muscle	8.9±0.7	8.9±0.9	14.3±1.2*
Brain	9.4±0.9	10.3±1.0	10.2±1.0
Tongue	3.1±0.3	3.3±0.4	3.8±0.3*
Bones	11.1±1.0	11.3±1.0	11.3±1.0
Eyes	1.9±0.25*	2.1±0.2	2.4±0.2
Salivary	7.1±0.6	7.6±0.8	6.5±0.5

All values (ml.min^{-1}) are given as means ± SEM; *, P<0.05 vs flow measured with 15 μm microsphere.

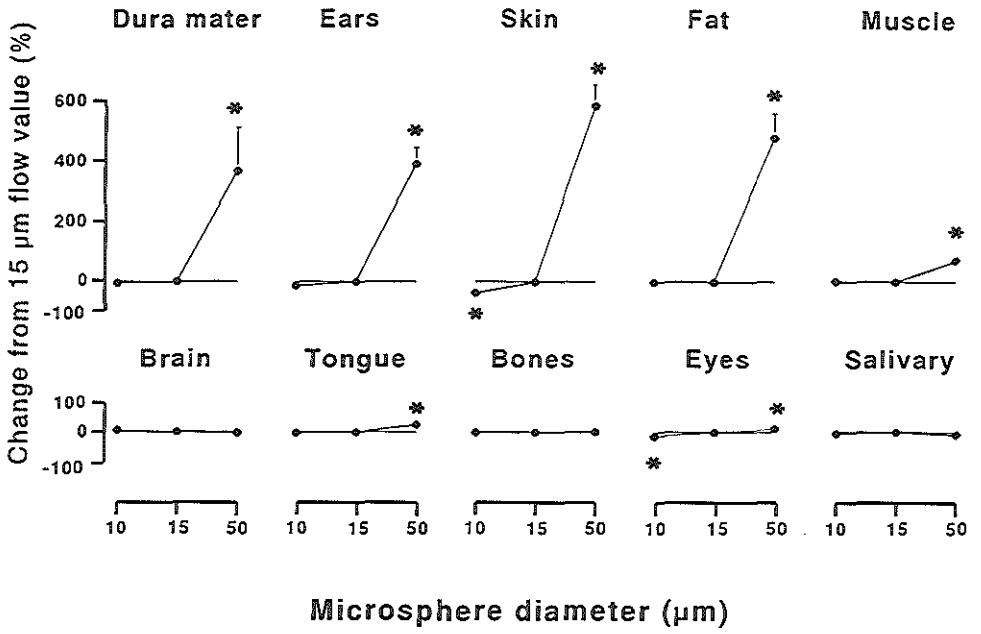


Figure 1. The localization of arteriovenous anastomoses in different cranial tissues, by differential entrapment of radioactive microspheres of 10, 15 and 50 μm diameter. All values (means ± SEM) have been given as percent difference from the flow value, measured with 15 μm spheres. *, P< 0.05 from 15 μm flow value.

microspheres of three sizes in the brain, tongue, bones and salivary glands (Table 1; Figure 1), it appears that these tissues contain no anastomoses with a diameter between 15 and 90 μm. From the present experiments it is, however, not possible to localize the shunts larger than 90 μm.

Effects of the antimigraine drugs

Systemic haemodynamics

The effects of i.v. injections of sumatriptan (0.3 mg.kg^{-1}), ergotamine (0.02 mg.kg^{-1}), dihydroergotamine (0.1 mg.kg^{-1}) or saline on heart rate and mean arterial blood pressure are shown in Table 2. After saline and dihydroergotamine small, but statistically significant, decreases in heart rate occurred. It is, therefore, not certain, whether the small change induced by dihydroergotamine reflects a direct action of the drug (Table 2). After ergotamine and dihydroergotamine mean arterial blood pressure increased by 38±8 and 34±5 %, respectively. Though not statistically significant, an increase in mean arterial pressure of 14±8 % was seen after sumatriptan.

Table 2. Effect of sumatriptan (0.3 mg.kg^{-1}), ergotamine (0.02 mg.kg^{-1}), dihydroergotamine (0.1 mg.kg^{-1}) or saline on heart rate (HR) and mean arterial blood pressure (MAP).

	HR (Beats.min^{-1})		MAP (mmHg)	
	Before	After	Before	After
Saline	89±6	87±5*	92±7	92±7
Sumatriptan	89±4	88±3	94±8	107±11
Ergotamine	87±6	85±6	88±4	120±7*
Dihydroergotamine	91±3	86±4*	101±6	134±4*

Values are given as mean ± SEM; *, $P < 0.05$ vs control.

Carotid blood flow and total arteriovenous anastomotic shunting

All three antimigraine drugs, sumatriptan, ergotamine and dihydroergotamine, reduced carotid blood flow (Table 3) by -30±7, -44±6 and -30±5 %, respectively. Although a much smaller, but statistically significant, reduction of carotid blood flow was seen after saline, most of the changes after the three antimigraine drugs appear to be specific for the drugs. These decreases were not caused by a decrease in capillary flow: drug-induced changes in total capillary blood flow values, determined with 15 μm microspheres, were only -1.6±1.3, 4.4±5.8, -3.7±8.0 and 6.6±5.7 ml.min^{-1},

Table 3. Effect of sumatriptan (0.3 mg.kg^{-1}), ergotamine (0.02 mg.kg^{-1}), dihydroergotamine (0.1 mg.kg^{-1}) or saline on carotid blood flow (CBF) and arterio-jugular venous oxygen saturation difference (AVOSD).

	CBF (ml.min^{-1})		AVOSD (%)	
	Before	After	Before	After
Saline	152±17	144±14*	5.4±1.8	5.8±1.9
Sumatriptan	157±20	106±9*	7.1±4.3	8.9±2.8
Ergotamine	181±36	98±18*	4.6±0.9	15.3±3.7*
Dihydroergotamine	170±24	117±18*	4.9±1.4	14.9±3.6*

Values are given as means ± SEM; *, P < 0.05 vs control.

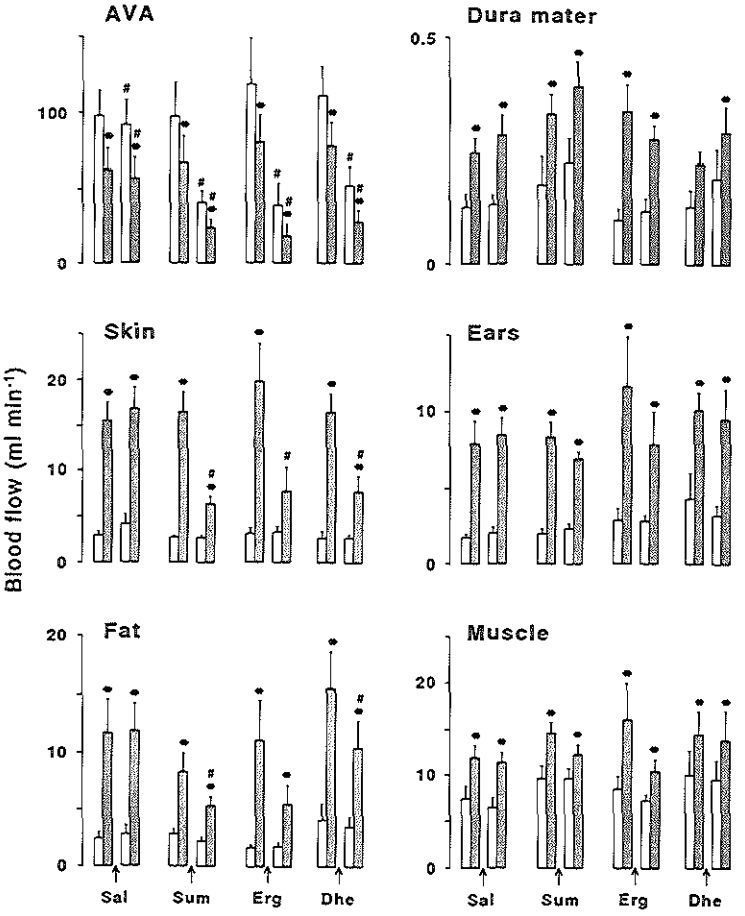


Figure 2. Effect of saline (Sal), sumatriptan (Sum, 0.3 mg.kg^{-1}), ergotamine (Erg, 0.02 mg.kg^{-1}) and dihydroergotamine (Dhe, 0.1 mg.kg^{-1}) on flow measured with 15 μm (empty bars) and 50 μm (cross-hatched bars) microspheres. Values as means ± SEM; *, P < 0.05 vs simultaneously injected 15 μm shere; #, P < 0.05 vs same sphere before treatment.

respectively, for saline, sumatriptan, ergotamine and dihydroergotamine. On the other hand, arteriovenous shunt flow, measured with microspheres of both 15 and 50 μm was decreased by the three antimigraine drugs (Figure 2). An increase in the difference between arterial and jugular venous oxygen saturation after ergotamine or dihydroergotamine is in keeping with an effect on arteriovenous anastomoses (Table 3).

Blood flow through smaller (28-90 μm) arteriovenous anastomoses was decreased by sumatriptan and tended to decrease, although not significantly, after ergotamine and dihydroergotamine (Table 4, Figure 2). A much larger decrease in shunting was observed in the shunts wider than 90 μm after all three agents (Table 4).

Table 4. Effect of sumatriptan (0.3 mg.kg^{-1}), ergotamine (0.02 mg.kg^{-1}), dihydroergotamine (0.1 mg.kg^{-1}) or saline on blood flow (ml.min^{-1}) through arteriovenous anastomoses with a diameter between 28 and 90 μm or anastomoses wider than 90 μm.

	28-90 μm		> 90 μm	
	Before	After	Before	After
Saline	35±5	35±5	61±14	56±13*
Sumatriptan	30±6	16±3*	66±17	24±5*
Ergotamine	38±14	20±7	79±18	18±8*
Dihydroergotamine	33±7	24±5	77±15	28±7*

Values are given as means ± SEM; *, $P < 0.05$ vs control.

Blood flow in tissues with arteriovenous anastomoses with a diameter between 16 and 28 μm

In skin and fat sumatriptan, ergotamine and dihydroergotamine decreased entrapment of 50 μm microspheres, while the entrapment of 15 μm microspheres remained unchanged (Figure 2). All three agents also attenuated the difference in blood flow measured with spheres of the two sizes in the skin, indicating a decrease in the blood flow through shunts with a diameter between 28 and 90 μm (Figure 2). In the fat, only dihydroergotamine significantly decreased the blood flow through these shunts, although a similar, non-significant, tendency was observed with sumatriptan and ergotamine (Figure 2). In spite of the abundance of shunts with a diameter between 28 and 90 μm in the dura mater and ears, they were not affected by any of the drugs (Figure 2). The slight amount of shunting in muscle, tongue and eyes was also not influenced by the drugs.

Blood flow in tissues without arteriovenous anastomoses with a diameter between 16 and 28 μm

In the bones, salivary glands and brain, where flow through shunts with a diameter between 28 and 90 μm was completely absent, administration of neither sumatriptan, ergotamine nor dihydroergotamine produced any effect, except for an increase in salivary gland blood flow, measured with 50 μm spheres after dihydroergotamine (Figure 3).

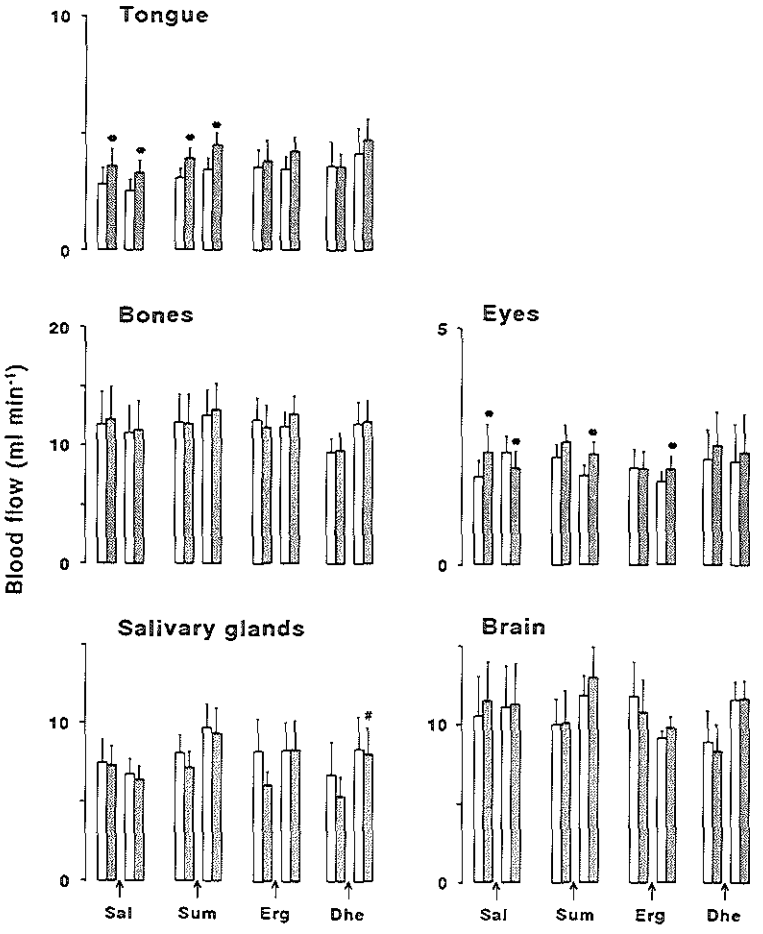


Figure 3. Effect of saline (Sal), sumatriptan (Sum, 0.3 mg.kg^{-1}), ergotamine (Erg, 0.02 mg.kg^{-1}) and dihydroergotamine (Dhe, 0.1 mg.kg^{-1}) on flow measured with 15 μm (empty bars) and 50 μm (cross-hatched bars) microspheres. Values as means ± SEM; *, $P < 0.05$ vs simultaneously injected 15 μm sphere; #, $P < 0.05$ vs same sphere before treatment.

Discussion

Use of microspheres of three sizes to measure arteriovenous anastomotic blood flow

The use of microspheres of different sizes to measure the distribution of carotid blood flow has already been extensively described by Saxena & Verdouw (1985a). A clear effect of 5-HT on arteriovenous anastomoses was observed in their experiments. From the early years of the use of radioactive microspheres to measure blood flow, non-entrapment of spheres in certain tissues has been observed (Kaihara et al., 1968; Hales, 1974). The amount of non-entrapment, ascribed to arteriovenous anastomotic blood flow (Hales, 1974), is dependent on microsphere size, the tissue studied and the use of anaesthesia (Forsyth & Hoffbrand, 1970). Dickhoner et al. (1978) examined with a video camera the behaviour of 9, 15 and 24 μm microspheres in the hamster cheek pouch, after intracardiac injection. They demonstrated that 9, 15 and 24 μm microspheres were trapped when arriving in blood vessels with a diameter of 11.5, 27.7 and 42.7 μm, respectively, for the three microspheres. Extrapolation of these results to our experiments indicates that the 10, 15 and 50 μm diameter microspheres are expected to pass to the venous side through blood vessels with a minimum diameter larger than 14, 28 and 90 μm, respectively. Such vessels are arteriovenous anastomoses, because capillaries have a diameter under 10 μm (for references see Saxena & Verdouw, 1985a). Since, in our experiments, no radioactivity could be detected in the heart or the kidneys, the microspheres reaching the venous side by arteriovenous anastomoses were completely trapped in the lungs. Therefore, the lung blood flow value obtained with a particular microsphere reflects the total amount of arteriovenous anastomotic shunting of that microsphere (Saxena & Verdouw, 1982). The tissue blood flow value, calculated with a certain microsphere, is a measure of the flow through blood vessels in which this particular microsphere is trapped. When a tissue is devoid of arteriovenous anastomoses, microspheres of all sizes yield similar blood flow values, which are equal to the tissue capillary flow (as demonstrated e.g. in the brain in the present experiments). An increase in tissue blood flow values with increasing microsphere size indicates that arteriovenous anastomoses are present in that tissue. Using the data from Dickhoner et al. (1978) the difference between the blood flow values measured with 10 and 15 μm microspheres can be used as an index of blood flow in arteriovenous anastomoses with a diameter between 14 and 28 μm. Similarly, the blood flow difference between 15 and 50 μm microspheres indicates blood flow in shunt vessels between 28 and 90 μm. Blood flow in still larger arteriovenous anastomoses, which may have a diameter up to 150 μm (Sherman, 1963), cannot be calculated for individual tissues, since the use of still larger microspheres is not

practical due to adverse influence on the circulation. However, the total blood flow in these largest shunts can be assessed by the lung entrapment of 50 μm spheres.

