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Blockade of porcine carotid vascular responses to sumatriptan by GR127935, a selective 5-HT_{1D} receptor antagonist

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1 It has previously been shown that the antimigraine drug, sumatriptan, a putative 5-HT_{1D} receptor agonist, decreases porcine common carotid and arteriovenous anastomotic blood flows, but slightly increases the arteriolar (capillary) blood flow to the skin and ears. Interestingly, such responses, being mediated by 5-HT_1-like receptors, are resistant to blockade by metergoline, which, in addition to displaying a very high affinity for (and occasionally intrinsic efficacy at) the 5-HT_{1D} receptor subtypes, blocks (with lower potency than methiothepin) some 5-HT_{1D} receptor-mediated vascular responses. These findings raise doubts whether sumatriptan-sensitive 5-HT_1-like receptors mediating changes in the distribution of porcine carotid blood flow are identical to cloned 5-HT_{1D} receptors. With the recent advent of the potent and selective 5-HT_{1D} receptor antagonist, GR127935, we have examined in the present study whether the carotid vascular effects of sumatriptan in the pig are amenable to blockade by GR127935.

2 In animals pretreated with saline, sumatriptan (30, 100 and 300 $\mu g\ kg^{-1}$, i.v.) reduced the total carotid and arteriovenous anastomotic blood flows in a dose dependent manner. In contrast, sumatriptan increased blood flow to the skin, ears and fat, although the total capillary fraction was not significantly affected.

3 While GR127935 pretreatment (0.25 and 0.5 $mg\ kg^{-1}$) itself slightly reduced the total carotid and arteriovenous anastomotic blood flows, carotid vasoconstrictor responses to sumatriptan were either partly (0.25 $mg\ kg^{-1}$) or completely (0.5 $mg\ kg^{-1}$) blocked by the compound. In GR127935 pretreated animals, the sumatriptan-induced increases in blood flow to the skin, ears and fat were also attenuated.

4 Taken together, the results suggest that arteriovenous anastomotic constriction and, possibly, arteriolar dilatation in the skin, ears and fat by sumatriptan are mediated by 5-HT_{1D} receptors. Therefore, vascular 5-HT_1-like receptors in the porcine carotid bed appear to be identical to 5-HT_{1D} receptors.

Keywords: Antimigraine drugs; arteriovenous anastomoses; carotid artery; GR127935; migraine; pig carotid vascular responses; sumatriptan

Introduction

Sumatriptan is a 5-HT_1-like receptor agonist (Humphrey *et al.*, 1988, 1990; Hoyer *et al.*, 1994) effective in the acute treatment of migraine headaches (The Subcutaneous Sumatriptan International Study Group, 1991; Ferrari & Saxena, 1993). Several studies have shown that the drug produces constriction of large cerebral and extracerebral blood vessels (e.g. Feniuk *et al.*, 1989; Caekebeke *et al.*, 1992; Villalón *et al.*, 1995), including porcine carotid arteriovenous anastomoses (Den Boer *et al.*, 1991b; 1992), as shown for the antimigraine drugs, ergotamine and dihydroergotamine (Den Boer *et al.*, 1991a; Villalón *et al.*, 1992). The constriction of porcine arteriovenous anastomoses by sumatriptan and, partly, by the ergot alkaloids is mediated via the 5-HT_1-like receptor because these effects are antagonized, either partially (ergot alkaloids) or fully (sumatriptan), by methiothepin, but not by ketanserin.

The 5-HT_1-like receptor mediating vasoconstriction has not yet been cloned and, therefore, its exact identity is in debate. In view of the high affinity of sumatriptan, but also of methiothepin, for 5-HT_{1D} receptors (Peroutka & McCarthy, 1989; Schoeffter & Hoyer, 1989; Beattie *et al.*, 1994), it is argued that sumatriptan-induced vasoconstriction is mediated by 5-HT_{1D} receptors (e.g. Hamel & Bouchard, 1991), implying that 5-HT_1-like and 5-HT_{1D} receptors are identical. In contrast, the IUPHAR 5-hydroxytryptamine (5-HT) receptor classification scheme (Hoyer *et al.*, 1994) recognizes the 5-HT_1-like receptor as a distinct entity, separate from any of the 5-HT_1 receptor subtypes, including 5-HT_{1D} receptors, identified by radioligand binding and cloning techniques. One of the main reasons for this distinction is that metergoline, which has an even higher affinity than methiothepin for 5-HT_{1D} receptors (Schoeffter *et al.*, 1988; Waeber *et al.*, 1988), does not or only weakly antagonizes sumatriptan-induced vasoconstrictor responses, both *in vivo* (Perren *et al.*, 1991; Den Boer *et al.*, 1992; Villalón *et al.*, 1990, 1995) and *in vitro* (Hamel & Bouchard, 1991; Perren *et al.*, 1991; Bax *et al.*, 1992; Deckert *et al.*, 1994).

