Lack of effect of the antimigraine drugs, sumatriptan, ergotamine and dihydroergotamine on arteriovenous anastomotic shunting in the dura mater of the pig

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1 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 other places in the dura mater, which is a putative site of the pain during a migraine attack.

2 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.

3 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 .

4 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.

5 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).

6 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.

7 Tissue blood flow measured with 15 μm microspheres remained unchanged after the three antimigraine drugs, implying a lack of effect on capillary flow.

8 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.

Keywords: Arteriovenous anastomoses; carotid blood flow; dihydroergotamine; dura mater; ergotamine; ergot alkaloids; 5-hydroxytryptamine receptors; migraine; sumatriptan

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.*, 1989; 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 is 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.*, 1990; Saxena & Den Boer, 1991) remain as possibilities. Indeed,

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sumatriptan increases vascular resistance in the human isolated, perfused dura mater (Humphrey *et al.*, 1991b). 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 $\times$ 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 at 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: $MAP = (SAP + 2 \times DAP)/3$. 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 μ m microspheres at baseline and of 15 and 50 μ m microspheres after drug treatment. These were labelled with either ¹⁴¹Ce (10 μ m), ¹¹³Sn (50 μ m), ¹⁰³Ru (50 μ m), ⁹⁵Nb (15 μ m) or ⁴⁶Sc (15 μ m; NEN Company, Dreieich, Germany). The order of 15 and 50 μ m spheres injected before and after the drugs was randomized. The approximate number of microspheres given per isotope was: 10 μ m, 700,000; 15 μ m, 300,000; 50 μ m, 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 programmes (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 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 μ m are trapped in blood vessels with a diameter of 11.5, 27.7 and 42.7 μ m, respectively. Therefore, in the present investigation blood flow values measured with the 10, 15 and 50 μ m microspheres in the lungs have been assumed to represent cranial shunt flow in arteriovenous anastomoses wider than approximately 14, 28 and 90 μ m, respectively. Similarly, in the cranial tissues, the difference between blood flow values measured with 10 and 15 μ m and with 15 and 50 μ m microspheres was assumed to represent the flow in local arteriovenous anastomoses with a diameter, respectively, between 14 and 28 μ m and between 28 and 90 μ m. 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 μ m microspheres was injected into the carotid artery. After another stabilization period of about 15 min, 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 use of a mixture of 15 and 50 μ m microspheres) were again determined.

Data presentation and statistical analysis

All data have been expressed as means $\pm$ s.e.mean. 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, U.K.), 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 in 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, $63 \pm 3\%$ of $10 \mu\text{m}$ microspheres, $61 \pm 3\%$ of $15 \mu\text{m}$ spheres and $41 \pm 3\%$ of $50 \mu\text{m}$ spheres were shunted to the venous side via arteriovenous anastomoses. Arteriovenous anastomotic blood flows, measured with the 10 , 15 and $50 \mu\text{m}$ microspheres, were 108 ± 11 , 105 ± 11 and $71 \pm 7 \text{ ml min}^{-1}$, respectively, so that $3 \pm 1 \text{ ml min}^{-1}$ passed through shunts with a diameter between 14 and $28 \mu\text{m}$, $34 \pm 4 \text{ ml min}^{-1}$ through shunts of 28 – $90 \mu\text{m}$ diameter and $71 \pm 7 \text{ ml min}^{-1}$ 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 $15 \mu\text{m}$ microspheres were less than those measured with $50 \mu\text{m}$ microspheres, thus implying the presence of arteriovenous anastomoses with a diameter between approximately 28 and $90 \mu\text{m}$ 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 $15 \mu\text{m}$ microspheres, so that a few arteriovenous anastomoses with a diameter between approximately 15 and $30 \mu\text{m}$ appear to be present in these tissues. Since there was no difference in the blood flow measured with the 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 $90 \mu\text{m}$. From the present experiments, it is, however, not possible to localize the shunts larger than $90 \mu\text{m}$.

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 $34 \pm 5\%$, respectively. Though not statistically significant, an increase in mean arterial pressure of $14 \pm 8\%$ was seen after sumatriptan.

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 $-30 \pm 5\%$, 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

Table 1 Tissue flow values in the pig, measured with 10 , 15 and $50 \mu\text{m}$ diameter radioactive microspheres

	$10 \mu\text{m}$	$15 \mu\text{m}$	$50 \mu\text{m}$
Dura mater	0.12 ± 0.02	0.13 ± 0.02	$0.28 \pm 0.02^*$
Ears	2.3 ± 0.3	2.7 ± 0.5	$9.4 \pm 1.0^*$
Skin	$2.1 \pm 0.1^*$	2.9 ± 0.3	$16.9 \pm 1.3^*$
Fat	2.6 ± 0.3	2.7 ± 0.4	$11.6 \pm 1.5^*$
Muscle	8.9 ± 0.7	8.9 ± 0.9	$14.3 \pm 1.2^*$
Brain	9.4 ± 0.9	10.3 ± 1.0	10.2 ± 1.0
Tongue	3.1 ± 0.3	3.3 ± 0.4	$3.8 \pm 0.3^*$
Bones	11.1 ± 1.0	11.3 ± 1.0	11.3 ± 1.0
Eyes	$1.9 \pm 0.3^*$	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 $\pm$ s.e.mean.
* $P < 0.05$ vs. flow measured with $15 \mu\text{m}$ microsphere.