Effects of the antimigraine drugs

It has been known for some time that the antimigraine drugs ergotamine and dihydroergotamine, when administered in anaesthetized animals, decrease arteriovenous anastomotic blood flow in the carotid vascular bed (Saxena, 1978; Saxena et al., 1983; Den Boer et al., 1991b). Since the new antimigraine agent sumatriptan also has this property (Perren et al., 1989; Den Boer et al., 1991a), interest in this field has been renewed. Arteriovenous anastomoses have been demonstrated in different cranial tissues, but the distribution is species dependent. Some arteriovenous anastomoses have a thermoregulatory function and they can be demonstrated e.g. in the human skin, the dog tongue and the rabbit ears (Hales & Molyneux, 1988). Others, for example those in the splanchnic region, are believed to play a role in blood pressure regulation (Hales & Molyneux, 1988). In the domestic pig such shunts have been functionally demonstrated in the skin and ears, using radioactive microspheres of four sizes (10, 15, 25 and 35 μm diameter; Saxena & Verdouw, 1985a).

A further location of arteriovenous anastomoses is the human dura mater (Rowbotham and Little, 1965; Kerber & Newton, 1973). This tissue is currently believed to play a role in the generation of the pain during a migraine attack. The three antimigraine agents used in this study can all inhibit the effects of stimulation of the trigeminal ganglion on the dura mater of rats. These effects include plasma protein extravasation and oedema caused by the release of vasoactive peptides (e.g. substance P, calcitonin-gene related peptide, neurokinin A) from trigeminal afferents (Markowitz et al., 1988; Buzzi & Moskowitz, 1990). An involvement of the trigeminal system, whether confined to the dura mater or not, is probably reflected by the increased level of calcitonin-gene related peptide in the jugular venous blood, which can be observed during a migraine attack and which can equally be normalized by sumatriptan (Goadsby et al., 1990; Goadsby & Edvinsson, 1991). The mechanism by which the antimigraine drugs inhibit this protein extravasation is not yet clear. A direct effect on the trigeminal afferents is possible, but a role for the vasoconstrictor effects of the antimigraine drugs cannot be excluded. In favour for this latter mechanism is the fact that these drugs have been shown to increase vascular resistance in the *in vitro*, perfused human dura mater (Humphrey et al., 1991a). It is, however, not known what the effects of these drugs are on the dural haemodynamics *in vivo*, and it is with this point that the present study is concerned. Since, in our model, the major effect of the antimigraine drugs is on the arteriovenous anastomoses, we have used intracarotid injection of radioactive

microspheres of different sizes to directly measure arteriovenous anastomotic blood flow in different tissues, including the dura mater. In concordance with the human dura mater (Rowbotham & Little, 1965; Kerber & Newton, 1973), arteriovenous anastomoses with a diameter between 28 and 90 μm could be functionally demonstrated in the porcine dura mater. This is different from the dog dura mater, where there was no difference in entrapment between 15 and 50 μm microspheres (Faraci et al., 1989), although, as in our experiments, the animals were anaesthetized with pentobarbitone, which is known to increase shunting (Forsyth & Hoffbrand, 1970). In addition to the dura mater, arteriovenous anastomoses were demonstrated in the ears, skin and fat; in muscle and eyes there was only a very small amount of shunted blood.

As in earlier studies, sumatriptan, ergotamine and dihydroergotamine all decreased arteriovenous shunt flow in the carotid circulation. Both smaller (28-90 μm) and larger ($>$90 μm) shunts were affected in this way. This decrease in arteriovenous anastomotic blood flow was confined to the skin and fat, while the shunting in the dura mater, ears, muscle and eyes was not affected by the drugs. Neither was there a change in capillary blood flow to any tissue that we studied. In view of the present results, it would seem unlikely that a haemodynamic effect of the antimigraine drugs on dural arteriovenous anastomoses is responsible for the inhibition of dural protein extravasation or the clinical effect of the antimigraine drugs, although one should keep in mind that drug effects may differ among species and preparations used. Sumatriptan has been shown to increase vascular resistance in isolated, perfused human dura mater (Humphrey et al., 1991a). This difference cannot be explained yet, but post-mortem damage to the dural vasculature or the different experimental set-up could be responsible.

The presence of a functional blood brain barrier may explain some differences in drug effects. For example, sumatriptan, which constricts pial vessels upon perivascular application in anaesthetized cats, fails to do so after intravenous administration (Humphrey et al., 1991b): this may be caused by its inability to cross the blood brain barrier (Sleight et al., 1990). However, the dura mater is devoid of an anatomical and functional blood-brain barrier, having fenestrated endothelium (Wislocki & Leduc, 1952; Knudsen et al., 1988).

A further point to consider is the size of the arteriovenous anastomoses. In the present study, 41 % of 50 μm microspheres was shunted to the lungs via arteriovenous anastomoses wider than 90 μm. The total flow in these large arteriovenous anastomoses was decreased by the antimigraine drugs, but these large shunts have not been localized. This would only be possible by using microspheres with a diameter larger than 50 μm. However, such microspheres will undoubtedly adversely influence haemodynamics. It is possible for these large shunts to have a different pharmacological reactivity than the

smaller ones. It should, however, be noted that the arteriovenous anastomoses in the human dura mater usually have a diameter between 50 and 90 μm, and that larger ones seem to be absent (Kerber & Newton, 1973).

A constrictor action of sumatriptan, ergotamine and dihydroergotamine has been demonstrated on large conducting arteries in the cranial vasculature, both *in vitro* (Müller-Schweinitzer & Weidmann, 1978; Parsons et al., 1989) and *in vivo* during migraine attacks (Friberg et al., 1991; Caekebeke et al., 1991). Such a constrictor action of these drugs on arteries, rather than on arteriovenous anastomoses, may be the basis of their antimigraine effect. Vasodilatation of extracranial arteries during the migraine attack and its reversal by ergotamine has been observed by Graham & Wolff (1938). Especially in the last decade the occurrence of vasodilatation has been severely questioned and both positive and negative studies have been published (for an overview, see Olesen et al., 1990). However, most of these studies were concerned only with changes in cerebral blood flow, and possible changes in the diameter of the larger arteries and arteriovenous anastomoses, which may not affect tissue blood flow, have remained largely unaddressed. Only very recently transcranial Doppler measurements of blood flow velocities in cranial arteries have provided an indirect estimate of arterial diameter (Caekebeke et al., 1991; Friberg et al., 1991). Whereas Friberg et al. (1991) found the middle cerebral artery to be dilated during the migraine headache, no such change was observed in the experiments of Caekebeke et al. (1991).

Therefore, it remains to be established, whether vasodilatation occurs during a migraine attack and which vascular beds and which segments of the vascular bed are involved. A role could be assigned to arteries or arteriovenous anastomoses, both of which are affected by the antimigraine drugs. The larger arteriovenous anastomoses ($>$ 90 μm diameter), which are constricted by the antimigraine drugs, have still to be localized. Furthermore, the role of the trigeminal system during a migraine attack has to be elucidated and the mechanism by which the antimigraine drugs inhibit the effects of stimulation of this system. It is interesting that this effect of the antimigraine drugs seems to be specific for the dura mater, since the protein extravasation in the conjunctiva, eyelids and lips are not affected by sumatriptan (Buzzi & Moskowitz, 1990).

Chapter 10

Comparative Effects of the Antimigraine Drugs Sumatriptan and Ergotamine on the Distribution of Cardiac Output in Anaesthetized Pigs

Summary

The haemodynamic effects of sumatriptan, a 5-HT_1-like receptor agonist, and ergotamine, an agonist at α-adrenergic, dopamine as well as 5-HT receptors, were compared using intracardiac injection of radioactive microspheres of different sizes in anaesthetized pigs. Ergotamine (0.02 $mg.kg^{-1}$) and sumatriptan (0.3 $mg.kg^{-1}$) decreased systemic vascular conductance and cardiac output. Only ergotamine raised arterial blood pressure. Both sumatriptan and ergotamine decreased arteriovenous anastomotic, but not capillary, blood flow in the head and body skin. Arteriovenous and capillary blood flow in the dura mater and nasal mucosa and capillary blood flow in the brain, kidneys, adrenals, intestine, heart, spleen and muscle remained unchanged. However, kidney conductance was decreased by both drugs, spleen conductance by sumatriptan and heart, liver and adrenal conductances were decreased by ergotamine. Thus, both sumatriptan and ergotamine constricted arteriovenous anastomoses in the skin, but not in the dura mater or nasal mucosa. Ergotamine constricted the vasculature more than sumatriptan, although both drugs may differentially decrease vascular conductances in some organs.

Introduction

Ergotamine, a classic drug for the treatment of the migraine attack, may produce unwanted vasoconstrictor effects. Although the drug displays some selectivity for the constriction of extracerebral cranial arteries (Saxena & De Vlaam-Schluter, 1974; Müller-Schweinitzer & Weidmann, 1978), in clinical doses signs of prolonged constriction of extracranial arteries, like a decrease in the toe-arm systolic gradient (Tfelt-Hansen, 1986), appear. Also, in anaesthetized animals vascular resistances in many organs are increased by ergotamine (Saxena & de Vlaam-Schluter, 1974; Perren et al., 1989). The vascular effects of ergotamine may be mediated by α-adrenergic, 5-HT and, possibly, still

unknown receptors on arteries, arterioles and arteriovenous anastomoses (Müller-Schweinitzer & Weidmann, 1978; Den Boer et al., 1991b).

The new antimigraine drug sumatriptan causes vasoconstriction of cranial arteries and arteriovenous anastomoses only via 5-HT_1-like receptors (Humphrey et al., 1988; Parsons et al., 1989; Den Boer et al., 1991a). This selectivity may explain the absence of an effect on toe-arm systolic gradients in man (Nielsen & Tfelt-Hansen, 1989) and the absence of an effect on blood pressure and coronary conductance in anaesthetized dogs (Feniuk et al., 1989b). However, constrictor 5-HT_1-like receptors exist also on extracranial arteries and arteriovenous anastomoses, and sumatriptan constricts human coronary arteries and rabbit renal arteries in vitro (Connor et al., 1989b; Chester et al., 1990; Blauw et al., 1991; Tadipatri et al., 1991). Moreover, sumatriptan constricts renal arterioles in anaesthetized dogs (Cambridge et al., 1991), although in anaesthetized cats only arteriovenous anastomoses were affected and not arterioles (Perren et al., 1989).

In the present study the systemic and regional haemodynamics effects of sumatriptan and ergotamine were compared in anaesthetized pigs. The distribution of cardiac output was measured with radioactive microspheres of different diameters, in order to study the effect of the drugs on capillary and arteriovenous anastomotic blood flow in a large number of tissues simultaneously.

Methods

General

After an overnight fast 15 domestic pigs (Yorkshire x Landrace; 16-22 kg) were anaesthetized with azaperone (120 mg, i.m.) and metomidate (150 mg, i.v.), intubated and connected to a respirator (Bear 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; Po_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of pentobarbitone sodium (Sanofi, Paris, France) at 20 $mg.kg^{-1}.h^{-1}$ for the first hour and thereafter 12 $mg.kg^{-1}.h^{-1}$).

The following catheters were placed: in the aortic arch via a femoral artery to withdraw arterial blood for determining blood gases (ABL-2, Radiometer, Copenhagen, Denmark); in the inferior vena cava via a femoral vein for the administration of drugs; in the other femoral artery for the withdrawal (10.5 $ml.min^{-1}$; Braun-Melsungen perfusor, Melsungen, Germany) of an arterial reference sample for calibration of the radioactive microsphere method; and in the left ventricle via a common carotid artery to inject

radioactive microspheres. A 7F Swan-Ganz thermodilution catheter (Braun Melsungen AG, Melsungen, Germany) was introduced into the pulmonary artery via a femoral vein to measure cardiac output. Blood pressures in the aortic arch, left ventricle and pulmonary artery were continually measured with Statham pressure transducers (P23 Dc, Hato Rey, Puerto Rico), and heart rate was triggered from the left ventricular pressure signal. Mean arterial blood pressure (MAP) was calculated from the systolic (SAP) and diastolic (DAP) arterial pressures: MAP = (SAP + 2 x DAP) / 3. Systemic vascular conductance (SVC) was calculated from cardiac output (CO) and MAP: SVC = CO / MAP.

During the experiment body temperature was kept above 37 °C and the animals were infused with saline to compensate for fluid loss.

Radioactive microsphere method

The distribution of cardiac output was determined with a mixture of 10, 15 and 50 μm microspheres at baseline and of 15 and 50 μm microspheres after drug treatment. The microspheres were labelled with either ^{141}Ce (10 μm), ^{113}Sn (50 μm), ^{103}Ru (50 μm), ^{95}Nb (15 μm) or ^{46}Sc (15 μm; NEN Company, Dreieich, Germany). The order of 15 and 50 μm spheres injected before and after the drugs was randomized. The approximate number of microspheres given per isotope was: 10 μm: 2,000,000; 15 μm: 1,000,000 and 50 μm: 100,000. The microspheres were vortexed for about 30 s and then injected into the left ventricle against the direction of the blood flow to ensure uniform mixing. An arterial reference sample (10.5 ml.min^{-1}) was withdrawn from the iliac artery, starting about 15 s before and continuing until 1 min after the injection of the microspheres. At the end of the experiment the animals were killed and the different tissues were dissected out, weighed and put in vials. The radioactivity in these vials and in the blood samples was counted for 10 min in a gamma-scintillation counter (Packard, Minaxi Autogamma 5000) using suitable windows for discriminating the different isotopes.

Tissue blood flow towards each tissue (Q_{tis}) was calculated with a set of specially developed computer programs (Saxena et al., 1980) from the tissue radioactivity (I_{tis}), the radioactivity (I_{ref}) and the withdrawal rate of the arterial reference sample (Q_{ref}) using the formula: $Q_{tis} = I_{tis} / I_{ref} \times Q_{ref}$. Tissue vascular conductance was calculated by dividing the tissue blood flow value by the mean arterial blood pressure. All blood flow and conductance values have been expressed per 100 g of tissue.

In all tissues, a difference between blood flow values of 10 and 15 μm microspheres was assumed to be an index of the blood flow in arteriovenous anastomoses with a diameter between 14 and 28 μm. Similarly, the difference between the blood flow values of 15 and 50 μm spheres was equated with the blood flow through shunts with a

diameter between 28 and 90 μm. The rationale for these assumptions, derived from the work of Dickhoner et al. (1978), is presented at the beginning of the discussion section. Lung radioactivity has been assumed to represent for the greatest part arteriovenous anastomotic blood flow, although a small part will be derived from the bronchial arteries (Wu et al., 1988).