Recently, a series of piperazinylbenzanilide derivatives with high affinity for and antagonist activity at 5-HT_{1D} receptors has been described (Clitherow *et al.*, 1994). One such derivative, GR127935, potently inhibited contralateral turning induced by unilateral infusion of the 5-HT_1 receptor agonist, GR56764 into the guinea-pig substantia nigra as well as sumatriptan-evoked inhibition of 5-HT release in the guinea-pig dorsal raphe nucleus (Clitherow *et al.*, 1994; Starkey & Skingle, 1994) and canine basilar artery contraction (Skingle *et al.*, 1993). The present study was designed to analyze whether the 5-HT_1-like receptor mediating changes in the distribution of common carotid artery blood flow by sumatriptan in the pig are amenable to blockade by GR127935. Preliminary results of this investigation have been communicated to the Joint Meeting of German and Dutch Pharmacological Societies (De Vries *et al.*, 1995).

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Methods

General

After an overnight fast, 14 domestic pigs (Yorkshire x Landrace; 10–15 kg) were anaesthetized with azaperone (160 mg, i.m.), midazolan hydrochloride (5 mg, i.m.) and metomidate (200 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; P_{CO_2}: 35–48 mmHg; P_{O_2}: 100–120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of pentobarbitone sodium at 20 mg kg^{-1} h^{-1}. With this anaesthetic regimen, arteriovenous anastomotic blood flow is considerably higher than that in pigs in a conscious state or under thiopentone anaesthesia (Den Boer *et al.*, 1993).

Catheters were placed in the inferior vena cava via the left femoral vein for the administration of drugs and in the aortic arch via the left femoral artery for the measurement of arterial blood pressure (P23 Dc pressure transducer; Statham, Hato Rey, Puerto Rico) and the withdrawal of arterial blood for determining blood gases (ABL-510, Radiometer, Copenhagen, Denmark).

The common carotid arteries, external jugular veins and vagus nerves were identified. After ligation, both vagi and the accompanying cervical sympathetic nerves were cut and a catheter was placed in the right external jugular vein for the withdrawal of venous blood samples. The right common carotid artery was dissected free and a needle was inserted against the direction of blood flow for the administration and uniform mixing of radioactive microspheres. Blood flow was measured in the right common carotid artery with a flow probe (internal diameter: 2.5 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands). Heart rate was measured with a tachograph (7P4 Grass Instrument Company, Quincy, Mass, U.S.A.) triggered by ECG signals.

Arterial blood pressure, heart rate and carotid blood flow were continuously monitored on a model 7 Grass polygraph. 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 carotid blood flow

The distribution of common carotid blood flow was determined with 15 ± 1 (s.d.) μm diameter microspheres labelled with ^{141}Ce, ^{113}Sn, ^{95}Nb, ^{103}Ru or ^{46}Sc (NEN Dupont, Boston, USA). For each measurement a suspension of about 200,000 microspheres labelled with one of the isotopes, was mixed and injected into the carotid artery. At the end of the experiment, the animal was killed 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. All data were processed by a set of specially designed programmes (Saxena *et al.*, 1980), on a personal computer.

The fraction of carotid blood flow distributed to the different tissues was calculated by multiplying the ratio of tissue and total radioactivities by the total common carotid blood flow at the time of the injection of microspheres. Since little or no radioactivity was detected in the heart and kidneys, all microspheres trapped in the lungs reached this tissue from the venous side after escaping via carotid arteriovenous anastomoses. Therefore, the amount of radioactivity in the lungs was used as an index of the arteriovenous anastomotic fraction of carotid blood flow (Saxena & Verdouw, 1982).

Experimental protocol

The experiments were started after a stabilization period of about 1 h. At baseline, heart rate, mean arterial blood pressure, carotid blood flow and its distribution as well as arterial and jugular venous blood gases were measured. Thereafter, the animals were divided into three groups which received i.v. infusions of either saline (5 ml; $n=5$), GR127935 (0.25 mg kg^{-1}; $n=4$) or GR127935 (0.5 mg kg^{-1}; $n=5$) over a period of 4–5 min. All variables were reassessed about 10 min after the end of the infusion. Subsequently, all three groups of animals received cumulative i.v. doses of sumatriptan (30, 100 and 300 μg kg^{-1}) every 20 min. Fifteen minutes after each dose of sumatriptan the haemodynamic variables were again assessed.

Data presentation and statistical analysis

All data have been expressed as means $\pm$ s.e.mean. The significance of the changes induced by pretreatment with saline or GR127935 was evaluated by the use of paired t test, while that of the change induced by the different doses of sumatriptan 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. The changes (from baseline values) caused by sumatriptan (30, 100 or 300 μg kg^{-1}) in the two groups of animals pretreated with GR127935 (0.25 or 0.5 mg kg^{-1}) were compared with those in the saline pretreated group at the same dose of sumatriptan by Student's t test. Statistical significance was accepted at $P<0.05$ (two-tailed).

Drugs

Apart from the anaesthetics, azaperone, metomidate (both from Janssen Pharmaceutica, Beerse, Belgium), midazolan hydrochloride (Hoffmann La Roche b.v., Mijdrecht, The Netherlands) and pentobarbitone sodium (Apharmo, Arnhem, The Netherlands), the drugs used in this study were: sumatriptan succinate and GR127935 (N-[methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1 2,4-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide hydrochloride; Glaxo Group Research, Ware, U.K.) and heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) to prevent clotting of the catheters. GR127935 was solubilized according to the instructions of the supplier by heating the dispersion in distilled water to about 70°C for 10 s and then allowing to cool down to room temperature. Sumatriptan was dissolved in physiological saline. All doses refer to the respective salts.

