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# Role of 5-$HT_1$-like receptors in the reduction of porcine cranial arteriovenous anastomotic shunting by sumatriptan

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**1** 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.

**2** 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.

**3** 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.

**4** 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.

**5** 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 subtypes of 5-$HT_{1D}$ binding sites (Sumner & Humphrey, 1989). Functionally, sumatriptan causes contraction of different isolated blood vessels (Humphrey *et al.*, 1989a; 1990; 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.*, 1989; 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.*, 1989; Perren *et al.*, 1989; MacLennan & Martin, 1990). In the dog this increase 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, 1990); 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, 1985), BEA 1654 (Verdouw *et al.*, 1985), 8-hydroxy-2-(di-n-propylamino) tetralin (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 whether indeed sumatriptan acted via a 5-$HT_1$-like receptor. 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; $P_{CO_2}$: 35–48 mmHg; $P_{O_2}$: 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 × DAP)/3. The common carotid arteries were dissected free and the cervical vagosympathetic trunks were cut. Blood flow was measured in one of

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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 programmes (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 1 h 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 μg $kg^{-1}$, 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 jugular venous blood gases were made.

### *Data presentation and statistical analysis*

All data have been expressed as means ± s.e.mean. 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 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; Glaxo Research Laboratories, Ware), 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%).

### *Changes in the systemic haemodynamics by sumatriptan*

The effects of i.v. infusions of sumatriptan on heart rate and blood pressure after pretreatment 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.

**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 ± s.e.mean. * $P < 0.05$ 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 $mg\,kg^{-1}$)

| | | *Sumatriptan* ($\mu g\,kg^{-1}$) | | | |
|---|---|---|---|---|---|
| *Pretreatment* | *Baseline* | 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 ± s.e.mean.
* $P < 0.05$ vs. baseline.
† $P < 0.05$ vs. the corresponding dose in saline pretreated animals.

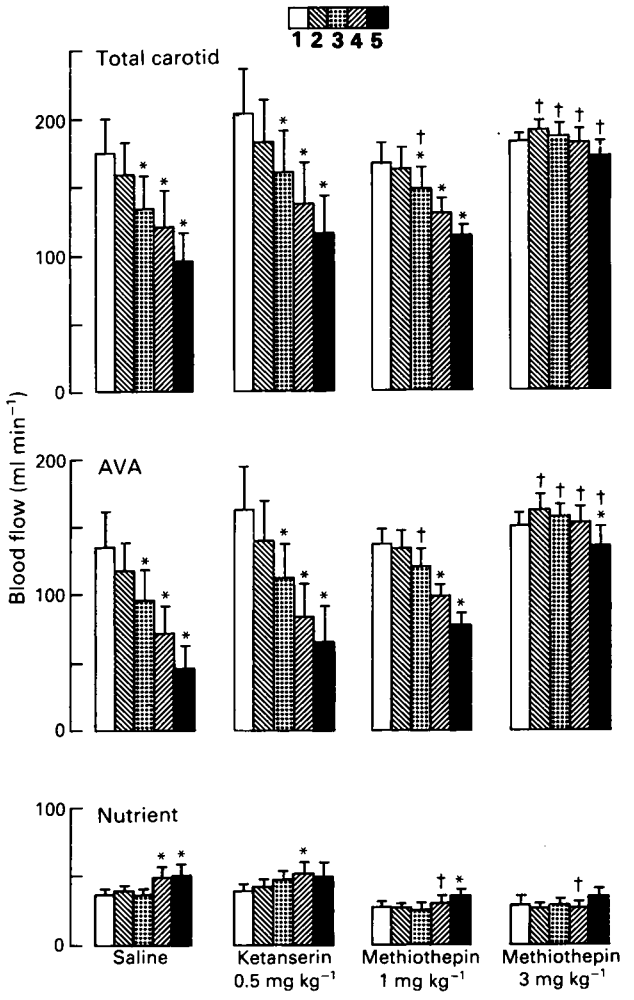


**Figure 1** Effect of sumatriptan i.v. on the total carotid blood flow and its arteriovenous anastomotic (AVA) and nutrient (tissue) fractions (ml $min^{-1}$) in pigs, pretreated i.v. with saline ($n = 8$), ketanserin 0.5 $mg\,kg^{-1}$ ($n = 6$), methiothepin 1 $mg\,kg^{-1}$ ($n = 7$) or methiothepin 3 $mg\,kg^{-1}$ ($n = 6$). (1) Before sumatriptan (after pretreatment); (2) sumatriptan 10 $\mu g\,kg^{-1}$; (3) sumatriptan 30 $\mu g\,kg^{-1}$; (4) sumatriptan 100 $\mu g\,kg^{-1}$ and (5) sumatriptan 300 $\mu g\,kg^{-1}$. All values have been presented as means with s.e.mean shown by vertical bars. * $P < 0.05$ vs baseline; † $P < 0.05$ vs the corresponding dose of sumatriptan in the saline pretreated animals.