Experimental protocol

After a stabilization period of about one hour, arterial, left ventricular and pulmonary blood pressure, heart rate, cardiac output and arterial blood gas values were determined. Then a mixture of 10, 15 and 50 μm microspheres was injected into the left ventricle. After another stabilization period of about 15 min, the animals received an intravenous injection of either sumatriptan (0.3 $mg.kg^{-1}$; n=6), ergotamine (0.02 $mg.kg^{-1}$; n=4) or saline (n=5). Fifteen min later all haemodynamic values were collected and blood gases and distribution of cardiac output (by using a mixture of 15 and 50 μm microspheres) were again determined.

Data presentation and statistical analysis

All data have been expressed as means $\pm$ SEM. The overall significance of the differences between the baseline flow values has been evaluated in the three groups together with a multivariate analysis of variance (repeated-measurements design). In the case of a significant F-value, individual differences were analyzed with a Student's t-test for paired data (two-tailed). The effect of the treatment was compared in every group separately with a repeated measurements analysis of variance with the factors size and treatment. Individual differences were analyzed with Student's t-test for paired data. Statistical significance was accepted at $P < 0.05$ (two-tailed).

Drugs

Apart from the anaesthetics, azaperone and metomidate (both from Janssen Pharmaceutica, Beerse, Belgium), the drugs used in this study were: sumatriptan succinate (gift: Glaxo group research, Ware, UK), ergotamine tartrate (Gynergeen ampoules, Wander-Pharma, Uden, The Netherlands) and heparin sodium (Thromboliquine, Organon Teknika B.V., Boxtel, The Netherlands) to prevent clotting of the catheters. Sumatriptan was dissolved in physiological saline. The ergotamine injection solution was diluted in saline. All doses refer to the respective salts.

Results

Presence of arteriovenous anastomoses in different tissues

In the dura mater, nasal mucosa and in the skin there was a difference between the blood flow measured with 15 and 50 μm microspheres, indicating blood flow in arteriovenous anastomoses with a diameter between approximately 28 and 90 μm (Table 1). This difference, and thus the extent of shunting, was greatest in the head skin, and decreased in the following order: body skin, nasal mucosa and dura mater (Table 1). In the dura mater and nasal mucosa a difference in entrapment between 10 and 15 μm microspheres was found, indicating additional arteriovenous anastomoses with a size between 15 and 28 μm (Table 1). No shunts were found in the brain, heart, muscles or any of the abdominal organs (Table 1).

Table 1 Comparison of baseline blood flow values measured with 10 μm, 15 μm and 50 μm microspheres (n=15).

	10 μm	15 μm	50 μm
Lungs†	209±27	222±34	102±28*
Head skin	3±1	3±0	39±9*
Body skin	4±1	4±1	25±4*
Dura mater	8±1*	13±3	25±4*
Nasal mucosa	27±4*	34±4	105±11*
Brain	37±2	36±2	41±3
Heart	112±10	115±12	122±11
Spleen	114±15	117±20	119±15
Muscle gluteus	7±1	6±1	7±1
Muscle back	5±1	5±1	6±1
Kidneys	213±9	228±9	242±11
Liver	17±2	16±2	17±2
Adrenals	133±11	145±14	134±15
Intestine	46±3	45±3	47±4

All values ($ml.min^{-1}.100g^{-1}$) are given as mean ± SEM. †, lung blood flow values, measured with 10, 15 and 50 μm spheres, provide an index of peripheral arteriovenous anastomotic blood flow through shunts with a diameter larger than approximately 14, 28 and 90 μm, respectively, with only a small contribution of bronchial blood flow. *, $P<0.05$ vs. 15 μm sphere flow value.

Table 2 Changes in systemic haemodynamics due to intravenous injection of saline (n=5), sumatriptan (0.3 mg.kg^{-1}; n=6) or ergotamine (0.02 mg.kg^{-1}; n=4).

	Saline		Sumatriptan		Ergotamine	
	Before	After	Before	After	Before	After
HR	92±6	88±4	97±8	91±6	95±6	86±7*
MAP	93±7	89±8	93±6	93±10	89±5	106±10*
CO	1.9±0.1	1.8±0.1	2.2±0.1	1.7±0.1*	2.1±0.2	1.5±0.2*
LVEDP	11±1	10±1	10±1	9±1	10±2	11±1
MPAP	26±3	31±3*	27±1	33±2*	23±2	32±3
SVC	20±1	20±1	24±2	20±3*	24±3	14±1*

All values as mean ± SEM; HR = Heart rate (beats.min^{-1}); MAP = Mean arterial blood pressure (mmHg); CO = Cardiac output (l.min^{-1}); LVEDP = Left ventricular end-diastolic pressure (mmHg); MPAP = Mean pulmonary artery pressure (mmHg); SVC = Systemic vascular conductance (ml.min^{-1}.mmHg^{-1}). *, $p<0.05$ vs control.

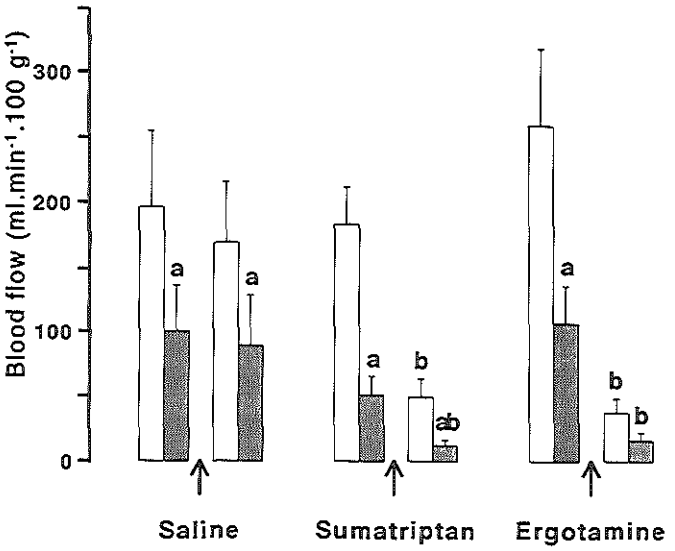


Figure 1. Effect of saline (n=5), sumatriptan (0.3 mg.kg^{-1}; n=6) or ergotamine (0.02 mg.kg^{-1}; n=4) on lung blood flow, measured with 15 μm (open bars) and 50 μm (cross-hatched bars) microspheres. Values are given as mean ± SEM. a, $P<0.05$ vs simultaneously injected 15 μm sphere; b, $P<0.05$ vs same sphere before treatment; c, $P<0.05$ vs saline induced change.

Effects of antimigraine drugs

Systemic haemodynamics

Both ergotamine and sumatriptan decreased cardiac output (by 30±4% and 22±3%, respectively) and systemic vascular conductance (by 35±7% and 19±8%, respectively; Table 2). Only in the case of ergotamine did this result in an increase in aortic blood pressure. Pulmonary artery pressure was increased in all three groups (including the saline-treated group) and may reflect the use of intracardiac injections of 50 μm microspheres in this model. As reported previously (Saxena & Cairo-Rawlins, 1979; Den Boer et al., 1989a), heart rate was slightly decreased by ergotamine.

Cardiac output distribution to lungs

Sumatriptan and ergotamine both reduced the number of 15 and 50 μm microspheres trapped in the lungs (Figure 1). Although both bronchial arteries and arteriovenous anastomoses (via the pulmonary arteries) supply microspheres to the lungs, bronchial artery blood flow is small (1.4 % of cardiac output in sheep) and contributes only to a small part of the microspheres trapped in the lungs (Wu et al., 1988). Therefore, the reduction in lung radioactivity by the antimigraine drugs reflects mostly an decrease in arteriovenous shunting in the body, both in smaller (28-90 μm diameter) and in larger (> 90 μm diameter) shunts.

Cardiac output distribution to tissues with arteriovenous anastomoses

In the head and body skin sumatriptan and ergotamine did not change the flow measured with 15 μm microspheres, indicating no change in capillary blood flow (Figure 2). The entrapment of 50 μm spheres, however, was decreased and the difference between flow values measured with 15 and 50 μm microspheres was attenuated, indicating a reduction of blood flow through arteriovenous anastomoses with a diameter between 28 and 90 μm in both skin regions (Figure 2). In the nasal mucosa sumatriptan induced no change in blood flow, but ergotamine decreased the 15-50 μm microsphere flow difference, indicating a decrease in arteriovenous anastomotic blood flow, and tended to increase capillary flow (Figure 2). In the dura mater neither agent induced any change in blood flow (Figure 2).

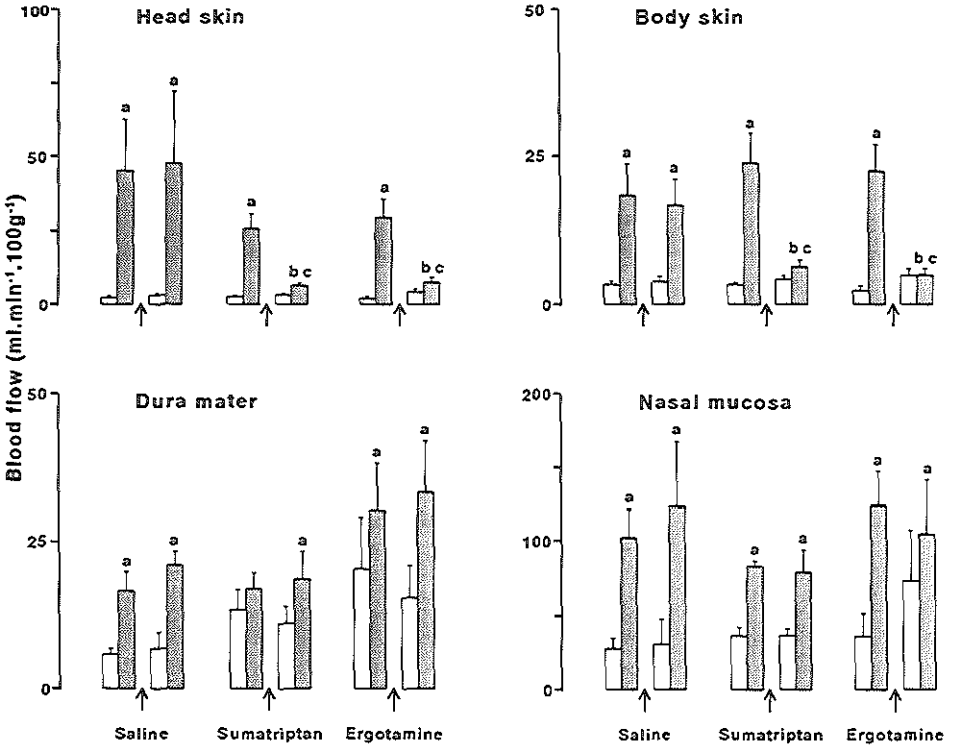


Figure 2. Effect of saline (n=5), sumatriptan (0.3 mg.kg^{-1}; n=6) or ergotamine (0.02 mg.kg^{-1}; n=4) on organ blood flows, measured with 15 μm (open bars) and 50 μm (cross-hatched bars) microspheres. Values are given as mean ± SEM. a, $P<0.05$ vs simultaneously injected 15 μm sphere; b, $P<0.05$ vs same sphere before treatment.

Cardiac output distribution to tissues without arteriovenous anastomoses

Liver and adrenal blood flows were significantly decreased after ergotamine (Figure 3). However, liver blood flow also tended, though not significantly, to decrease after saline and sumatriptan. Neither sumatriptan nor ergotamine affected blood flow to the brain, heart, spleen, gluteal muscle, kidneys or intestine (Figures 3 and 4). When changes in vascular conductances were compared to those induced by saline, however, relative small decreases in kidney conductances were observed after both sumatriptan and ergotamine, a decrease in spleen conductance after sumatriptan and in heart, liver and adrenal conductances after ergotamine (Table 3). These indicate moderate vasoconstriction in the respective vascular bed, when compared to saline treatment.

Table 3 Changes in vascular conductances ($ml.min^{-1}.100g^{-1}$) due to intravenous injection of saline (n=5), sumatriptan (0.3 $mg.kg^{-1}$; n=6) or ergotamine (0.02 $mg.kg^{-1}$; n=4).

	Saline		Sumatriptan		Ergotamine	
	Before	After	Before	After	Before	After
Brain	0.39±0.04	0.45±0.05	0.38±0.03	0.45±0.06	0.36±0.07	0.34±0.05
Heart	1.22±0.31	1.58±0.63	1.24±0.27	1.15±0.16	1.22±0.16	1.04±0.11†
Spleen	0.98±0.20	1.28±0.32	1.60±0.57	1.17±0.35†	1.27±0.12	1.11±0.16
Kidneys	2.25±0.24	2.71±0.43	2.71±0.25	2.41±0.24†	2.20±0.15	1.98±0.19†
Liver	0.18±0.03	0.14±0.02	0.18±0.03	0.10±0.02*	0.13±0.03	0.06±0.02*†
Adrenals	1.50±0.24	1.78±0.25	1.80±0.34	1.53±0.17	1.42±0.20	0.83±0.11*†
Intestine	0.44±0.09	0.50±0.11	0.46±0.02	0.49±0.05	0.55±0.08	0.46±0.07

All values as mean ± SEM. *, P<0.05 vs. before drug; †, P<0.05 vs. change in saline group.

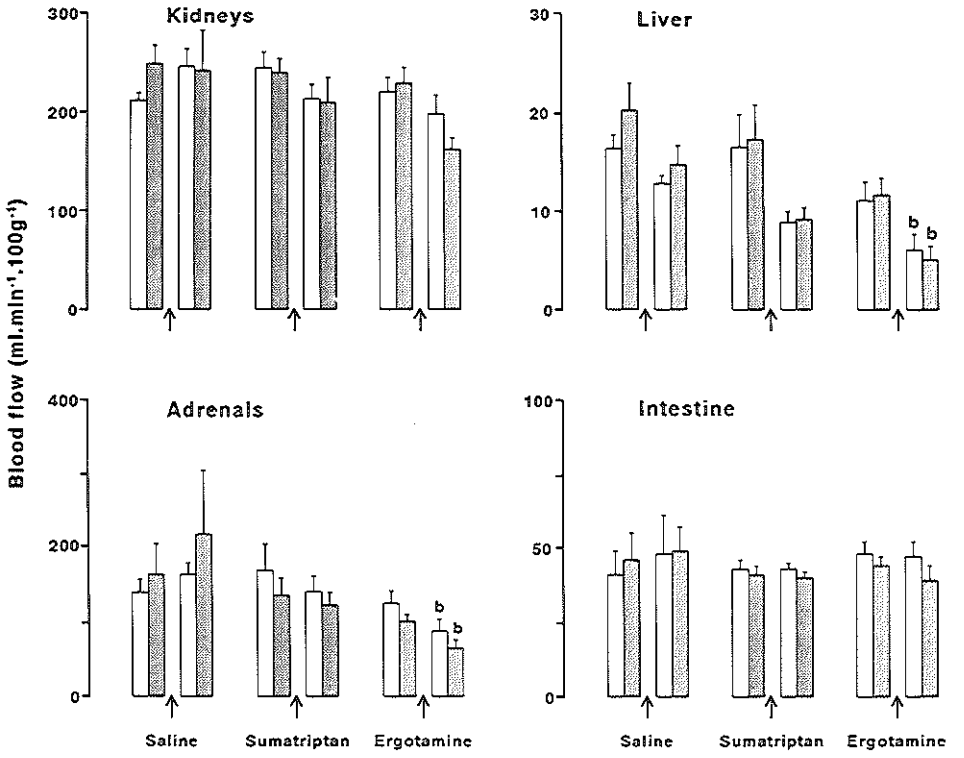


Figure 3. Effect of saline (n=5), sumatriptan (0.3 $mg.kg^{-1}$; n=6) or ergotamine (0.02 $mg.kg^{-1}$; n=4) on organ blood flows, measured with 15 μm (open bars) and 50 μm (cross-hatched bars) microspheres. Values are given as mean ± SEM. b, P<0.05 vs same sphere before treatment.