Results

Effects of GR127935

The changes in systemic and carotid haemodynamics and in arterio-jugular venous oxygen saturation difference elicited by saline and GR127935 are shown in Table 1. Pretreatment with saline did not affect any of the variables measured. At the highest dose (0.5 mg kg^{-1}), GR127935 slightly, but significantly, reduced heart rate (4 ± 1%) and mean arterial blood pressure (10 ± 2%). While capillary blood flow did not change, both doses (0.25 and 0.5 mg kg^{-1}) of GR127935 decreased total carotid blood flow (13 ± 1% and 20 ± 3%, respectively) by a selective action on its arteriovenous anastomotic fraction, which was decreased by 22 ± 5% and 30 ± 6%, respectively. The decreases in arteriovenous anastomotic blood flow were accompanied by increases in the corresponding resistance. In keeping with these findings, the difference in arterial and jugular venous oxygen saturation was significantly increased by the highest dose of GR127935. However, it has to be remarked that the baseline value in this group was lower than in the other two groups (Table 1).

Effect of sumatriptan in saline and GR127935 pretreated groups

Systemic haemodynamics Bolus injections of sumatriptan (30–300 $\mu g\ kg^{-1}$, i.v.) elicited a slight, but significant, decrease in heart rate in both saline and GR127935 pretreated animals. Mean arterial blood pressure was not changed by sumatriptan, except in the animals pretreated with 0.25 $mg\ kg^{-1}$ GR127935 where the highest dose of sumatriptan decreased arterial pressure by 13±1% (Table 2).

Arterio-jugular venous oxygen saturation difference In the saline pretreated animals, sumatriptan (100 and 300 $\mu g\ kg^{-1}$, i.v.) increased the aterio-jugular venous oxygen saturation difference by 147±63% and 229±96%, respectively. Table 2 shows that in animals pretreated with GR127935 this effect was either markedly reduced (0.25 $mg\ kg^{-1}$) or completely blocked (0.5 $mg\ kg^{-1}$).

Carotid haemodynamics As shown in Figures 1 and 2, sumatriptan (30, 100 or 300 $\mu g\ kg^{-1}$, i.v.) elicited a dose-dependent decrease in both the total carotid and arteriovenous anastomotic blood flows, but the total capillary fraction was not significantly increased. The decreases in total carotid and arteriovenous anastomotic blood flows by sumatriptan (maximal decrease 52±6% and 76±4%, respectively) were attenuated by 0.25 $mg\ kg^{-1}$ of GR127935 (maximal decrease 15±2% and 27±4%, respectively) or abolished by 0.5 $mg\ kg^{-1}$ of GR127935 (maximal decrease 9±10% and 14±12%, respectively).

The distribution of carotid blood flow to the head tissues in the three groups of animals is depicted in Figure 3. Sumatriptan did not significantly modify the fraction of carotid blood flow distributed to the brian, eyes, muscles, bones and salivary glands; similar results were observed in the tongue and dura mater (data not shown). In contrast, sumatriptan markedly increased blood flow to the skin (maximum increase 166±99%) and ears (maximum increase 234±111%) and, slightly, to the fat (maximum increase 96±42%); these effects of sumatriptan were attenuated in animals pretreated with GR127935 (Figures 3 and 4).

Discussion

General

The mechanisms involved in vascular constriction and blood flow reduction by 5-HT are complex and can be mediated by 5-HT_1-like and/or 5-HT_2 receptors depending on, amongst other factors, the species, the blood vessel under study and the degree of sympathetic vascular tone (Saxena & Villalón, 1990; 1991). Unlike 5-HT, sumatriptan, which has a negligible affinity for 5-HT_2 receptors (Humphrey *et al.*, 1988; 1990; Peroutka & McCarthy, 1989), reduces porcine common carotid and arteriovenous anastomotic blood flows exclusively by 5-HT_1-like receptors (Den Boer *et al.*, 1991b).

Previous findings obtained by the use of several 5-HT receptor agonists and antagonists suggested that 5-HT_1-like receptors were unrelated to the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} (now 5-HT_{2C}) and 5-HT_{1D} subtypes (for references, see Saxena & Villalón, 1990). In agreement with this view, Den Boer *et al.* (1992) contended that the sumatriptan-sensitive 5-HT_1-like receptor mediating constriction of porcine arteriovenous anastomoses was apparently unrelated to the 5-HT_{1D} subtype,

Table 1 Changes in heart rate, mean arterial blood pressure, difference in arterial and jugular venous oxygen saturation (A-V SO_2), total carotid blood flow, arteriovenous anastomotic (AVA) blood flow, AVA resistance and capillary blood flow caused by either saline (n = 5) or GR127935 (0.25 and 0.5 $mg\ kg^{-1}$; n = 4 and n = 5, respectively)