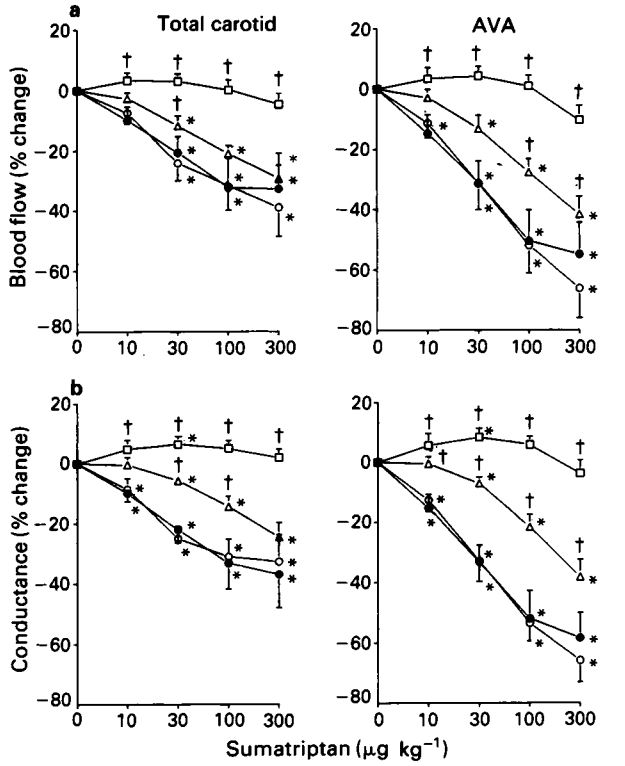


**Figure 2** Effect (% change from baseline) of sumatriptan i.v. on the total carotid and arteriovenous anastomotic (AVA) blood flow (a) and conductance (b) in pigs, pretreated i.v. with either saline (○, $n = 8$), ketanserin 0.5 $mg\,kg^{-1}$ (●, $n = 6$), methiothepin 1 $mg\,kg^{-1}$ (△, $n = 7$) or methiothepin 3 $mg\,kg^{-}$ (□, $n = 6$). All values have been presented as means with s.e.mean shown by vertical bars. * $P < 0.05$ vs baseline. † $P < 0.05$ vs the corresponding dose of sumatriptan in the saline pretreated animals.

## *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 (non-nutrient) and arteriolar (nutrient) blood flow are shown in Figure 1 (absolute values) and Figure 2a (% 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 ± 10%. Total carotid blood flow had decreased by only 39 ± 9%, because nutrient (tissue) blood flow had increased by 36 ± 18%. 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 $\mu g\,kg^{-1}$ 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. As depicted in Figure 2b, ketanserin did not modify, but methiothepin antagonized the sumatriptan-induced increases in both total carotid and arteriovenous anastomotic conductance.