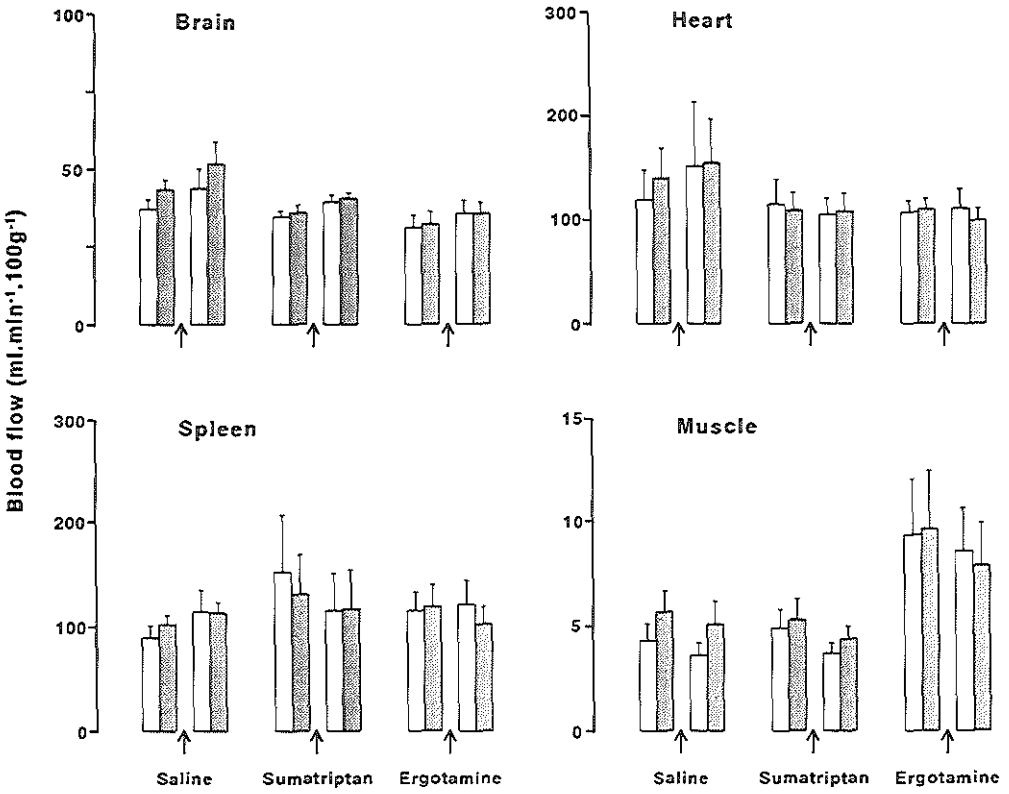


Figure 4. Effect of saline (n=5), sumatriptan (0.3 mg.kg^{-1}; n=6) or ergotamine (0.02 mg.kg^{-1}; n=4) on organ blood flows, measured with 15 μm (open bars) and 50 μm (cross-hatched bars) microspheres. Values are given as mean ± SEM.

Discussion

Use of microspheres of three sizes to measure arteriovenous anastomotic blood flow

The use of microspheres of different sizes to measure the distribution of cardiac output has already been extensively described (Saxena & Verdouw, 1985a). In these experiments an effect of 5-HT on arteriovenous anastomotic blood flow was observed. In early experiments with radioactive microspheres to measure blood flow, non-entrapment of spheres was observed in certain tissues (Hales, 1974; Heymann et al., 1977). This non-entrapment depends on microsphere size, the tissue studied and the type of anaesthesia used (Forsyth & Hoffbrand, 1970), and has been ascribed to arteriovenous anastomotic blood flow (Hales, 1974). Dickhoner et al. (1978) demonstrated that in the

hamster cheek pouch 9, 15 and 24 μm microspheres are trapped when arriving in blood vessels with a diameter of 11.5, 27.7 and 42.7 μm, respectively. Extrapolation of these results to our experiments indicates that the 10, 15 and 50 μm diameter microspheres will not pass through capillaries, which have a diameter under 10 μm (Saxena & Verdouw, 1982). However, they will escape to the venous side through arteriovenous anastomoses with a minimum diameter larger than 14, 28 and 90 μm, respectively and get trapped in the lung vasculature. Therefore, the blood flow measured with the microspheres in the lungs is the sum of bronchial and arteriovenous anastomotic blood flow. Wu et al. (1988), using combined right and left atrial injections of radioactive microspheres in sheep, found bronchial arterial blood flow to be 1.4% of cardiac output. In our experiments 21% of the microspheres injected into the heart were detected in the lungs, so that more than 90 % of the microspheres that are trapped in the lungs passed via arteriovenous anastomoses. The tissue blood flow values, calculated with a certain microsphere, is a measure for the flow through blood vessels in which a particular microsphere is trapped. When a tissue lacks arteriovenous anastomoses, microspheres of all sizes yield similar blood flow values, representing tissue blood flow, as demonstrated e.g. in the brain in the present experiments (see also Saxena & Verdouw, 1985a). An increase in tissue blood flow values with increasing microsphere size indicates that arteriovenous anastomoses are present in that tissue, since microspheres of smaller sizes are presumably have escaped to the venous side via arteriovenous anastomoses to be ultimately trapped by lung capillaries. The difference between the tissue blood flow values measured with 15 and 50 μm microspheres is therefore an index of blood flow in arteriovenous anastomoses with a diameter 28-90 μm. Blood flow in larger arteriovenous anastomoses, which may have a diameter up to 150 μm (Sherman, 1963) cannot be measured for individual tissues, since the use of still larger microspheres is not practical due to adverse influence on the circulation.

Effect of antimigraine drugs

Ergotamine and sumatriptan decreased cardiac output and systemic vascular conductance in the present experiments, indicating vasoconstriction. Only ergotamine raised arterial blood pressure. The effect of ergotamine on total vascular resistance is well known (Saxena & De Vlaam-Schluter, 1974; Johnston & Saxena, 1978; Schamhardt et al., 1979; Tfelt-Hansen et al., 1983; Perren et al., 1989; Feniuk et al., 1989b), but the magnitude of this increase varies greatly. Similarly, blood pressure responses vary: both an unchanged (Johnston & Saxena, 1978) as well as large increases (Perren et al., 1989) in blood pressure have been reported after ergotamine. In man intravenous injection of ergotamine usually increases arterial blood pressure and total peripheral resistance and

decreases cardiac output. However, these effects are usually transient, while the drug causes prolonged constrictions of large, non-resistance arteries (Tfelt-Hansen et al., 1980).

The decrease of vascular conductance by ergotamine was partly due to constriction of cutaneous arteriovenous anastomoses and partly to constriction of arterioles. Ergotamine potently constricted both cranial and peripheral cutaneous arteriovenous anastomoses. It is not known if ergotamine preferentially constricts cranial cutaneous arteriovenous anastomoses compared with those of the head, as has been observed with arteries (Müller-Schweinitzer & Weidmann, 1978), but in the present experiments no difference was found. Surprisingly, arteriovenous shunting in the dura mater and nasal mucosa was unaffected by the drug, indicating a different receptor profile from those in the skin. Although arteriovenous anastomoses with a diameter larger than 90 μm, where equally a marked reduction in blood flow occurred, could not be localized, the arteriovenous anastomoses in the dura mater do generally not exceed 90 μm (Kerber & Newton, 1973). When compared to saline treatment, ergotamine also reduced arteriolar conductances to the heart, kidneys, liver and adrenals. Only in the liver and the adrenals did this cause a significant decrease in blood flow. A decrease in hepatic blood flow has also been observed in man after an intravenous dose of ergotamine, which, however, was transient in nature, unlike the prolonged constrictor property on larger arteries (Tfelt-Hansen et al., 1983). Although cardiac conductance was decreased by ergotamine in the present experiments, cardiac blood flow was maintained. This is in agreement with the fact that, even in the presence of a coronary artery stenosis in anaesthetized pigs, ergotamine fails to decrease myocardial blood flow (Schamhardt et al., 1979). On the other hand, in cats ergotamine causes clear decreases in coronary blood flow, but in these experiments blood pressure was not raised to compensate for a decrease in coronary conductance (Johnston & Saxena, 1978).

Despite a similar effect of sumatriptan on arteriovenous anastomoses, the overall vasoconstrictor effect of this drug was less than of ergotamine, and comparable to the effect found in anaesthetized cats (Perren et al., 1989). Most of this vasoconstrictor property seems to be localized with the arteriovenous anastomoses, as has been shown before in the carotid circulation of cats and pigs (Perren et al., 1989; Den Boer et al., 1991a). Like ergotamine, sumatriptan only affected arteriovenous anastomoses in the skin, while those in the dura mater and the nasal mucosa were not affected. However, as mentioned before, no information could be obtained on arteriovenous anastomoses wider than 90 μm. Sumatriptan did not change arteriolar resistance in the dura mater either. This finding contrasts with the increase in vascular resistance, which sumatriptan induces in the human dura mater in vitro (Humphrey et al., 1991a). However, postmortem

changes or the effect of denervation on the isolated dural vessels may be an explanation for this difference.

Sumatriptan also slightly decreased kidney and spleen conductance in comparison to saline. The isolated renal arteries of the rabbit are known to possess sumatriptan-sensitive 5-HT_1-like receptors (Van Heuven-Nolsen et al., 1990; Martin & MacLennan, 1990; Tadipatri et al., 1991) and sumatriptan has been shown to decrease renal conductance in anaesthetized dogs (Cambridge et al., 1991). No effect was seen on the cardiac conductance, in contrast to in vitro observations (Connor et al., 1989b; Chester et al., 1990). It should, however, be noted that in the in vitro experiments large conduit coronary arteries were studied, while in our experiments the vascular conductance was determined by the constrictor state of the arterioles. Whether the increase in renal and spleen conductance occurs in man and, if so, is clinically relevant remains to be determined.

Chapter 11

Evidence for a role of Noradrenaline in the Regulation of Carotid Arteriovenous Shunting in the Pig

Summary

Noradrenaline and 5-hydroxytryptamine are both present in perivascular nerves of cranial blood vessels. Although noradrenaline is generally assumed to be the main regulatory neurotransmitter of arteriovenous anastomoses, in the dilated cranial arteriovenous anastomoses of pentobarbitone-anaesthetized pigs 5-hydroxytryptamine is a powerful vasoconstrictor, while these vessels are less sensitive to noradrenaline. It is therefore a tempting question whether noradrenaline or 5-hydroxytryptamine is responsible for the constricted state of these arteriovenous anastomoses, which can be observed in conscious pigs.

This was further investigated in fentanyl-thiopentone-anaesthetized pigs, in which there is also a low basal AVA shunting. The distribution of carotid blood flow in AVA and capillary flow was determined using carotid injection of 15μ radioactive microspheres. After baseline measurements, consecutive antagonism of α_1, α_2, 5-HT_1 and 5-HT_2 receptors was obtained in six animals by consecutive injection of prazosin (0.02 $mg.kg^{-1}$), phentolamine (1 $mg.kg^{-1}$), ketanserin (0.5 $mg.kg^{-1}$) and methiothepin (3 $mg.kg^{-1}$). After each injection a new batch of microspheres was given. Because some of these drugs affected blood pressure, also conductances (flow/mean blood pressure) were calculated. Six other animals received saline at the same time intervals and confirmed the stability of the preparation.

Prazosin increased total carotid flow and conductance. This was caused by a large increase in AVA shunting, both in absolute values and in percent of carotid blood flow. Part of this increase in shunting occurred at the expense of extracerebral tissue flow, which decreased; brain flow, however, was preserved. Neither phentolamine, ketanserin or methiothepin had any significant further effect on cranial haemodynamics, although AVA flow tended to decrease again with methiothepin.

It is concluded that a tonic, probably noradrenergic, stimulation of the vascular α_1-receptor, is responsible for the normal tone in arteriovenous anastomoses. A role for 5-hydroxytryptamine could, at least in this model, not be ascertained.

Introduction

Arteriovenous anastomoses, known to play a part in temperature regulation in different species, have been shown to be partly subjected to a sympathetic noradrenergic control (Hales & Molyneux, 1988). In sheep, the dilatation of arteriovenous anastomoses following an elevation of central body temperature is accompanied by a decrease in sympathetic nerve activity (Hales et al., 1978) and a decrease in perivascular noradrenaline immunofluorescence (Molyneux & Hales, 1982). Furthermore, in conscious dogs arteriovenous anastomotic blood flow is increased by prazosin (Hof & Hof, 1989) and in conscious sheep arteriovenous anastomotic blood flow is enhanced by phentolamine and potently reduced by noradrenaline (Hales et al., 1982). Anaesthesia with pentobarbitone causes arteriovenous anastomoses to dilate, presumably as a result of inhibition of the sympathetic nerves (Forsyth & Hoffbrand, 1970).

Likewise, in conscious pigs cranial arteriovenous anastomoses (AVAs) are in a constricted state (Van Woerkens et al., 1990), while anaesthesia with pentobarbitone causes the arteriovenous anastomoses to be dilated (chapters 6-10 of this thesis). Unexpectedly, both noradrenaline and stimulation of the cervical sympathetic nerves had minimal influence on arteriovenous anastomoses in the porcine cranial circulation, questioning a role of the sympathetic nerves in this species (Verdouw et al., 1984a). Furthermore, there is some evidence in favour of a neurotransmitter role for 5-hydroxytryptamine in cranial blood vessels. This amine seems to be co-stored with noradrenaline in sympathetic nerves (Scatton et al., 1985), and there in also evidence for a genuine 5-hydroxytryptaminergic innervation arising from the dorsal raphe nuclei (Marco et al., 1985). Lastly, 5-hydroxytryptamine is a potent vasoconstrictor of dilated arteriovenous anastomoses (Saxena et al., 1986).

Therefore the aim of our study was to investigate the role of noradrenaline and 5-hydroxytryptamine in the regulation of the cranial arteriovenous anastomoses in the pigs. In contrast to former experiments, the pigs were anaesthetized with fentanyl and thiopentone, yielding constricted AVAs, in order to mimic haemodynamically the conscious state (Verdouw, personal communication).

Methods

General

The experiments were performed on 12 domestic pigs (Yorkshire x Landrace; 16-22 kg) after an overnight fast. Anaesthesia was induced with i.m. injection of ketamine (20 $mg.kg^{-1}$) and midazolam (0.5 $mg.kg^{-1}$). After i.v. injection of thiopentone (5 $mg.kg^{-1}$) the animals were intubated and connected to a respirator (Bear 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; Po_2: 100-120 mmHg). Anaesthesia was maintained with intravenous infusion of a mixture of fentanyl (12-40 $\mu g.kg^{-1}.hr^{-1}$) and thiopentone (3-10 $mg.kg^{-1}.hr^{-1}$). Due to differences in sensitivity of each animal to the anaesthetics, the optimal infusion rate was adjusted, using two criteria: absence of the blinking reflex (indicating sufficient anaesthesia) and a jugular venous oxygen saturation lower than 70% (indicating constricted AVAs).