	Saline		*GR127935* (0.25 $mg\ kg^{-1}$)		*GR127935* (0.5 $mg\ kg^{-1}$)	
	Before	*After*	*Before*	*After*	*Before*	*After*
Heart rate (beats min^{-1})	99±5	97±4	96±5	96±5	93±2	89±3[a]
Mean arterial blood pressure (mmHg)	105±3	102±4	97±1	99±5	103±5	93±4[a]
A-V SO_2 (%)	8.9±3.2	8.3±3.1	8.2±3.4	9.2±3.2	5.2±1.9	9.6±3.8[a]
Total carotid blood flow (ml min^{-1})	132±11	133±14	121±2	106±3[a]	125±13	101±11[a]
AVA blood flow (ml min^{-1})	107±12	106±16	91±6	71±7[a]	98±11	69±8[a]
AVA resistance (mmHg min ml^{-1})	1.0±0.1	1.0±0.1	1.1±0.1	1.4±0.1[a]	1.1±0.1	1.4±0.1[a]
Capillary blood flow (ml min^{-1})	24±4	27±4	30±4	35±4	27±3	32±5

All values have been presented as means±s.e.mean; [a]$P<0.05$ vs baseline.

Table 2 Changes in heart rate, mean arterial blood pressure and difference in arterial and jugular venous oxygen saturation caused by cumulative doses of sumatriptan in animals pretreated with either saline (n = 5) or GR127935 (0.25 and 0.5 $mg\ kg^{-1}$; n = 4 and n = 5 respectively)

		Sumatriptan ($\mu g\ kg^{-1}$)		
Pretreatment	*Baseline*	30	100	300
Heart rate (beats min^{-1})				
Saline	97±4	94±4	94±4[a]	92±5[a]
GR127935 (0.25 $mg\ kg^{-1}$)	96±5	94±5	93±5[a]	92±5[a]
GR127935 (0.5 $mg\ kg^{-1}$)	89±3	88±3[a]	86±3[a]	85±3[a]
Mean arterial blood pressure (mmHg)				
Saline	102±4	103±6	103±6	96±6
GR127935 (0.25 $mg\ kg^{-1}$)	99±5	97±5	93±6	86±4[a]
GR127935 (0.5 $mg\ kg^{-1}$)	93±4	91±4	91±3	87±3
Arteriovenous difference in oxygen saturation (%)				
Saline	8,3±3.1	10.7±3.2	15.9±3.2[a]	20.1±3.7[a]
GR127935 (0.25 $mg\ kg^{-1}$)	9.2±3.2	9.8±2.1	9.6±3.2	12.5±2.4[a]
GR127935 (0.5 $mg\ kg^{-1}$)	9.6±3.8	9.2±3.6	11.6±4.4	12.8±5.2

All values have been presented as means±s.e.mean, [a]$P<0.5$ vs baseline.

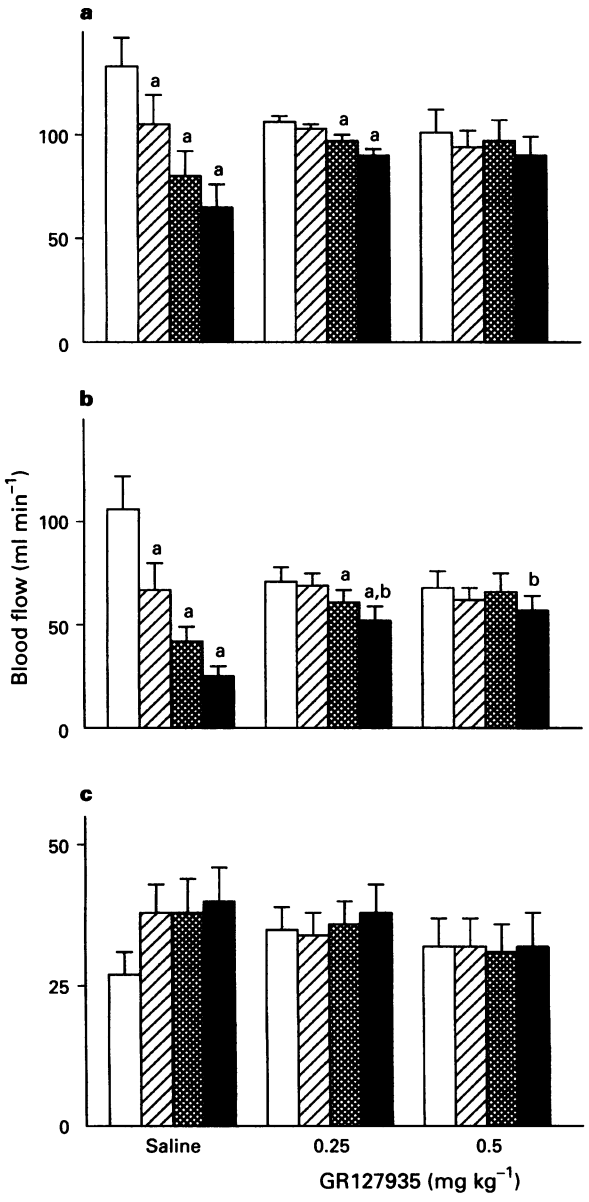

Figure 1 Effect of sumatriptan on the total carotid blood flow (a) and its arteriovenous anastomotic (AVA) (b) and nutrient (capillary) (c) fractions in pigs, pretreated with either saline ($n=5$) or GR127935 (0.25 and 0.5 mg kg^{-1}; $n=4$ and $n=5$, respectively). From left to right the columns signify values before sumatriptan (after pretreatment with saline or GR127935) and after sumatriptan (30, 100 and 300 μg kg^{-1}). All values are presented as means ± s.e.mean. $^{a}P<0.05$ vs baseline; $^{b}P<0.05$ vs the corresponding dose in the saline pretreated animals.