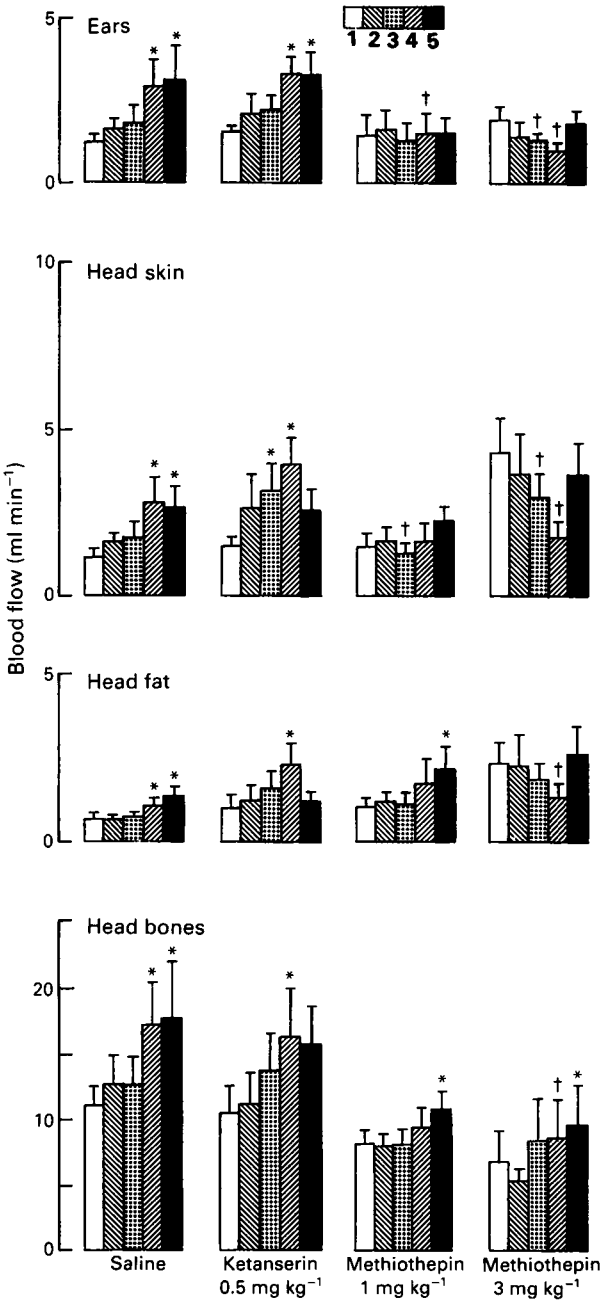


**Figure 3** Effect of sumatriptan i.v. on the regional carotid blood flow distributed to the ears, skin, fat and bones (ml $min^{-1}$) in pigs, pretreated i.v. with either saline ($n = 8$), ketanserin 0.5 mg $kg^{-1}$ ($n = 6$), methiothepin 1 mg $kg^{-1}$ ($n = 7$) or methiothepin 3 mg $kg^{-1}$ ($n = 6$). (1) Before sumatriptan (after pretreatment); (2) sumatriptan 10 $\mu$g $kg^{-1}$; (3) sumatriptan 30 $\mu$g $kg^{-1}$; (4) sumatriptan 100 $\mu$g $kg^{-1}$ and (5) sumatriptan 300 $\mu$g $kg^{-1}$. All values have been presented as means with s.e.mean shown by vertical bars. * $P < 0.05$ vs baseline; † $P < 0.05$ vs the corresponding dose of sumatriptan in the saline pretreated animals.