Catheters were placed in the inferior vena cava via a femoral vein for the administration of drugs and in the aortic arch via a femoral artery, connected to a Statham pressure transducer (P23 Dc, Hato Rey, Puerto Rico) for the measurement of arterial blood pressure and the withdrawal of arterial blood for determining blood gases (ABL-2, Radiometer, Copenhagen, Denmark). Mean arterial blood pressure (MAP) was calculated from the systolic (SAP) and diastolic (DAP) arterial pressures:
MAP = (SAP + 2 x DAP) / 3. The common carotid arteries were dissected free and blood flow was measured in one of the common carotid arteries with a flow probe (internal diameter: 2.5 or 3 mm) connected to a sine-wave electromagnetic flow meter (Transflow 600-system, Skalar, Delft, The Netherlands). Heart rate was measured with a tachograph triggered from the blood pressure or the flow signal, depending on their shape. A 0.5 mm (external diameter) needle, connected to a polyethylene tubing was inserted into the common carotid artery against the direction of the blood flow for the administration of radioactive microspheres. At the same side the jugular vein was cannulated in order to obtain venous blood samples for determining blood gases.

During the experiment body temperature was kept at about 37 °C and the animals were continuously infused with saline to compensate for fluid losses.

Distribution of common carotid blood flow

The distribution of common carotid blood flow was determined with 15±1 (S.D.) μm diameter microspheres labelled with either ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Company, Dreieich, Germany). For each measurement a suspension of about 200,000 microspheres, labelled with one of the, randomly assigned, isotopes, was mixed and injected into the carotid artery against the direction of the blood flow to ensure uniform mixing. At the end of the experiment the animals were killed and the heart, kidneys, lungs and the different cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 5-10 min in a gamma-scintillation counter (Packard, Minaxi Autogamma 5000) using suitable windows for discriminating the different isotopes.

The ratio between the radioactivity in a particular tissue and the total radioactivity was calculated with a set of specially developed computer programs (Saxena et al., 1980). By multiplying this ratio with the total carotid blood flow value at the time of the injection, blood flow to the tissues (capillary blood flow) was determined. No radioactivity could be detected in the heart or the kidneys, so all microspheres reaching the venous side by arteriovenous anastomoses were trapped in the lungs. Therefore, the amount of radioactivity in the lungs was used as an index for the arteriovenous anastomotic part of the common carotid blood flow.

Experimental protocol

The experiments were started after a stabilization period of about 1 h. After measuring heart rate, mean arterial blood pressure, carotid blood flow and its distribution and arterial and jugular venous blood gases at baseline, in six pigs consecutive antagonism of α_1, α_2, 5-HT_2 and 5-HT_1 receptors was obtained by *sequential* intravenous administration of prazosin (0.02 $mg.kg^{-1}$), phentolamine (1 $mg.kg^{-1}$), ketanserin (0.5 $mg.kg^{-1}$) and methiothepin (3 $mg.kg^{-1}$) at 20 min intervals (Bradley et al., 1986; Cavero & Roach, 1980). After each antagonist a new batch of microspheres was given. Six other animals received saline at the same time intervals to confirm the stability of the preparation.

Data presentation and statistical analysis

All data have been expressed as means ± SEM. The significance of the differences between the variables within one group was evaluated with Duncan's new multiple range test, once an analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel and Torrie, 1980). Statistical significance was accepted at $P<0.05$ (two-tailed).

Drugs

The following drugs were used: ketamine hydrochloride (Kombivet, Etten-Leur, The Netherlands), midazolam hydrochloride (Dormicum, Hoffmann-La Roche, Mijdrecht, The Netherlands), thiopentone sodium (Nesdonal, Rhône-Poulenc, Amstelveen, The Netherlands), fentanyl citrate (Janssen, Beerse, Belgium), prazosin hydrochloride (Bufa-Chemie, Castricum, The Netherlands), phentolamine methanesulfonide (Regitine, Ciba-Geigy, Arnhem, The Netherlands), ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium), methiothepin maleate (Hoffman La Roche B.V., Mijdrecht, The Netherlands) and heparin sodium (Thromboliquine, Organon Teknika B.V., Boxtel, The Netherlands). Thiopentone sodium was dissolved in saline. Fentanyl citrate, ketanserin tartrate and methiothepin maleate were dissolved in propyleneglycol and subsequently diluted with saline (1:10). Prazosin was dissolved in glucose (5%) in distilled water. The other drugs were purchased as solutions. All doses refer to the respective salts.

Results

Systemic haemodynamics

Prazosin caused a decrease in mean arterial blood pressure of -17±5%, from an initial value of 103±7 mmHg (Table 1). During the remainder of the experiment this decrease was gradually reversed until after methiothepin the initial blood pressure was reached 100±7 mmHg. In the saline group blood pressure remained unchanged as well as heart rate in both experimental groups (Table 1).

Table 1. Systemic haemodynamics and blood gas values.

Saline group					
	Baseline	Saline 1	Saline 2	Saline 3	Saline 4
HR	87±6	88±6	85±6	85±8	87±7
MAP	99±7	101±7	97±7	99±8	100±8
AVSOD	33±5	30±5	35±6	33±6	34±6
Antagonist group					
	Baseline	Prazosin	Phentolamine	Ketanserin	Methiothepin
HR	79±6	77±9	80±9	81±7	92±9
MAP	103±7	85±5*	89±6*	98±8	100±7
AVSOD	29±3	9±3*	7±3*	10±3*	14±5*

Data have been given as means ± SEM; HR, heart rate (beats.min^{-1}); MAP, mean arterial pressure (mmHg); AVSOD, arterio-jugular venous oxygen saturation difference (%); *, $P<0.05$ vs baseline.

Carotid haemodynamics

Prazosin increased total carotid blood flow by 137±54 % and total carotid conductance by 181±60% (Figure 1). This was caused by a massive increase in the fraction passing through the arteriovenous anastomoses (1060±440% and 1250±470%, respectively for flow and conductance), while total capillary flow was even decreased by 36±4% (Figure 1). Since brain flow was not significantly changed, this decrease in capillary flow occurred mainly in the extracerebral tissues. Conductance in the total capillary bed and also of the extracerebral tissues was unchanged (Figure 1), indicating that the decrease in blood flow was entirely due to the decrease in blood pressure.

Neither phentolamine, ketanserin nor methiothepin had any *further* significant effect on cranial haemodynamics, although AVA flow tended to decrease again with methiothepin.

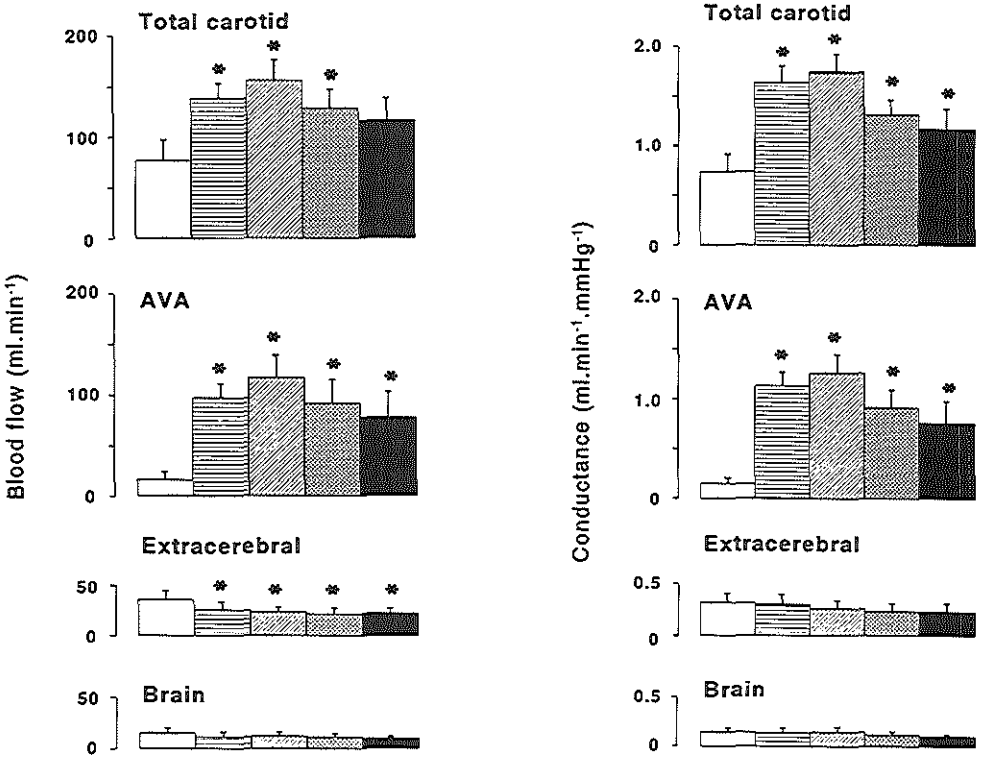


Figure 1. Effect of cumulative doses of different receptor antagonists on total carotid blood flow and its distribution (left panel) and the respective conductances (right panel) over arteriovenous anastomoses (AVA), extracerebral tissues and brain. From left to right the bars signify: Baseline, prazosin (0.02 $mg.kg^{-1}$), Phentolamine (1 $mg.kg^{-1}$), Ketanserin (0.5 $mg.kg^{-1}$) and Methiothepin (3 $mg.kg^{-1}$). Data are given as mean ± SEM; *, $P < 0.05$ vs baseline.

Arterio-jugular venous oxygen saturation difference

Administration of prazosin markedly decreased the difference in oxygen saturation between arterial and jugular venous blood (Table 1). Additional administration of phentolamine, ketanserin and methiothepin had no further significant effect on the blood gases. In the saline group no changes occurred in the saturation differences, confirming the stability of this parameter (Table 1).

Discussion

Due to the lack of really selective antagonists at 5-hydroxytryptamine receptors, sequential administration of antagonists with increasing non-selectivity was chosen for this study. The first antagonist administered was prazosin, a selective α_1-adrenoceptor antagonist (Cavero & Roach, 1980). Consecutive administration of phentolamine, a non-selective α-adrenoceptor antagonist (Cavero et al., 1979) added α_2-adrenoceptor antagonism; further administration of ketanserin, an antagonist of α_1 and 5-HT_2 receptors (Leysen et al., 1981), added 5-HT_2 antagonism and final administration of methiothepin, a non-selective α-adrenoceptor and 5-HT receptor antagonist (Bradley et al., 1986) added 5-HT_1 receptor antagonism.

The present experiments establish a role for α_1 receptors in the maintenance of the tone in cranial AVAs in our model, since prazosin greatly increased arteriovenous anastomotic blood flow. The decrease in the oxygen saturation difference between arterial and jugular venous blood is in keeping with dilatation of arteriovenous anastomoses. Noradrenaline, released from sympathetic nerve terminals, is presumably the endogenous neurotransmitter regulating AVA tone. This is in concordance with observations in dogs (Hof & Hof, 1989) and sheep (Hales et al., 1982). In spite of its presence in cranial blood vessels (Marco et al., 1985), a role for 5-hydroxytryptamine could not be ascertained in this model, since neither ketanserin nor methiothepin caused any further significant change in carotid haemodynamics. It seems, therefore, quite unlikely that 5-hydroxytryptamine has a major role in maintaining the tone in cranial arteriovenous anastomoses in the pig. However, from these experiments it is not possible to exclude a modulatory role of 5-hydroxytryptamine, released from sympathetic or tryptaminergic nerves, on neural release of noradrenaline, since prazosin and phentolamine already blocked the noradrenaline effects. A modulatory role of 5-hydroxytryptamine on the sympathetic nervous system has been shown under different experimental procedures: both enhancement and inhibition of the sympathetic system has been observed (McGrath, 1977). Furthermore, 5-hydroxytryptamine has been shown

capable of enhancing noradrenaline-induced vasoconstriction at the level of the vessel wall (De La Lande et al., 1966), so that a modulatory role may be expected here. In the present model such roles can only be demonstrated by using selective 5-HT antagonists, which at the present time are not available. While prazosin can be regarded as a selective α_1 antagonist (Cavero & Roach, 1980), all 5-HT_1 antagonists that can be used to inhibit the effects of 5-HT on arteriovenous anastomoses cause some degree of 5-HT_2 and α antagonism (Leysen et al., 1981).

The role of noradrenaline in maintaining constrictor tone in the AVAs seems in contradiction with earlier experiments (Verdouw et al., 1984), where noradrenaline was a poor constrictor of the AVAs. Two explanations for this discrepancy can be proposed. Unlike in most other blood vessels, in arteriovenous anastomoses the sympathetic nerves penetrate deeply into the vascular wall (Molyneux, 1970). It might be that noradrenaline released by these sympathetic nerves constricts these vessels, while intraluminally administered noradrenaline does not. This is, however, difficult to reconcile with the constriction of arteriovenous anastomoses in the hind limb of the sheep by intra-arterially injected noradrenaline (Hales et al., 1982), although species differences may exist. A second explanation for the poor noradrenaline response in the experiments of Verdouw et al. (1984) is the use of the anaesthetic, azaperone, which displays α_1-adrenoceptor antagonism (Fukuchi et al., 1988).

Therefore, it would be worthwhile to repeat these experiments in the absence of azaperone, but in the presence of pentobarbitone, for which non-specific blunting of the autonomic nervous system (Rall, 1990) appears to be the mechanism of dilatating arteriovenous anastomoses, rather than α-adrenoceptor antagonism.

Chapter 12

Summary and General Discussion

Introduction

(Chapter 1)

The vascular effects of the antimigraine drugs, sumatriptan, ergotamine and dihydroergotamine were investigated in this thesis. This work was conducted for two main reasons: (i) a disturbance within the cranial blood vessels has been implicated in the pathogenesis of migraine; and (ii) the antimigraine drugs mentioned above are powerful vasoconstrictor agents, a phenomenon which is likely to be important in their antimigraine activity.

In the present thesis the effect of these drugs on arteriovenous anastomoses and arterioles was determined with injection of radioactive microspheres both in the carotid artery and in the left ventricle of anaesthetized pigs. Except in chapter 11 pentibarbitone-anaesthesia was used and the animals were subjected to bilateral vago-sympathectomy. Furthermore, suitable pharmacologic agents were used to characterize the receptors involved in the vascular effects of the antimigraine drugs. Lastly, an attempt was made to determine the neurotransmitter which is responsible for the maintenance of physiological tone in the arteriovenous anastomoses.

Pathophysiology of migraine and action of antimigraine drugs

(Chapters 2 and 3)

The widely variable clinical picture and lack of uniform criteria in classifying migraine, reflected in the way that patient populations were assembled for scientific studies, has prompted the International Headache Society to publish in 1988 criteria for diagnosing and classifying migraine. Two common forms of migraine are distinguished: migraine without and migraine with aura. The migraine headache lasts from 4-72 hours, but may sometimes be absent. The headache is accompanied by malaise, photo- and phonophobia, nausea and, frequently, vomiting. An aura, usually preceding the headache, may comprise sensory and motor phenomena and does not exceed 60 minutes. This subdivision of migraine is also important for research purposes, since some of the features differ among the two groups.