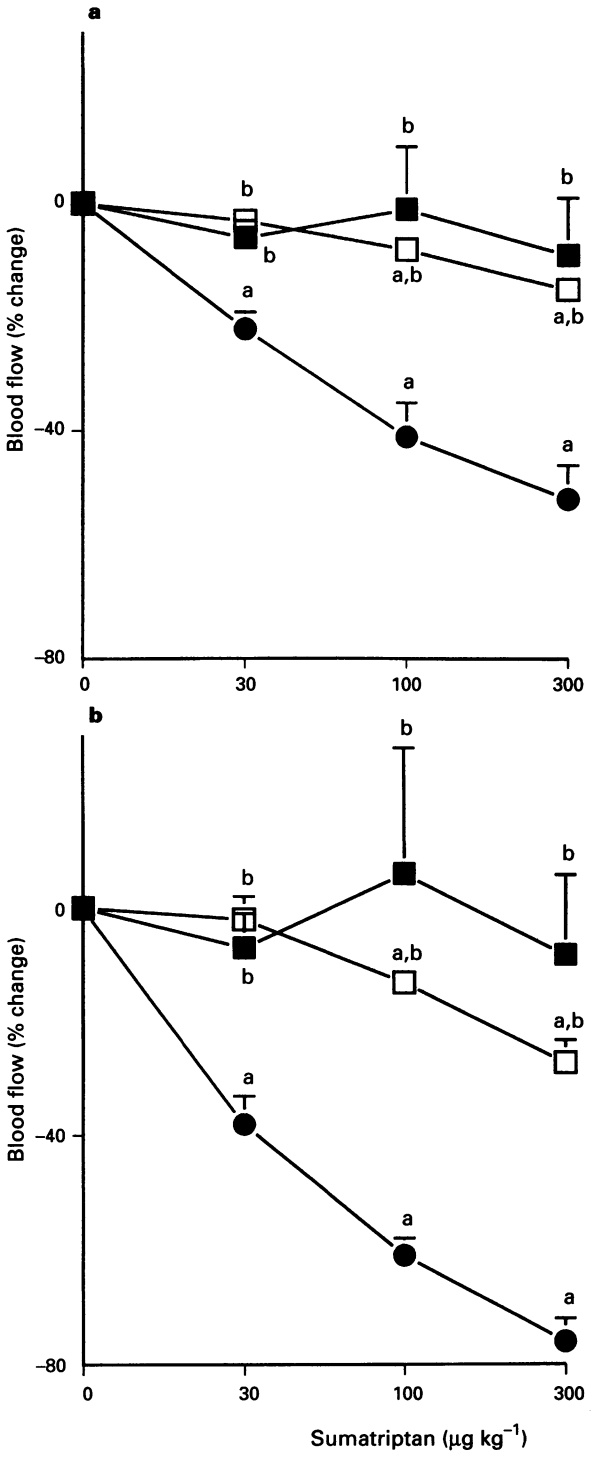

Figure 2 Percentage changes from baseline values by sumatriptan in the total carotid (a) and arteriovenous anastomotic blood flow (b) in pigs, pretreated with either saline (●; $n=5$) or GR127935 (0.25 mg kg^{-1}, □, $n=4$ and 0.5 mg kg^{-1}, ■ $n=5$). All values are presented as means ± s.e.mean. $^{a}P<0.05$ vs baseline; $^{b}P<0.05$ vs the corresponding dose in saline pretreated animals.

mainly because of the resistance to antagonism by metergoline, which displays the highest affinity for 5-HT$_{1D}$ receptors (Waeber *et al.*, 1988). Although metergoline is capable of antagonizing some 5-HT$_{1D}$ receptor-mediated vascular responses (e.g. Schoeffter & Hoyer, 1990; Hamel & Bouchard, 1991; Bax *et al.*, 1992; Jansen *et al.*, 1993; Deckert *et al.*, 1994; Villalón & Terrón, 1994), it is important to note that in these studies, the antagonist potency of metergoline did not correlate with its affinity at 5-HT$_{1D}$ receptors (Waeber *et al.*, 1988) and, in some cases, metergoline showed a non-competitive antagonism (e.g. Hamel & Bouchard, 1991) or even intrinsic efficacy at 5-HT$_{1D}$ receptors (Schoeffter *et al.*, 1988; Miller *et al.*, 1992). The advent of GR127935, which is a potent and selective ligand for 5-HT$_{1D}$ receptors and antagonizes a number of responses elicited by 5-HT$_{1D}$ receptor agonists (Clitherow *et al.*, 1995), offers us the possibility of investigating its effect on sumatriptan-induced changes. Indeed, in addition to the implications discussed below, our results show that GR127935 decreased the arteriovenous anastomotic blood flow by itself and antagonized sumatriptan-induced changes in porcine carotid haemodynamics, implying a common site of action. The use of metergoline and/or methiothepin against the agonist effects of GR127935 may provide further insight into the nature of receptors involved.