Figure 1 The localization of arteriovenous anastomoses in different cranial tissues, by differential entrapment of radioactive microspheres of 10 , 15 and $50 \mu\text{m}$ diameter. All values (means $\pm$ s.e.mean) have been given as the percentage difference from the flow value, measured with $15 \mu\text{m}$ spheres. * $P < 0.05$ from $15 \mu\text{m}$ flow value.

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) in the pig

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

Values are given as mean $\pm$ s.e.mean.
* $P < 0.05$ versus control.

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 difference in arterial and jugular venous oxygen saturation (AVOSD) in the pig

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

Values are given as mean $\pm$ s.e.mean.
* $P < 0.05$ versus control.

decrease in capillary flow: drug-induced changes in total capillary blood flow values, determined with $15 \mu\text{m}$ microspheres, were only -1.6 ± 1.3 , 4.4 ± 5.8 , -3.7 ± 8.0 and $6.6 \pm 5.7 \text{ ml min}^{-1}$, respectively, for saline, sumatriptan, ergotamine and dihydroergotamine. On the other hand, arteriovenous shunt flow, measured with microspheres of both 15 and $50 \mu\text{m}$ 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 – $90 \mu\text{m}$) 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 $90 \mu\text{m}$ after all three agents (Table 4).

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 $15 \mu\text{m}$ (open columns) and $50 \mu\text{m}$ (cross-hatched columns) microspheres. Values as means $\pm$ s.e.mean (vertical bars). * $P < 0.05$ vs simultaneously injected $15 \mu\text{m}$ sphere; # $P < 0.05$ vs same sphere before treatment.

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}) of the pig through arteriovenous anastomoses with a diameter between 28 and $90 \mu\text{m}$ or anastomoses wider than $90 \mu\text{m}$

	$28-90 \mu\text{m}$		$> 90 \mu\text{m}$	
	Before	After	Before	After
Saline	35 ± 5	35 ± 5	61 ± 14	$56 \pm 13^*$
Sumatriptan	30 ± 6	$16 \pm 3^*$	66 ± 17	$24 \pm 5^*$
Ergotamine	38 ± 14	20 ± 7	79 ± 18	$18 \pm 8^*$
Dihydroergotamine	33 ± 7	24 ± 5	77 ± 15	$28 \pm 7^*$

Values are given as mean $\pm$ s.e.mean.

* $P < 0.05$ versus control.

Blood flow in tissues with arteriovenous anastomoses with a diameter between 16 and $28 \mu\text{m}$ In skin and fat, sumatriptan, ergotamine and dihydroergotamine decreased entrapment of $50 \mu\text{m}$ microspheres, while the entrapment of $15 \mu\text{m}$ 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 $90 \mu\text{m}$ (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 $90 \mu\text{m}$ 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 $28 \mu\text{m}$ In the bones, salivary glands and brain, where flow through shunts with a diameter between 28 and $90 \mu\text{m}$ was completely absent, administration of neither sumatriptan, ergotamine nor dihydroergotamine produced any effect, except for an increase in salivary gland blood flow, measured with $50 \mu\text{m}$ spheres after dihydroergotamine (Figure 3).

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 (1985). A clear effect of 5-hydroxytryptamine (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 (Kaijara *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 $24 \mu\text{m}$ microspheres in the hamster cheek pouch, after intracardiac injection. They demonstrated that 9 , 15 and $24 \mu\text{m}$ microspheres were trapped when arriving in blood vessels with a diameter of 11.5 , 27.7 and $42.7 \mu\text{m}$, respectively, for the three microspheres. Extrapolation of these results to our experiments indicates that the 10 , 15 and $50 \mu\text{m}$ diameter microspheres are expected to pass to the venous side through blood vessels with a minimum diameter larger than 14 , 28

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 $15 \mu\text{m}$ (open columns) and $50 \mu\text{m}$ (cross-hatched columns) microspheres. Values as means $\pm$ s.e.mean (vertical bars). * $P < 0.05$ vs simultaneously injected $15 \mu\text{m}$ sphere; # $P < 0.05$ vs same sphere before treatment.

and 90 μm , respectively. Such vessels are arteriovenous anastomoses, because capillaries have a diameter under 10 μm (for references see Saxena & Verdouw, 1985). 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.*, 1991a). Since the new antimigraine agent, sumatriptan, also has this property (Perren *et al.*, 1989; Den Boer *et al.*, 1991b), 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, with radioactive microspheres of four sizes (10, 15, 25 and 35 μm diameter; Saxena & Verdouw, 1985).

A further location of arteriovenous anastomoses is the human dura mater (Rowbotham & 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 of 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.*, 1991b). 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 measure directly 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 human isolated, perfused dura mater (Humphrey *et al.*, 1991b). 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.*, 1991a): 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 use of 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 from 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 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 vasodilata-

tion 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 ($>90\ \mu\text{m}$ 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).

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