In the past, vascular (pioneered by Thomas Willis in 1664), neurogenic (nineteenth century) and humoral (5-hydroxytryptamine, the sixties) aetiologies of migraine have been proposed. Graham & Wolff (1938) were the first to really perform experiments on migraine patients during the attack. They explained the aura symptoms as resulting from ischaemia due to cerebral vasoconstriction and the headache as a result of following extracerebral vasodilatation. Indeed, in later studies on the aura phase cerebral oligaemia was repeatedly demonstrated, but this finding did not prove the ischaemic theory, for the reduction of brain blood flow did not follow vascular boundaries or reach ischaemic levels and sometimes continued into the headache phase. Therefore, the oligaemia was thought to be secondary to a basic neurologic disturbance. However, the method used to measure brain flow (^{133}Xe-inhalation) poses some problems in reliably estimating low flow levels so the issue whether ischaemia occurs during the migraine aura has not yet been settled. The notion that the headache is caused by vasodilatation was strengthened by Ray and Wolff (1940) who demonstrated that some cranial blood vessels and the dura mater are the only painful intracranial structures. A possible involvement of arteriovenous anastomoses was proposed by Heyck (1969) on the basis of an increased oxygen saturation in the jugular venous blood on the headache side and its reversal by dihydroergotamine. Since cerebral blood flow was shown to be unchanged in migraine without aura and the decrease in cerebral blood flow in the aura phase even persisted into the headache phase, the headache cannot be explained by changes in cerebral perfusion. Rather, dilatation of larger intracranial arteries, that do not regulate cerebral vascular resistance, or of extracranial arteries is currently being considered. The estimation of vascular diameter by measuring arterial blood flow velocity with the transcranial Doppler-method has led to conflicting results. In the only study measuring extracranial blood flow during the migraine headache phase with ^{133}Xe, an increase was observed although the reliability of the extracranial blood flow values measured with ^{133}Xe is questionable due to interference with intracranial radiation. In conclusion, whether dilatation of extracerebral arteries occurs during migraine is a matter which also remains to be settled.

A neurovascular theory on migraine was strengthened by the demonstration in the jugular venous blood during the migraine attack of increased levels of CGRP (calcitonin gene-related peptide), which is derived from trigeminal nerve fibres and a strong vasodilator. Furthermore, stimulation of certain brain stem regions, like the 5-hydroxytryptamine-containing dorsal and median raphe nuclei, markedly influences cranial haemodynamics.

For migraine treatment a prophylactic or an attack-related approach is available. This thesis deals only with specific, non-analgesic drugs used for abolishing the attack.

Ergotamine: Ergotamine is an ergot alkaloid and, like most other ergot alkaloids, has a complex pharmacology, stimulating or antagonizing 5-HT, dopamine and noradrenaline receptors, while an additional interaction with still unclassified receptors is likely. That the effect of ergotamine involved vasoconstriction has for the first time been proposed by Graham and Wolff (1938). They reported a decrease in pulsatility of the temporal artery after administration of ergotamine. Further research on this compound have disclosed both vasoconstrictor and vasodilator actions, although vasoconstriction usually predominates. A short-lasting increase in vascular resistance in arterioles and small arteries is followed by a long lasting constriction of larger arteries, which latter action may be involved in the therapeutic effect. A constrictor effect of ergotamine on arteriovenous anastomoses in the cranial circulation, is another possibility to be considered.

Recent studies at Harvard University have disclosed another possible mechanism: the inhibition by ergotamine of the 'neurogenic inflammation', which they proposed to be involved in migraine. It is not yet well established if this is a direct neural action of the drug or a result of vasoconstriction.

Dihydroergotamine: The basic pharmacologic features of dihydroergotamine resemble those of ergotamine, despite small differences, so a similar mechanism as for ergotamine is very likely in the antimigraine action of the drug.

Sumatriptan: Sumatriptan is a recently developed antimigraine drug and, unlike the ergot alkaloids, a selective agonist of 5-HT_1-like receptors. Both vasoconstrictor (of arteries and arteriovenous anastomoses) and neural inhibitor effects of sumatriptan have been reported. These effects are qualitatively similar to those of the ergot alkaloids, although the vasoconstrictor effects tend to be milder and the receptor mechanisms may be different. Therefore, both a vascular and a neural mechanism could participate in the therapeutic effect of sumatriptan, though a direct action of sumatriptan on sensory nerve fibres could, however, not be established in isolated arteries. Sumatriptan is more effective than the ergot alkaloids in relieving the associated symptoms of the migraine attack, the reason of which is unknown.

Anatomical and physiological aspects of the cranial circulation
(Chapter 4)

Craniovascular anatomy: The cranial arterial supply is derived from two carotid and two vertebral arteries. In man most of the brain blood flow is derived from the internal carotid and vertebral arteries and in the pig from the external carotid and the vertebral

arteries. The vertebral arteries are more important in pigs. Collaterals in the cerebral circulation (The Circle of Willis and the rostral epidural retia mirabile) are well developed in pigs in contrast to man. The six major end-arteries perfusing the brain are localized in the pia mater and contribute for a large part to the cerebrovascular resistance.

One possible consequence of the existing differences between the species is an underestimation of brain flow in the present experiments, since the contribution from the vertebral arteries has not been taken into account. A special feature of the cerebral circulation is the blood-brain barrier.

The dura mater is a well vascularized tissue, supplied for a large part by the external carotid arteries via the middle meningeal arteries, but the internal carotid and vertebral arteries also contribute to dural perfusion. No blood-brain barrier is present in the dura mater, which further contains many arteriovenous anastomoses both in man. In both species the extracranial tissues are exclusively supplied by branches of the external carotid artery.

Craniovascular physiology and pharmacology: The tight autoregulation and metabolic regulation (mediated by extracellular potassium- and hydrogen-ions) of blood flow is a unique feature of the cerebral circulation. This metabolic regulation may be uncoupled during the migraine aura. Neural mechanisms seem to play only a secondary role in the cerebral circulation, of which the resistance vessels are scarcely innervated. In contrast, the larger cerebral arteries are densely innervated, by both vasodilator and vasoconstrictor nerves. Constrictor neurotransmitters are noradrenaline, adenosine triphosphate and neuropeptide Y in sympathetic nerves and 5-hydroxytryptamine, either in sympathetic nerves or genuine tryptaminergic nerves. Dilator neurotransmitters are acetylcholine and vasoactive intestinal polypeptide in cholinergic nerves arising from the sphenopalatine and otic ganglia and substance P and calcitonin gene-related peptide in sensory nerves. For all these neurotransmitters receptors are found on the cerebral arteries, but the receptor density seems to decline when the blood vessels become smaller.

The effect of the sympathetic nervous system on the cerebral perfusion and its function are uncertain, due to conflictive reports. It may have a role in the protection of the cerebral vascular bed against hypertension. Strikingly, the porcine cerebral arteries seem to lack α-adrenoceptors, in contrast to human cerebral arteries. There is a continuing debate on whether vascular 5-hydroxytryptamine, which in large cerebral arteries is a very potent vasoconstrictor, is contained in sympathetic neurones or in genuinely serotonergic neurons originating in the dorsal raphe nuclei. Both 5-HT_1 and 5-HT_2 receptors contribute to the contractile response to 5-hydroxytryptamine. In contrast, smaller cerebral arteries and arterioles are dilated by the amine. No physiological role has yet been ascribed to serotonergic nerves in the cerebral circulation,

although, when released from platelets, the amine may be responsible for the vascular spasm accompanying subarachnoidal haemorrhage.

The parasympathetic system is involved in the vasodilatation observed after experimental stimulation of different intracranial structures. The physiological role of this system is still unknown, although it has been proposed to be involved in coupling cerebral blood flow to metabolic requirements. Furthermore, it has been implied in the pathogenesis of migraine.

Arteriovenous anastomoses can be divided into those located on body surfaces and those in deep tissues. The superficial arteriovenous anastomoses play a role in the regulation of body temperature, and seem to be under tight control of the sympathetic nervous system, although porcine arteriovenous anastomoses are relatively insensitive to sympathetic stimulation. Dilator cholinergic and dopaminergic and peptidergic systems have also been claimed on some arteriovenous anastomoses.

The function of deep arteriovenous anastomoses e.g in the dura mater remains intriguing in view of the possible role of the dura mater in the migraine attack. Therefore, the effect of antimigraine drugs on dural arteriovenous anastomoses has been investigated in this thesis. The dura mater has a sympathetic and a modest parasympathetic innervation with unknown functions and a trigeminal innervation with a putative sensory role. Lastly, a modest serotonergic innervation was observed. However, conflictive results have been obtained on the effect of this amine on dural blood flow.

Summary of the present investigations
(Chapters 6-11)

Chapter 6. In this study the carotid haemodynamic effects of the antimigraine drug sumatriptan were tested. Sumatriptan reduced dose-dependently arteriovenous anastomotic blood flow; on the other hand capillary blood flow increased with the highest doses. The reduction in the carotid arteriovenous anastomotic ("shunt") blood flow were mediated by a 5-HT_1-like receptor, because methiothepin, but not ketanserin, antagonized the responses. The selective reduction in arteriovenous anastomotic blood flow produced by sumatriptan may reflect its antimigraine action, thought to involve vasoconstriction of those cranial vessels, be they "shunt" vessels or not, which are distended and inflamed during a migraine attack.

Chapter 7. The nature of the 5-hydroxytryptamine receptors involved in reducing arteriovenous shunting and increasing cutaneous capillary flow in the carotid vascular bed

of the pig was investigated in this study, especially whether one or both of these receptors belong to the 5-HT_{1D} subtype. The animals received either an intracarotid infusion of 5-hydroxytryptamine or cumulative intravenous doses of sumatriptan, a 5-HT_1-like agonist. The effects on the distribution of carotid blood flow were determined in the absence and in the presence of metergoline, an antagonist of the 5-HT_{1D} receptor as well as of the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_2 receptors. Both 5-HT and sumatriptan reduced carotid arteriovenous anastomotic blood flow. 5-HT and, to a lesser extent, sumatriptan also increased capillary blood flow towards some tissues. Metergoline by itself did not affect the distribution of porcine carotid blood flow. It attenuated the constrictor response, but increased the vasodilator response to 5-HT, in a manner similar to the 5-HT_2 receptor antagonists cyproheptadine and ketanserin in earlier experiments. These effects seem, therefore, to be related to the blockade of 5-HT_2 receptors by metergoline. On the other hand, metergoline had no significant effect against the responses to sumatriptan. Therefore, neither the constrictor nor the dilator carotid 5-HT_1-like receptors seem to belong to the known 5-HT_1 binding subtypes, including the 5-HT_{1D} subtype.

Chapter 8. Using a high dose of methiothepin (3 $mg.kg^{-1}$) we have investigated whether 5-hydroxytryptamine receptors are responsible for the reduction in carotid arteriovenous anastomotic shunting by ergotamine and dihydroergotamine. The ergot alkaloids reduced dose-dependently arteriovenous anastomotic blood flow and methiothepin partly antagonized this effect. This indicates that the effects of ergotamine and dihydroergotamine are partly mediated by methiothepin-sensitive receptors, which probably are either 5-HT_1 or 5-HT_2 receptors or α-adrenoceptors. An important part of the effect of the ergot alkaloids, however, is left after methiothepin and is likely to be mediated by other, perhaps novel, receptors.

Chapter 9. We have localized the arteriovenous anastomoses of the carotid vascular bed of the pig by simultaneous intracarotid injections of radiolabelled microspheres of three different sizes (10, 15 and 50 μm), which provides an index of blood flow via arteriovenous anastomoses larger than approximately 14, 27 and 90 μm diameter, respectively. The effects of sumatriptan (0.3 mg kg^{-1}), ergotamine (0.02 mg kg^{-1}), dihydroergotamine (0.1 mg kg^{-1}) were studied by repeating the injections of 15 and 50 μm spheres after the treatments. The 10 and 15 μm microsphere values were not different, so arteriovenous anastomoses with a diameter between 14 and 27 μm were absent. Arteriovenous anastomoses with a diameter between 27 and 90 μm, were found predominantly in the dura mater, ears, skin and fat. In total, all three drugs reduced the blood flow in the smaller arteriovenous anastomoses (diameter between 27 and 90 μm), and, to a higher extent, in larger shunts (wider than 90 μm). Locally, blood flow in the

smaller arteriovenous shunts was reduced in the skin and fat, but not in the dura mater or ears. It is not possible to determine in which tissues blood flow in the larger arteriovenous anastomoses was reduced. Capillary blood flow remained unchanged after the three drugs. Therefore, the only evident effect of these antimigraine drugs on carotid haemodynamics was a decrease in blood flow in arteriovenous anastomoses; the ones with a diameter smaller than 90 μm were affected in the skin and fat, but not in the dura mater or ears.

Chapter 10. The haemodynamic effects of sumatriptan, a 5-HT_1-like receptor agonist, and ergotamine, an agonist at α-adrenergic, dopamine as well as 5-HT receptors, were compared using intracardiac injection of radioactive microspheres of different sizes (see chapter 9). Ergotamine (0.02 $mg.kg^{-1}$) and sumatriptan (0.3 $mg.kg^{-1}$) decreased systemic vascular conductance and cardiac output. Only ergotamine raised arterial blood pressure. Both sumatriptan and ergotamine decreased arteriovenous anastomotic, but not capillary, blood flow in the head and body skin. Arteriovenous and capillary blood flow in the dura mater and nasal mucosa and capillary blood flow in the brain, kidneys, adrenals, intestine, heart, spleen and muscle remained unchanged. However, kidney conductance was decreased by both drugs, spleen conductance by sumatriptan and heart, liver and adrenal conductances were decreased by ergotamine. Thus, both sumatriptan and ergotamine constricted arteriovenous anastomoses in the skin, but not in the dura mater or nasal mucosa. Ergotamine constricted the vasculature more than sumatriptan, although both drugs may differentially decrease vascular conductances in some organs.

Chapter 11. We have investigated whether noradrenaline or 5-hydroxytryptamine is responsible for the maintenance of the physiological tone in the cranial arteriovenous anastomoses in the pig, using anaesthesia with fentanyl and thiopentone during which basal shunting is low. Consecutive antagonism of α_1, α_2, 5-HT_1 and 5-HT_2 receptors was obtained by cumulative administration of prazosin (0.02 $mg.kg^{-1}$), phentolamine (1 $mg.kg^{-1}$), ketanserin (0.5 $mg.kg^{-1}$) and methiothepin (3 $mg.kg^{-1}$). Prazosin caused a large increase in arteriovenous anastomotic blood flow. Extracerebral tissue flow decreased, but brain flow was preserved. Neither phentolamine, ketanserin or methiothepin had any significant further effect on cranial haemodynamics. It is concluded that an α_1-receptor, with probably noradrenaline as the endogenous ligand, is responsible for the normal tone in arteriovenous anastomoses. A role for 5-HT in the maintenance of vascular tone in the arteriovenous anastomoses could not be ascertained, at least in this model.