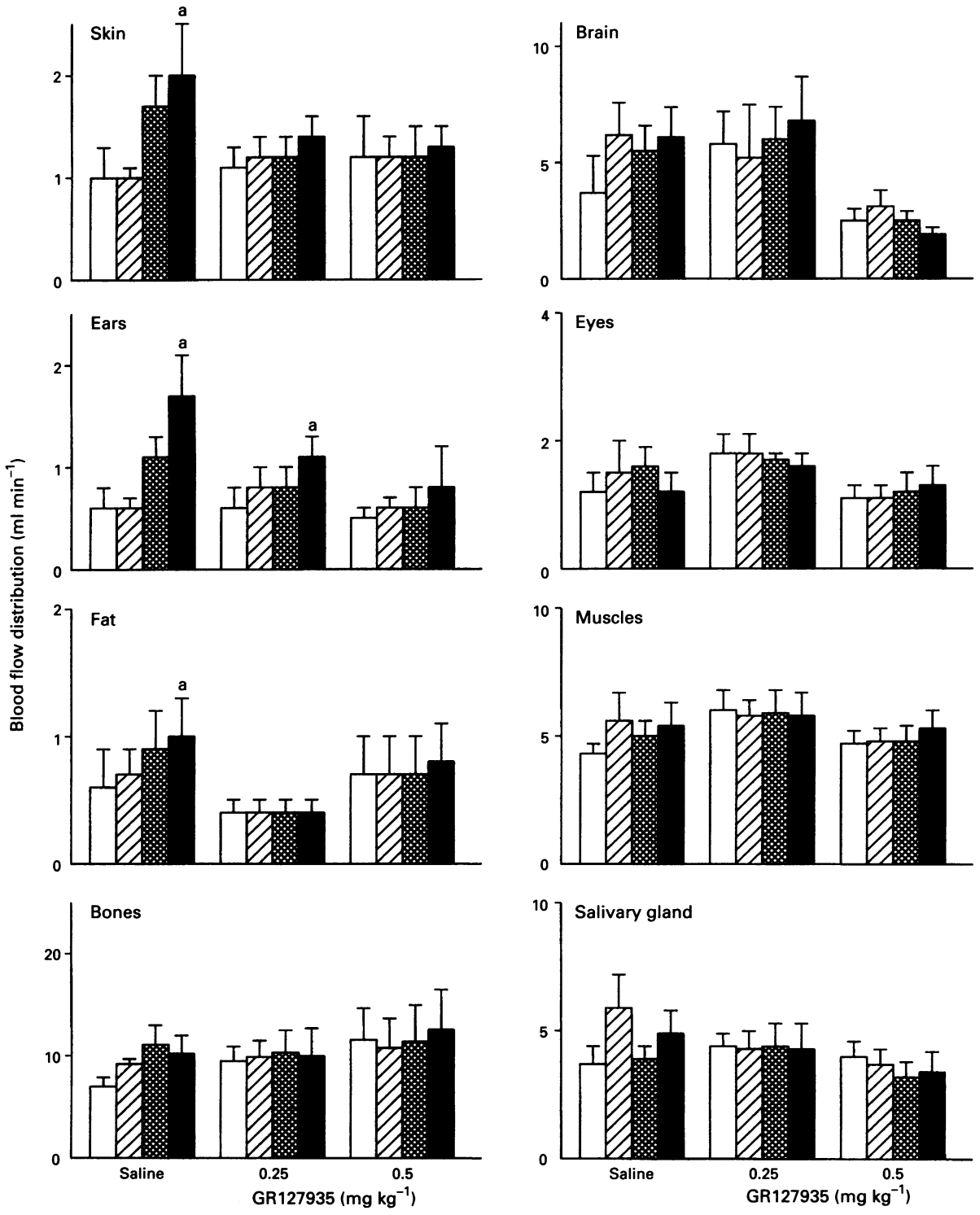

Figure 3 Effect of sumatriptan on the distribution of carotid blood flow to different cranial tissues in pigs, pretreated with either saline ($n=5$) or GR127935 (0.25 and 0.5 mg kg^{-1}; $n=4$ and $n=5$, respectively). From left to right the columns signify values before sumatriptan (after pretreatment with saline or GR127935) and after sumatriptan (30, 100 and 300 μg kg^{-1}). All values are presented as means ± s.e.mean. $^{a}P<0.05$ vs baseline.

Effect of GR127935 on systemic and carotid haemodynamics

GR127935 elicited a slight reduction in heart rate and mean arterial blood pressure, but we have no clear-cut explanation for it. The compound also decreased by itself the total carotid blood flow and, as described with sumatriptan (see below), this decrease was exclusively confined to the arteriovenous anastomotic fraction. Since these effects were observed with a concomitant increase in the arteriovenous anastomotic resistance (Table 1), GR127935 seems to act as an agonist at the receptors mediating contraction of arteriovenous anatomoses. Consistent with this effect, recent studies in cells with human cloned receptors showed that GR127935 can behave as an agonist at 5-HT$_{1D}$ receptor subtypes (Pauwels & Colpaert, 1995; Watson *et al.*, 1995).