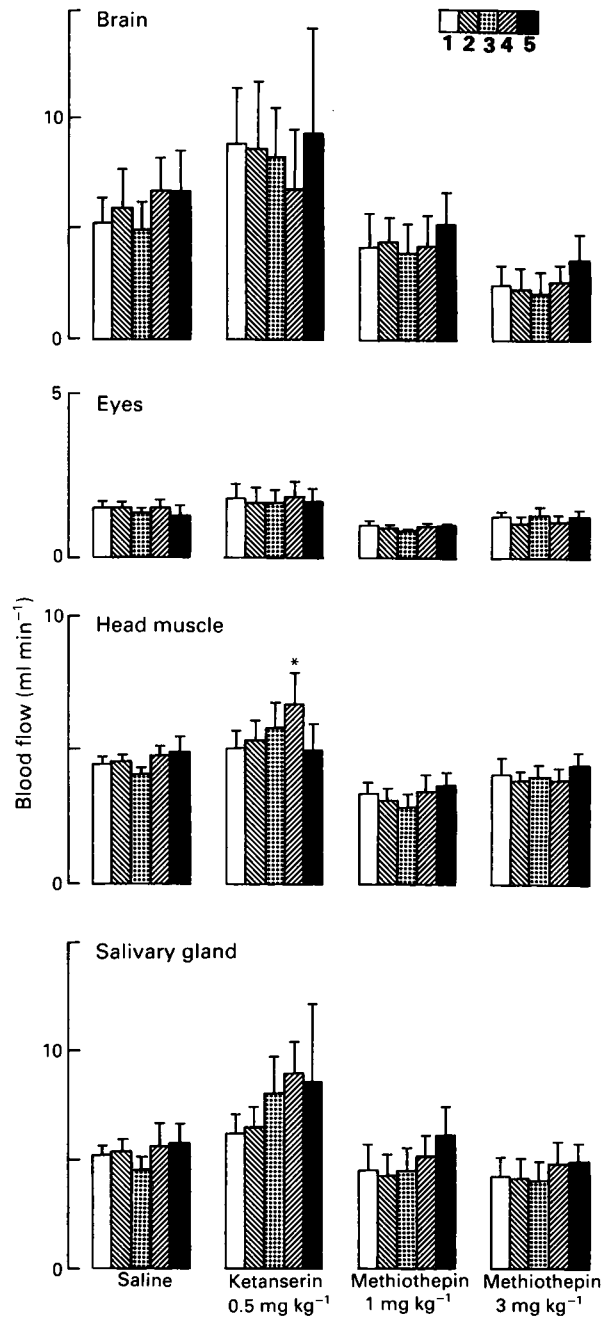


**Figure 4** Effect of sumatriptan i.v. on the regional carotid blood flow distributed to the brain, eyes, head muscle and salivary gland (ml $min^{-1}$) in pigs, pretreated i.v. with either saline ($n = 8$), ketanserin 0.5 mg $kg^{-1}$ ($n = 6$), methiothepin 1 mg $kg^{-1}$ ($n = 7$) or methiothepin 3 mg $kg^{-1}$ ($n = 6$). (1) Before sumatriptan (after pretreatment), (2) sumatriptan 10 mg $kg^{-1}$; (3) sumatriptan 30 $\mu$g $kg^{-1}$; (4) sumatriptan 100 $\mu$g $kg^{-1}$ and (5) sumatriptan 300 $\mu$g $kg^{-1}$. All values have been presented as means with s.e.mean shown by vertical bars. * $P < 0.05$ vs baseline. No significant differences between the corresponding doses of saline and (2) the drug were found.

The effects of sumatriptan on the distribution of arteriolar, nutrient blood flow to the different tissues are shown in Figures 3 and 4. 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.

### *Changes in 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 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 $mg\,kg^{-1}$)

| | | *Sumatriptan* ($\mu g\,kg^{-1}$) | | | |
|---|---|---|---|---|---|
| *Pretreatment* | *Baseline* | 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 ± s.e.mean. * $P < 0.05$ vs baseline.
No significant differences between the corresponding doses of saline and the drug were found.

## 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.*, 1989). 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 $\alpha_1$-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. However, it should 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 (Perrin *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.*, 1989).

### *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, 1990). 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 to 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; for a full inhibition of the response of sumatriptan a dose of 3 $mg\,kg^{-1}$ of methiothepin was needed. A possible reason for 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.*, 1984; 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. In view of vagosymapthectomy carried out in the present experiments, this vasodilatation is unlikely to be due to inhibition of noradrenaline release from sympathetic neurones by sumatriptan (Humphrey *et al.*, 1988; 1990). It is possibly a consequence of the decrease in arteriovenous anastomotic flow with resultant diversion of blood flow to the tissues. However, a small effect of sumatriptan via the vascular 5-$HT_1$-like receptors mediating vasodilatation cannot be ruled out (Bradley *et al.*, 1986; Saxena & Villalón, 1990). Previous experiments had shown that the reduction of arteriovenous anastomotic blood flow, as for example in case of indorenate, is not necessarily associated with an increase in the nutrient fraction of the carotid blood flow (Villalón *et al.*, 1990) and sumatriptan can elicit endothelium-dependent relaxation in the pig coronary artery (Schoeffter & Hoyer, 1989a). 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, 1985), both of which induce massive vasodilatation 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 receptors 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 shunting by RU 24969, a 5-$HT_{1A}$ and 5-$HT_{1B}$ receptor agonist, is not amenable to blockade by (±)-pindolol (Bom et al., 1989c), 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 the very high potency of 5-carboxamidotryptamine in the present experimental model (Saxena & Verdouw, 1985).

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 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, 1990) 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 has been shown to antagonize a putative 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 brain subtypes characterized so far. Rather the receptor would seem to be the same as that characterized in the dog saphenous vein and intracerebral vessels in a variety of species (Perren et al., 1991).

### *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 proved little more effective against sumatriptan in the present study than it did against ergotamine earlier (Bom et al., 1989a). In view of these new data with sumatriptan, the question of involvement of the 5-$HT_1$-like receptor 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 of 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 dilatation 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 those employed in patients against migraine attacks or those required to cause a substantial decrease in arteriovenous anastomotic blood flow. Furthermore, it is possible 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; Buzzi & Moskowitz, 1990) does not necessarily 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.

In conclusion this study shows that sumatriptan reduces porcine carotid blood flow by selectively affecting its arteriovenous anastomotic fraction and that these effects are mediated by a 5-$HT_1$-like receptor. The reduction in arteriovenous anastomotic blood flow may play a role in its antimigraine efficacy. Alternatively, since the cerebral anatomy in man is somewhat different from that in domestic animals, this action may reflect a similar selective cranial vasoconstrictor action in man on the particular vessels, yet to be identified, thought to be distended and inflamed during a migraine attack. These vessels may be the meningeal vessels and evidence has already been provided that sumatriptan will constrict the human dural vasculature (Humphrey et al., 1989b), where arteriovenous anastomoses have been demonstrated (Rowbotham & Little, 1965; Kerber & Newton, 1973).

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