General discussion

In this thesis, the antimigraine drug sumatriptan was shown to increase total resistance in the carotid vascular bed of the pig. This was caused by a constriction of arteriovenous anastomoses, rather than of arterioles. In this sense, sumatriptan behaves similarly to the ergot alkaloids, ergotamine and dihydroergotamine, which are also used against migraine attacks. The main question is whether such a constrictor action at arteriovenous anastomoses contributes to the antimigraine effect of these drugs. Dilatation of blood vessels is known to occur at least in some cases during the headache phase of the migraine attack. Distention of extracranial arteries (e.g. increased pulsation of the superficial temporal artery and dilatation of conjunctival blood vessels) is observed in roughly one-third of the patients, the "red migraineurs". In these patients compression of the superficial temporal artery usually alleviates the headache. In another third of the patients the intracranial vasculature appears to be affected, because the headache was not relieved by compressing the superficial temporal artery, but, conversely, was aggravated by an increase in intracranial pressure. A dilatation of arteriovenous anastomoses had been postulated after the observation of an increase in the jugular venous oxygen saturation during the migraine headache. Such an increased oxygen saturation should not necessarily be due to dilatation of arteriovenous anastomoses, but may also be observed with a decreased cerebral oxygen consumption. However, if arteriovenous anastomoses are involved, those in the skin are most likely to contribute to the headache in the "red migraineurs". Indeed, cutaneous arteriovenous anastomoses are innervated by sensory nerves. One would, however, expect the pain produced in these cutaneous shunts to have the sharp and well localized character of cutaneous pain and that is not usually the case in migraine. In migraineurs with a predominant intracranial vascular component, the arteriovenous anastomoses in the dura mater could be involved, especially since around the intradural blood vessels this tissue is pain-sensitive. In our investigation, however, only the cutaneous and not the dural arteriovenous anastomoses were constricted by the antimigraine drugs. Therefore, a therapeutic effect of constriction of arteriovenous anastomoses during migraine is questionable. For a definite proof, however, methods to accurately measure arteriovenous anastomotic blood flow in patients should be developed. In the hand and forearm this has been achieved by injection of radioactive albumin microspheres. For obvious reasons, this method cannot be used to study the cranial circulation. With the laser-Doppler method total cutaneous blood flow can be measured, but distinguishing between arteriovenous anastomotic and capillary blood flow is not possible.

The antimigraine drugs studied in this thesis equally constrict large and medium-sized arteries. This may well be involved in the antimigraine activity. Some arteries have been shown to be dilated during the attack and pain can readily be induced in sites often involved in migraine attacks by inflating balloons in intracranial arteries. The receptors responsible for the arterial effect seem to be similar to those of the arteriovenous anastomoses. In the case of sumatriptan the constrictor effects are mediated by 5-HT_1-like receptors. This is, however, a heterogeneous group and the particular receptor involved in vascular constriction cannot be completely equated with one of the presently known 5-HT_1-like receptor subtypes. The receptor mechanism of the constrictor effect of the ergot alkaloids is still completely unknown, but the effect does not appear to be mediated by a single receptor type, but rather by a combination of known and still unknown receptors.

Although these antimigraine drugs appear to have some selectivity for the cranial vasculature, the effects are by no means limited to the head. Constriction was also observed in cutaneous arteriovenous anastomoses in other parts of the body as well as in some organs, like the heart and the kidneys. In the case of the ergot alkaloids vasoconstriction outside the head is a well known phenomenon, which may even lead to peripheral gangrene or, possibly, myocardial infarctions. Although the vascular effects of sumatriptan appeared to be milder, the drug has equally the potential to constrict arteries outside the head through 5-HT_1-like receptors and caution should be observed when using this drug in patients suffering from peripheral vascular disease or coronary heart disease.

The last part of this thesis is concerned with the nature of the neurotransmitter responsible for the normal constricted state of the cranial arteriovenous anastomoses in conscious pigs. In order to study this the conscious state was mimicked by a special anaesthesia with minimal influence on arteriovenous anastomoses. In this model, a role for α_1 adrenergic receptors was demonstrated, implying a tonic constriction by noradrenaline released from sympathetic nerves.

Hoofdstuk 13

Samenvatting en Algemene Beschouwing

Introductie

(Hoofdstuk 1)

In dit proefschrift zijn de vasculaire effecten van de antimigraine-middelen sumatriptan, ergotamine en dihydroergotamine bekeken. De redenen voor dit onderzoek zijn tweeërlei: (i) men denkt dat de hoofdpijn bij migraine veroorzaakt wordt door een stoornis in craniële bloedvaten; (ii) de bovengenoemde antimigraine-middelen zijn sterke vaatvernauwers, wat tot hun therapeutisch effect zou kunnen bijdragen.

In dit proefschrift werden de effecten van de bovengenoemde middelen op arterioveneuze anastomosen en arteriolen bepaald door middel van injectie van radioactieve microsferen in varkens onder narcose. Op de experimenten in hoofdstuk 11 na werd pentobarbital als narcoticum gebruikt en werden de proeven verricht na bilaterale cervicale vagosympathotomie. De microsferen werden hetzij in een a. carotis communis hetzij in de linker ventrikel van het hart ingespoten. Voorts zijn passende receptorantagonisten gebruikt om de bij de effecten van de antimigrainemiddelen betrokken receptoren te karakteriseren. In de laatste plaats is geprobeerd de neurotransmitter die verantwoordelijk is voor handhaving van de fysiologische tonus in de arterioveneuze anastomosen te bepalen.

Pathofysiologie van migraine en werking van antimigraine-middelen

(Hoofdstukken 2 en 3)

De zeer wisselende klinische presentatie van migraine en het gebrek aan eenduidige diagnostische criteria voor dit syndroom heeft ertoe geleid dat in het verleden patiëntengroepen voor klinisch onderzoek vaak zeer verschillend werden samengesteld. Dit heeft de 'International Headache Society' ertoe bewogen om in 1988 duidelijke criteria te publiceren om migraine te diagnostiseren en te classificeren. Men onderscheidt twee vaak voorkomende vormen: migraine zonder en migraine met aura. De migrainehoofdpijn moet 4 tot 72 uren duren, maar kan soms achterwege blijven. De hoofdpijn wordt begeleid door malaise, foto- en fonofobie, misselijkheid en soms braken.

Indien een aura aanwezig is, gaat deze gewoonlijk aan de hoofdpijn vooraf, kan uit sensore of motore verschijnselen bestaan, en duurt gewoonlijk niet langer dan 60 minuten. De bovengenoemde onderverdeling van migraine is belangrijk voor onderzoeksdoeleinden, aangezien de twee groepen ook in andere opzichten verschillen.

In het verleden zijn vasculaire (Thomas Willis in 1664), neurogene (negentiende eeuw) en humorale (serotonine) theorieën voor migraine geponeerd. Graham en Wolff (1938) waren de eersten die wezenlijk onderzoek verrichten met migrainepatiënten. Zij verklaarden de aurasymptomen als zijnde een gevolg van ischaemie door cerebrale vasoconstrictie en de hoofdpijn als gevolg van extracerebrale vasodilatatie. Inderdaad kon in latere studies een verminderde hersendoorbloeding tijdens de aura aangetoond worden. Dit bewees de ischaemie-theorie echter niet, aangezien deze verminderde doorbloeding zich niet aan vaatgrenzen hield, niet groot genoeg was om de ischaemie te verklaren en soms voortduurde tot in de hoofdpijnfase. Daarom werd eerder gedacht aan een primaire neurologische stoornis met secundair vasculaire veranderingen. Echter, de methode die gebruikt is om de cerebrale doorbloeding te meten (inhalatie van ^{133}Xe) is niet erg nauwkeurig bij het meten van lage waarden, zodat de vraag of inderdaad ischaemie optreedt tijdens de aura nog niet afdoende beantwoord is. Het idee dat de hoofdpijn veroorzaakt wordt door vaatverwijding werd onderbouwd door Ray en Wolff (1940) die aantoonden dat sommige craniële bloedvaten en de dura mater in de buurt van deze vaten de enige pijngevoelige intracraniële structuren zijn. Een mogelijke betrokkenheid van arterioveneuze anastomosen werd voorgesteld door Heyck (1969) op basis van een toename in de jugulair veneuze zuurstofverzadiging aan de hoofdpijnzijde en de normalisering hiervan na toediening van dihydroergotamine. Omdat de cerebrale doorbloeding bij migraine zonder aura niet verandert en de afname van de cerebrale doorbloeding, die gezien wordt tijdens de aura, soms voortduurt tot in de hoofdpijnfase, kan de hoofdpijn niet verklaard worden door veranderingen in de hersendoorbloeding. Daarentegen vermoedt men tegenwoordig dat verwijding van grotere intracraniële bloedvaten, die de vaatweerstand en dus de doorbloeding niet beïnvloeden, een rol speelt. De schatting van de vaatdiameter door het meten van de bloedstroomsnelheid in sommige intracraniële arteriën met behulp van de transcraniële Doppler-methode heeft tot tegenstrijdige resultaten geleid. In de enige studie waarin extracraniële doorbloeding gedurende de hoofdpijnfase is gemeten met ^{133}Xe, werd een toename gevonden, hoewel de betrouwbaarheid van deze methode twijfelachtig is vanwege interferentie door straling vanuit de hersenen. Daarom moet de vraag of inderdaad een intra- of extracraniële vaatverwijding optreedt gedurende de hoofdpijnfase nog steeds opgelost worden.

Een neurovasculaire pathogenese van migraine werd waarschijnlijker na de vondst in het jugulair veneuze bloed van een verhoogde spiegel van CGRP (calcitonin

gene-related peptide), dat afkomstig is van sensore trigeminusvezels en verder een zeer sterk vaatverwijdend effect heeft. Verder werd aangetoond dat elektrische stimulatie van sommige hersenkernen, zoals de serotonine-bevattende nucleus raphe dorsalis en medianus, een sterke invloed heeft op de craniële haemodynamiek.

Voor de behandeling van migraine is een profylactische of een aanvalsafhankelijke benadering mogelijk. In dit proefschrift worden alleen de specifieke, niet-analgetische middelen om de aanval te couperen besproken.

Ergotamine: Ergotamine is een ergot-alkaloïde en heeft, net als de meeste ander stoffen van deze klasse, een complexe farmacologie. De effecten van deze stof kunnen zowel veroorzaakt worden door stimulering als door antagonisme van 5-HT, dopamine of adrenerge receptoren, terwijl een bijkomende interactie met nog onbekende receptoren vermoed wordt. Dat vaatvernauwing bij het effect van ergotamine betrokken zou zijn werd voor het eerst gepostuleerd door Graham en Wolff (1938). Zij vermeldden een afname in pulsatiliteit van de a. temporalis aan de hoofdpijnzijde na toediening van deze stof. Verder onderzoek met ergotamine heeft zowel vaatvernauwende als vaatverwijdende eigenschappen aan het licht gebracht, hoewel vaatvernauwing gewoonlijk de overhand heeft. Meestal wordt een kort durende toename van de vaatweerstand in arteriolen en kleine arteriën gevolgd door een langdurige vaatvernauwing van grotere arteriën. Dit laatste zou betrokken kunnen zijn bij het therapeutische effect van ergotamine, dat zeer langdurig werkzaam is.

Recent onderzoek aan de Harvard Universiteit in Boston heeft een ander mogelijk werkingsmechanisme van ergotamine aan het licht gebracht: de remming van de neurogene ontsteking in de vaatwand die volgens deze onderzoekers zou optreden gedurende de migraineaanval. Het is echter nog niet bekend of dit een direct neuraal effect van ergotamine is of dat het een gevolg is van vaatvernauwing.

Dihydroergotamine: De basale farmacologische eigenschappen van dihydroergotamine lijken op die van ergotamine, niettegenstaande kleine verschillen, zodat een vergelijkbaar werkingsmechamisme voor beide stoffen te verwachten is.

Sumatriptan: Sumatriptan is een recent ontwikkeld antimigrainemiddel en deze stof is, in tegenstelling tot de ergot alkaloïden, een selectieve agonist van 5-HT_1-achtige receptoren. Van deze stof zijn zowel vaatvernauwende als neurogene ontstekingsremmende eigenschappen aangetoond. Deze zijn kwalitatief te vergelijken met die van de ergot alkaloïden, hoewel de vaatvernauwende eigenschappen milder zijn en de receptormechanismen waarschijnlijk verschillen. Daarom zouden zowel een vasculair als een neuraal mechanisme betrokken kunnen zijn bij het therapeutische effect van sumatriptan, hoewel een direct effect van sumatriptan op zenuwuiteinden in geïsoleerde arteriën niet aangetoond kon worden. Sumatriptan is effectiever in het onderdrukken van

de begeleidende symptomen van de migraineaanval dan de ergot alkaloïden, waarvoor de reden onbekend is.

Anatomische en fysiologische aspecten van de doorbloeding van het hoofd
(Hoofdstuk 4)

Craniovasculaire anatomie: De arteriële vaatvoorziening van het hoofdgebied wordt verzorgd door de twee aa. carotis en de twee aa. vertebrales. In de mens worden de hersenen voornamelijk door de aa. carotis internae en de aa. vertebrales van bloed voorzien en in het varken door de aa. carotis externae en vertebrales. Het aandeel van de aa. vertebrales is groter bij varkens dan bij de mens. Collateralen tussen het carotide en het vertebrale systeem (Cirkel van Willis, rostrale epidurale retia mirabile) zijn bij varkens, in tegenstelling tot bij de mens, sterk ontwikkeld. De zes grote eind-arteriën die de hersenen van bloed voorzien lopen voor een deel in de pia mater en deze dragen voor een groot deel bij aan de cerebrovasculaire weerstand.

De bovengenoemde soortverschillen hebben waarschijnlijk tot gevolg dat in de hier beschreven experimenten de hersendoorbloeding onderschat is omdat de bijdrage van de vertebrale arteriën niet is meegerekend. Een speciaal kenmerk van de cerebrale circulatie is het bestaan van de bloed-hersen barrière.

De dura mater is een vaatrijk weefsel en wordt voornamelijk door de aa. meningeae mediae van bloed voorzien, hoewel de aa. carotis internae en vertebrales ook een bijdrage leveren. De dura mater bevindt zich buiten de bloed-hersen barrière. In dit weefsel worden verder vele arterioveneuze anastomosen gevonden. De extracraniële weefsels worden in beide soorten geheel door takken van aa. carotis externae van bloed voorzien.

Craniovasculaire fysiologie and farmacologie: De strikte autoregulatie en metabole regulatie (gemedieerd door extracellulaire kalium- en waterstofionen) is een specifiek kenmerk van de cerebrale circulatie, die tijdens de migraineaura echter verstoord schijnt te zijn. Zenuwen lijken slechts van secundair belang te zijn voor de regulatie van de cerebrale bloedvoorziening, waarvan de weerstandsvaten ook weinig geïnnerveerd zijn. Daarentegen bezitten de grotere cerebrale arteriën een dichte innervatie door zowel vaatverwijdende als vaatvernauwende zenuwen. Vaatvernauwende neurotransmitters zijn noradrenaline, adenosine trifosfaat en neuropeptide Y in sympathische zenuwen en het serotonine, dat zich ofwel in sympathische zenuwen, ofwel in serotonerge zenuwen bevindt. Vaatverwijdende neurotransmitters zijn acetylcholine en vasoactief intestinaal polypeptide in cholinerge zenuwen afkomstig van het ganglion sphenopalatinum en het ganglion oticum en substance P en calcitonin gene-related peptide in de sensore zenuwen

van de n. trigeminus. Voor al deze neurotransmitters zijn receptoren op de cerebrale arteriën beschreven, maar hun dichtheid neemt af met de vaatdiameter.

De werking van het sympathische zenuwstelsel op de cerebrale bloedvoorziening en diens functie hierbij zijn onzeker tengevolge van tegenstrijdige gegevens. Het zou een rol spelen bij de bescherming van het cerebrale vaatbed tegen hypertensie. Merkwaardigerwijze lijken de cerebrale arteriën van het varken geen α-receptoren te bezitten in tegenstelling tot die van de mens. Er bestaat verder een voortdurende controverse of het serotonine, dat zich in de vaatwand bevindt en in grotere hersenarteriën een sterk vaatvernauwende werking heeft, gelokaliseerd is in sympathische zenuwen of gevonden wordt in serotonerge zenuwen die van de dorsale raphe-kernen afkomstig zouden zijn. Zowel 5-HT_1 als 5-HT_2 receptoren dragen bij tot het vaatvernauwende effect van deze stof. Daarentegen verwijdt deze stof de kleinere cerebrale arteriën en arteriolen. De functie van deze serotonine-bevattende zenuwen in het hersenvaatbed is nog goeddeels onbekend, hoewel serotonine, afkomstig uit bloedplaatjes, een rol lijkt te spelen bij de vaatspasme die bij een subarachnoidale bloeding wordt gezien.