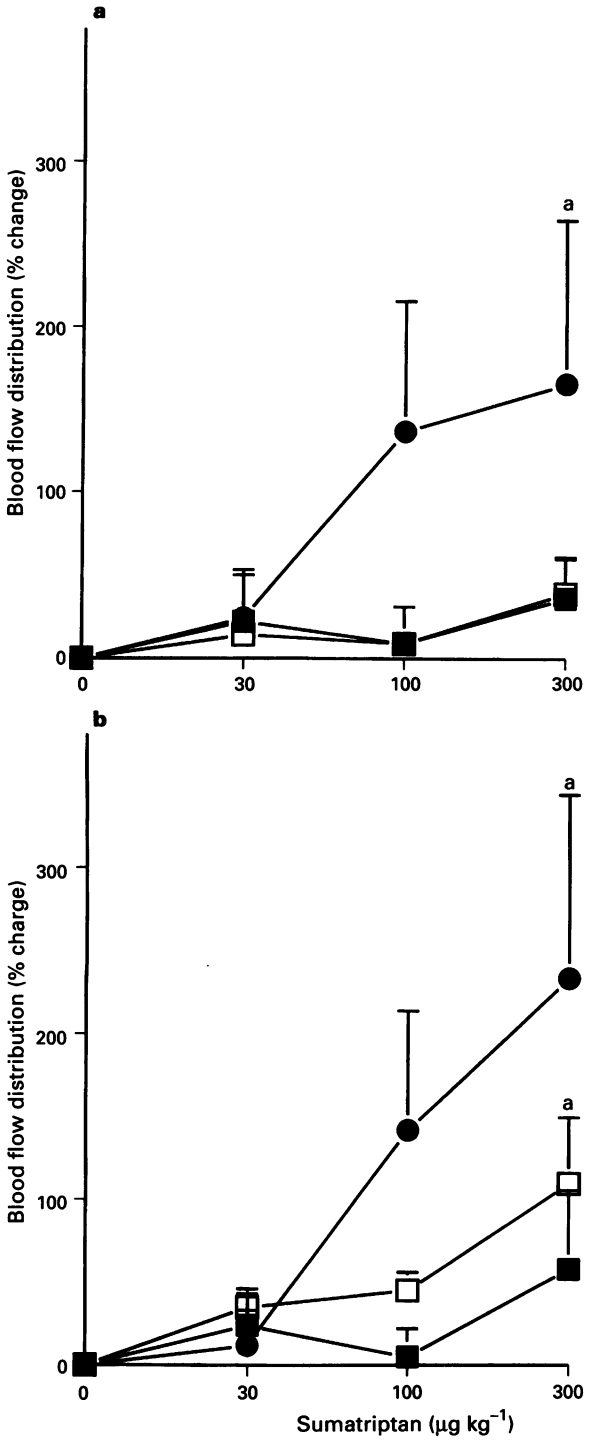

Figure 4 Percentage changes from baseline values by sumatriptan in the skin (a) and ear (b) blood flow in pigs, pretreated with either saline (●; $n=5$) or GR127935 (0.25 mg kg^{-1}, □, $n=4$ and 0.5 mg kg^{-1}, ■, $n=5$). All values are presented as means ± s.e.mean. $^{a}P<0.05$ vs baseline; $^{b}P<0.05$ vs the corresponding dose in saline pretreated animals.

Systemic haemodynamic changes after sumatriptan

As previously reported by other authors (Feniuk *et al.*, 1989; Den Boer *et al.*, 1991b, 1992), sumatriptan produced a slight, but significant, decrease in heart rate; this is likely to be an effect of the drug, since in similar experiments no changes in heart rate were observed after four consecutive bolus injections of saline (Den Boer *et al.*, 1991b). The mechanism invovled in the rather small decrease in heart rate by sumatriptan is not clear, but may be related to presynaptic inhibition of sympathetic neruones (Humphrey *et al.*, 1988; 1990) or central 5-HT_{1A} receptor activation (Dreteler *et al.*, 1989; Saxena & Villalón, 1990). However, on the other hand, the bradycardiac effects of sumatriptan were also observed in animals pretreated with GR127935 (Table 2) and sumatriptan, which has an appreciable affinity for the 5-HT_{1A} receptor (Peroutka & McCarthy, 1989; Beattie *et al.*, 1994), does not easily penetrate into the central nervous system (see Saxena & Tfelt-Hansen, 1993). In any case, bradycardia following the use of sumatriptan in patients seems to be of little clinical relevance (Saxena & Tfelt-Hansen, 1993).

Significantly, the fact that sumatriptan did not produce important changes in mean arterial blood pressure in the saline or GR127935 pretreated groups (Table 2) implies that the drug has a more selective vasoconstrictor action on cranial blood vessels than, for example, ergotamine, which elicits a hypertensive response (Den Boer *et al.*, 1991a).