Het parasympathische systeem speelt een rol bij de vaatverwijding die wordt gezien tijdens stimulatie van sommige intracraniële structuren. Hoewel de rol van dit systeem evenmin duidelijk is, is er een aanwijzing dat het betrokken zou kunnen zijn bij de coördinatie van hersendoorbloeding en metabole behoefte. Verder heeft men een rol van dit systeem bij migraine gepostuleerd.

Bij arterioveneuze anastomosen kan man een onderscheid maken tussen diegene in oppervlakkige en diegene in diepe lichaamsweefels. De oppervlakkige arterioveneuze anastomosen zijn betrokken bij de regulatie van de lichaamstemperatuur en staan waarschijnlijk voornamelijk onder controle van de sympathicus, hoewel die van het varken juist erg ongevoelig voor sympathische stimulatie lijken te zijn. Er is ook een aanwijzing voor het bestaan van dilatatoire dopaminerge en peptiderge systemen op arterioveneuze anastomosen.

De rol van de diepe arterioveneuze anastomosen, zoals bijvoorbeeld in de dura mater aangetroffen worden, blijft intrigerend gezien de mogelijke rol van deze structuur bij de migraineaanval. Daarom zijn in dit proefschrift ook de effecten van antimigrainemiddelen op deze durale arterioveneuze anastomosen bekeken. Verder bezit de dura mater een sympathische en een bescheiden parasympathische innervatie, waarvan de rol niet bekend is, en een innervatie door de trigeminus met een waarschijnlijk sensore functie. Als laatste zijn er aanwijzingen voor een bescheiden serotonerge innervatie. Tegenstrijdige effecten van dit amine op de durale bloedvoorziening zijn echter beschreven.

Samenvatting van de huidige onderzoeken
(Hoofdstukken 6 t/m 11)

Hoofdstuk 6. In deze studie werden de effecten van het antimigrainemiddel sumatriptan op de carotide circulatie onderzocht. Sumatriptan veroorzaakte een dosis-afhankelijke daling in de doorbloeding van arterioveneuze anastomosen; capillaire doorbloeding nam juist toe bij de hoogste doseringen. De afname in de doorbloeding van de carotide arterioveneuze anastomosen werd veroorzaakt door stimulatie van 5-HT_1-achtige receptoren, omdat methiothepine, maar niet ketanserine, dit effect blokkeerde. De selectieve afname van de doorbloeding van arterioveneuze anastomosen zou een afspiegeling kunnen zijn van de antimigraine werking van sumatriptan, waarbij men denkt dat constrictie van verwijde en ontstoken bloedvaten (arterioveneuze anastomosen of andere) een rol speelt.
Hoofdstuk 7. De aard van de 5-HT receptoren, die betrokken zijn bij de afname van de doorbloeding van de arterioveneuze anastomosen en bij de toename van de capillaire huiddoorbloeding, werd in deze studie onderzocht, met nadruk op de mogelijke betrokkenheid van het 5-HT_{1D} subtype. De dieren ontvingen ofwel een infuus van serotonine in een a. carotis communis, ofwel cumulatieve intraveneuze doseringen van sumatriptan, een 5-HT_1-achtige agonist. Hun effecten op de verdeling van de carotide doorbloeding werden bepaald in de afwezigheid en de aanwezigheid van metergoline, een antagonist van de 5-HT_{1D} receptor en eveneens van 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} en 5-HT_2 receptoren. Zowel serotonine als sumatriptan verminderden de doorbloeding van arterioveneuze anastomosen. Daarbij verhoogden serotonine en, in mindere mate, sumatriptan de capillaire doorbloeding van enkele weefsels. Metergoline zelf had geen invloed op de verdeling van de carotide doorbloeding. Wel veroorzaakte het een vermindering van het constrictoire effect, maar een vergroting van het dilatatoire effect van serotonine, net als de 5-HT_2 antagonisten cyproheptadine en ketanserine in vroegere experimenten. Daarom lijken deze effecten verband te houden met 5-HT_2-blokkade door metergoline. Anderzijds had metergoline geen invloed op de effecten van sumatriptan. Daarom lijken noch de constrictoire, noch de dilatatoire carotide 5-HT_1-achtige receptoren tot een van de bekende subtypen te horen, inclusief de 5-HT_{1D} receptor.
Hoofdstuk 8. Gebruik makend van een hoge dosering methiothepine (3 $mg.kg^{-1}$), hebben we onderzocht of serotoninereceptoren betrokken zijn bij de afname van de doorbloeding van arterioveneuze anastomosen ten gevolge van ergotamine en dihydroergotamine. De ergot-alkaloïden veroorzaakten een dosis-afhankelijke vermindering van de doorbloeding van arterioveneuze anastomosen. Methiothepine blokkeerde deze effecten slechts ten dele. Dit geeft aan dat de effecten van ergotamine deels veroorzaakt worden door

stimulatie van methiothepine-gevoelige receptoren, waarschijnlijk 5-HT_1, 5-HT_2 of α-adrenerge receptoren. Een belangrijk deel van het effect bleef echter over na methiothepine en werd dus waarschijnlijk door andere, misschien nog onbekende, receptoren gemedieerd.

Hoofdstuk 9. In dit hoofdstuk werd de lokalisatie van de arterioveneuze anastomosen in het carotide vaatbed bepaald door tegelijkertijd radioactieve microsferen met drie verschillende doorsneden (10, 15 en 50 μm) in de a. carotis communis te injecteren. Deze meten de doorbloeding van arterioveneuze anastomosen die wijder zijn dan ongeveer 14, 27 en 90 μm, respectievelijk. De effecten van sumatriptan (0,03 mg.kg^{-1}), ergotamine (0,02 mg.kg^{-1}) en dihydroergotamine (0,1 mg.kg^{-1}) werden onderzocht door na toediening van een van deze stoffen de injectie van 15 en 50 μm microsferen te herhalen. De waarden gevonden met 10 en 15 μm sferen waren niet verschillend, zodat arterioveneuze anastomosen met een diameter tussen 14 en 27 μm ontbraken. Anastomosen met een diameter tussen 27 en 90 μm werden voornamelijk gevonden in de dura mater, oren, huid, en vetweefsel. Alle drie de stoffen veroorzaakten een vermindering in de totale doorbloeding van kleinere arterioveneuze anastomosen (diameter tussen 27 en 90 μm) en nog meer in grotere anastomosen (wijder dan 90 μm). Lokaal werd de doorbloeding van kleinere arterioveneuze anastomosen verminderd in de huid en in het vetweefsel, maar niet in de dura mater of de oren. Het is niet mogelijk om de weefsels te bepalen waar de afname van de doorbloeding van de grotere arterioveneuze anastomosen plaats vond. De capillaire doorbloeding bleef onveranderd na de drie middelen. Daarom was het enige effect van deze antimigrainemiddelen op de carotide haemodynamiek een afname in de doorbloeding van arterioveneuze anastomosen; degene in de huid en het vetweefsel met een diameter kleiner dan 90 μm waren hier gevoelig voor, echter niet in de dura mater of de oren.

Hoofdstuk 10. De haemodynamische effecten van sumatriptan, een 5-HT_1-achtige receptor agonist, en van ergotamine, een agonist van α-adrenerge, dopamine en 5-HT receptoren, werden vergeleken door middel van intracardiale injectie van radioactieve microsferen met verschillende diameter (zie hoofdstuk 11). Ergotamine (0.02 mg.kg^{-1}) and sumatriptan (0.3 mg.kg^{-1}) veroorzaakten een vermindering in systemische vasculaire conductantie en hartminuutvolume. Alleen ergotamine veroorzaakte een bloeddrukstijging. In de hoofd- en de lichaamshuid verminderden zowel sumatriptan als ergotamine de doorbloeding van arterioveneuze anastomosen, maar niet van capillairen. De arterioveneuze en capillaire doorbloeding van de dura mater en het neusslijmvlies en de capillaire doorbloeding van de hersenen, nieren, bijnieren, dunne darm, hart, milt en skeletspieren veranderden niet. Daarentegen nam de nierconductantie af ten gevolge van beide middelen, de miltconductantie ten gevolge van sumatriptan en de hart-, lever- en

bijnierconductantie ten gevolge van ergotamine. De conclusie is, dat zowel sumatriptan als ergotamine constrictie van arterioveneuze anastomosen veroorzaken in de huid, echter niet in de dura mater of het neusslijmvlies. Over het geheel genomen gaf ergotamine een sterkere vasoconstrictie dan sumatriptan, hoewel de effecten van beide stoffen per vaatbed lijken te verschillen.

Hoofdstuk 11. In dit hoofdstuk is onderzocht of noradrenaline of serotonine verantwoordelijk is voor handhaving van de fysiologische tonus van craniële arterioveneuze anastomosen in het varken. Hiervoor werd gebruik gemaakt van narcose met fentanyl en thiopental, waarbij de basale doorbloeding van arterioveneuze anastomosen laag is. Opeenvolgend werden α_1-, α_2-, 5-HT_2- en 5-HT_1 receptoren geblokkeerd door cumulatieve toediening van prazosine (0,02 mg.kg^{-1}), fentolamine (1 mg.kg^{-1}), ketanserine (0,5 mg.kg^{-1}) en methiothepine (3 mg.kg^{-1}). Prazosine veroorzaakte een forse toename in de doorbloeding van de arterioveneuze anastomosen. De doorbloeding van de extracerebrale weefsels nam af maar de hersendoorbloeding bleef gelijk. Fentolamine, ketanserine noch methiothepine had enige verdere invloed op de craniële haemodynamiek. Daarom kan geconcludeerd worden dat een α_1-receptor, vermoedelijk met noradrenaline als endogene agonist, verantwoordelijk is voor de normale tonus in de craniële arterioveneuze anastomosen. Een rol voor serotonine kon niet bevestigd worden, althans niet in dit model.

Algemene beschouwing

In dit proefschrift is aangetoond dat het antimigrainemiddel sumatriptan een toename geeft in de totale weerstand van het carotide vaatbed van het varken. Dit wordt veroorzaakt door een constrictie van arterioveneuze anastomosen en niet van arteriolen. In dit opzicht gedraagt sumatriptan zich net als de ergot alkaloïden ergotamine en dihydroergotamine, welke ook tegen migraineaanvallen gebruikt worden. Een belangrijke vraag is, of zo'n effect op arterioveneuze anastomosen een bijdrage levert aan het therapeutische effect van deze middelen. Dat vaatverwijding kan optreden gedurende de hoofdpijnfase van een migraineaanval, is bekend. Verwijding van extracraniële arteriën (b.v. verhoogde pulsaties van de a. temporalis superficialis en verwijding van conjunctivale bloedvaten) wordt bij ongeveer een op de drie patiënten gezien, de zogenoemde "red migraineurs". In deze patiënten verlicht druk op de a. temporalis superficialis gewoonlijk de hoofdpijn. Bij een ander derde deel van de patiënten lijken intracraniële vaten betrokken te zijn, aangezien de hoofdpijn niet vermindert door druk op de a. temporalis superficialis maar daarentegen toeneemt tijdens verhoging van de

intracraniële druk. Een verwijding van arterioveneuze anastomosen is gepostuleerd na de vondst van een verhoogde jugulair veneuze zuurstofverzadiging gedurende de migrainehoofdpijn. Zo'n toename in de zuurstofverzadiging is echter niet noodzakelijkerwijze een gevolg van verwijding van arterioveneuze anastomosen, maar kan ook gezien worden bij een verminderd cerebraal zuurstofverbruik. Indien arterioveneuze anastomosen betrokken zouden zijn bij de hoofdpijn, zou dit zeker bij de "red migraineurs" de huidanastomosen betreffen. Inderdaad is een sensore innervatie van deze anastomosen aangetoond. Aan de andere kant zou men verwachten dat pijn, veroorzaakt door deze huidanastomosen, scherper en beter gelokaliseerd zou zijn dan meestal het geval is bij migraine. In migrainepatiënten met een overwegend intracraniële vaatcomponent zouden de arterioveneuze in de dura mater betrokken kunnen zijn, zeker omdat dit weefsel rond de intradurale bloedvaten pijngevoelig is. Echter, in onze proeven werd gezien dat de antimigrainemiddelen alleen de cutane en niet de durale arterioveneuze anastomosen deed samentrekken. Daarom lijkt een therapeutisch effect van constrictie van arterioveneuze anastomosen onzeker. Om dit echter te bewijzen zouden betrouwbare methoden ontwikkeld moeten worden om de doorbloeding van arterioveneuze anastomosen in patiënten te meten. In de hand en onderarm is dit gedaan door middel van injectie van radioactieve albuminemicrosferen. Het is duidelijk dat deze methode niet gebruikt kan worden om de craniële circulatie te meten. Met de laser-Doppler methode kan de totale huiddoorbloeding gemeten worden maar met deze methode kan geen onderscheid gemaakt worden tussen capillaire en arterioveneus anastomotische doorbloeding.

De antimigrainemiddelen die in dit proefschrift zijn onderzocht veroorzaken eveneens een vernauwing van grote en middelgrote arteriën. Het is goed mogelijk dat dit voor het antimigraineffect van belang is. Men heeft verwijding van sommige arteriën aangetoond gedurende een migraineaanval en men kan pijn opwekken op plaatsen, waar vaak bij migraine pijun aangegeven wordt, door het opblazen van een balloncatheter in sommige intracraniële arteriën. De receptoren die betrokken zijn bij het arteriële effect van de antimigrainemiddelen zijn waarschijnlijk gelijk aan die in de arterioveneuze anastomosen. In het geval van sumatriptan zijn 5-HT_1-achtige receptoren betrokken. Dit is echter een heterogene groep en ook in de arteriën kan de betrokken receptor niet gelijkgesteld worden met een van de huidig bekende subtypen. Het receptormechanisme van het constrictoire effect van de ergot-alkaloïden is nog steeds niet geheel bekend, hoewel het effect niet door een enkele receptor maar daarentegen door een combinatie van bekende en onbekende receptortypen gemedieerd schijnt te worden.

Hoewel deze antimigrainemiddelen een zekere selectiviteit voor het craniële vaatbed lijken te bezitten, blijven de effecten geenszins beperkt tot het hoofd. Ook in

andere delen van het lichaam werd constrictie van arterioveneuze anastomosen in de huid gezien evenals in sommige organen als het hart en de nieren. Voor de ergot alkaloïden is vaatvernauwing buiten het hoofd een bekend verschijnsel dat zelfs tot een hartinfarct of perifere gangrenen kan leiden. Hoewel de vaateffecten van sumatriptan milder lijken te zijn, heeft dit middel ook de potentie om buiten het hoofd vaatvernauwing te veroorzaken door stimulatie van 5-HT_1-achtige receptoren en men moet voorzichtig zijn met het voorschrijven van dit middel aan patiënten met coronaire hartziekte of perifere vaatziekten.

Het laatste gedeelte van dit proefschrift is gewijd aan de neurotransmitter die verantwoordelijk is voor de normale tonus van de craniële arterioveneuze anastomosen in wakkere varkens. Om dit te bestuderen werd de wakkere toestand nagebootst door een bijzondere narcose met weinig invloed op de arterioveneuze anastomosen. In dit model werd een rol voor α_1-adrenerge receptoren aangetoond, zodat het sympathische zenuwstelsel met de neurotransmitter noradrenaline voor deze tonus verantwoordelijk lijkt.

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