Carotid haemodynamic changes after sumatriptan

Sumatriptan elicited a dose-dependent reduction in the total carotid blood flow, which was exclusively due to a decrease in its arteriovenous anastomotic fraction; consistent with this finding, sumatriptan also increased the arterio-jugular venous oxygen saturation difference. The reductions in the total and arteriovenous anastomotic blood flows as well as the accompanying increase in the arterio-jugular venous oxygen saturation difference by sumatriptan were reduced (0.25 mg kg^{-1}) or abolished (0.5 mg kg^{-1}) in animals pretreated with GR127935. Although it cannot be entirely ruled out that sumatriptan and GR127935 both act on an 'unknown' receptor, taking into account that both sumatriptan (Peroutka & McCarthy, 1989; Schoeffter & Hoyer, 1989; Beattie *et al.*, 1994) and GR127935 (Skingle *et al.*, 1993; Clitherow *et al.*, 1994; Pauwels & Colpaert, 1995; Watson *et al.*, 1995) have high affinities for 5-HT_{1D} receptors, our results suggest that the sumatriptan-induced vasoconstriction of carotid arteriovenous anastomoses is mediated by 5-HT_{1D} receptors, reinforcing the view that 5-HT_1-like and 5-HT_{1D} receptors may be identical. Nevertheless, it may be recalled that sumatriptan (pK_i: 7.63) and ergotamine (pK_i: 6.76) also display affinity for the cloned human 5-HT_{1F} receptor (Adham *et al.*, 1993). However, the involvement of 5-HT_{1F} receptor seems not very likely because (i) GR127935 has a substantially lower affinity for 5-HT_{1F} (pK_i: 7.1; H. Connor, personal communication) than for 5-$HT_{1D\alpha}$ (pK_i: 8.9) or 5-$HT_{1D\beta}$ (pK_i: 9.9) receptors (Skingle *et al.*, 1993), (ii) sumatriptan is several fold less potent than ergotamine (not more potent as might be expected from their affinities for the 5-HT_{1F} receptor) on porcine arteriovenous anastomoses (see Den Boer *et al.*, 1991a, b), and (iii) sumatriptan is more potent at the 5-$HT_{1D\alpha}$ (pK_i: 8.5) or 5-$HT_{1D\beta}$ (pK_i: 8.1) than at the 5-HT_{1F} (pK_i: 7.6) receptor (Beattie *et al.*, 1994).

As reported earlier from our laboratory (Den Boer *et al.*, 1991b), sumatriptan conspicuously increased blood flows to the skin and ears, without any alteration in the total capillary blood flow. Although, the increase in the skin and ear blood flow was largely attenuated in animals pretreated with GR127935, it can be argued that the dilatation of the skin and ear arterioles is an indirect consequence of the closure of arteriovenous anastomoses by sumatriptan. On the other hand, Schoeffter & Hoyer (1990) have reported 5-HT receptors similar to the 5-HT_{1D} receptor subtype mediate endothelium-dependent relaxations of porcine isolated coronary artery.

Nature of 5-HT_{1D} receptors mediating constriction of porcine carotid arteriovenous anastomoses

5-HT_{1D} receptors have been cloned and they consist of two different subtypes, namely 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors (Hamblin & Metcalf, 1991; Weinshank *et al.*, 1992). Interestingly, the 5-$HT_{1D\alpha}$ receptor mRNA has been found to be expressed in human trigeminal ganglia (Rebeck *et al.*, 1994),

while the 5-$HT_{1D\beta}$ mRNA, but not the 5-$HT_{1D\alpha}$ receptor mRNA, is expressed in human or bovine cerebral arteries (Hamel *et al.*, 1993); therefore, the 5-$HT_{1D\beta}$ receptor is thought to mediate contractile responses in these vessels.

The use of GR127935, which has similar affinities at 5-$HT_{1D\alpha}$ (pK_i: 8.9) and 5-$HT_{1D\beta}$ (pK_i: 9.9) receptors (Skingle *et al.*, 1993), does not allow us to infer that one or both 5-HT_{1D} subtypes are involved in the vasoconstrictor effect of sumatriptan on porcine cranial arteriovenous anastomoses (and, possibly, in the vasodilatation of arterioles). However, using cells with human cloned receptors, GR127935 has been described as acting as a full (Pauwels & Colpaert, 1995) or partial (Watson *et al.*, 1995) agonist at the 5-$HT_{1D\alpha}$ receptor, and as a silent (Pauwels & Colpaert, 1995) or partial (Watson *et al.*, 1995) antagonist at the 5-$HT_{1D\beta}$ receptor. In view of the presence of 5-$HT_{1D\beta}$ and not 5-$HT_{1D\alpha}$ receptor mRNA in blood vessels (Hamel *et al.*, 1993) and the findings that GR127935 itself induced arteriovenous anastomotic blood flow but antagonized sumatriptan-induced reductions in arteriovenous anastomotic blood flow, it is possible that the 5-$HT_{1D\beta}$ receptor subtype mediates the constriction of porcine cranial arteriovenous anastomoses. Notwithstanding, one must await the development of subtype-selective 5-HT_{1D} receptor agonists and antagonists for more definitive evidence.

In conclusion, the results of the present experiments imply that the constriction of porcine carotid arteriovenous anastomoses by the 5-HT_1-like receptor agonist, sumatriptan, which is antagonized by the 5-HT_{1D} receptor ligand GR127935, is mediated by 5-HT_{1D} receptors. It would, therefore, appear that vascular 5-HT_1-like receptors, which are yet to be cloned, are identical to 5-HT_{1D} receptors. In view of the putative pathophysiological role of arteriovenous anastomotic dilatation in migraine (Saxena, 1990), the constriction of these non-nutrient vessels by sumatriptan via a 5-HT_{1D} receptor mechanism may be, at least in part, responsible for the therapeutic effect of the drug in migraine.

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