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Characterization of 5-HT receptors mediating constriction of porcine carotid arteriovenous anastomoses; involvement of 5-$HT_{1B/1D}$ and novel receptors

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1 It was previously shown that porcine cranial arteriovenous anastomoses (AVAs) constrict to 5-hydroxytryptamine (5-HT), ergotamine, dihydroergotamine, as well as sumatriptan and that sumatriptan acts exclusively via 5-$HT_{1B/1D}$ receptors. The present study was devoted to establish the contribution of 5-$HT_{1B/1D}$ receptors in the constriction of AVAs elicited by 5-HT (in presence of 0.5 mg kg^{-1} ketanserin), ergotamine and dihydroergotamine in anaesthetized pigs.

2 Intracarotid infusion of 5-HT (2 μg kg^{-1} min^{-1}) and intravenous doses of ergotamine (2.5–20 μg kg^{-1}) and dihydroergotamine (3–100 μg kg^{-1}) reduced AVA and increased nutrient blood flows and vascular conductances. The vasodilator response to 5-HT, observed mainly in the skin and ear, was much more prominent than that of the ergot alkaloids.

3 Treatment with the 5-$HT_{1B/1D}$ receptor antagonist GR127935 (0.5 mg kg^{-1}, i.v.) significantly attenuated both ergot-induced AVA constriction and arteriolar dilatation, whereas GR127935 only slightly affected the carotid vascular effects of 5-HT.

4 The results suggest that 5-HT constricts carotid AVAs primarily via receptors, which seem to differ from those (5-$HT_{1B/1D}$) stimulated by sumatriptan. The ergot alkaloids produce AVA constriction for a substantial part via 5-$HT_{1B/1D}$ receptors, but also stimulate unidentified receptors. Both these non-5-$HT_{1B/1D}$ receptors may be targets for the development of novel antimigraine drugs.

5 The moderate vasodilator response to the ergot derivatives seems to be mediated, at least in part, by 5-$HT_{1B/1D}$ receptors, whereas the arteriolar dilatation caused by 5-HT may be mediated by other, possibly 5-HT_7 receptors.

Keywords: 5-HT; 5-$HT_{1B/1D}$ receptors; antimigraine drugs; arteriovenous anastomoses; carotid artery; dihydroergotamine; ergotamine; GR127935; sumatriptan

Introduction

In previous studies in vagosympathectomized, anaesthetized pigs, we have shown that intracarotid infusions of 5-hydroxytryptamine (5-HT) lead to a redistribution of carotid blood flow towards tissue arterioles at the expense of arteriovenous anastomoses (AVAs; Saxena & Verdouw, 1982; Saxena *et al*., 1986; Den Boer *et al*., 1992). These changes are dose-dependent and recover within minutes of stopping 5-HT infusions (Saxena & Verdouw, 1982). The vasoconstrictor action of 5-HT on AVAs is mainly mediated by 5-HT_1-like receptors with some contribution from 5-HT_2 receptors (Saxena *et al*., 1986). The constriction of porcine AVAs by the antimigraine drugs sumatriptan and, partly, by the ergot alkaloids (ergotamine and dihydroergotamine) is also mediated by 5-HT_1-like receptors, because these effects are antagonized, either partially (ergot alkaloids) or fully (sumatriptan), by methiothepin, but not by ketanserin (Den Boer *et al*., 1991a,b).

It is now recognized that the term '5-HT_1-like'-includes several different receptor subtypes. The vascular 5-HT_1-like receptor appears to be identical to recombinant 5-$HT_{1B/1D}$ receptors, since the vasoconstrictor responses to sumatriptan, which has a high affinity for 5-$HT_{1B/1D}$ receptors (Peroutka & McCarthy, 1989; Beattie *et al*., 1994), are antagonized by GR127935, a selective 5-$HT_{1B/1D}$ receptor antagonist (Clitherow *et al*., 1994; De Vries *et al*., 1996; Pauwels, 1996; Skingle *et al*., 1996). Although potent and selective antagonists at either the 5-HT_{1B} or 5-HT_{1D} receptors are not available, the sumatriptan-induced vasoconstriction is most probably mediated by the 5-HT_{1B} receptor. Indeed, mRNA for the 5-HT_{1B} but not 5-HT_{1D} receptor has been located in cranial blood vessels (Hamel & Bouchard, 1991; Hamel *et al*., 1993; Bouchelet *et al*., 1996).

On the basis of above, the present investigation was undertaken to establish the contribution of 5-$HT_{1B/1D}$ receptors in the reduction of porcine carotid AVA blood flow induced by the endogenous ligand, 5-HT, and by the ergot alkaloids, ergotamine and dihydroergotamine, both potent antimigraine agents. For this purpose, we analysed the carotid vasoconstrictor effects of intracarotid infusions of 5-HT and i.v. doses of the ergot compounds before and after treatment with GR127935 or equivalent volumes of physiological saline. To eliminate 5-HT_2 receptor-mediated AVA constriction (Verdouw *et al*., 1984b), the animals receiving 5-HT were systematically pretreated with ketanserin (0.5 mg kg^{-1}).

Methods

General

After an overnight fast, 36 domestic pigs (Yorkshire × Landrace; 10–15 kg) were anaesthetized with azaperone (160 mg,

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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, AVA blood flow is considerably more than in pigs in a conscious state or under thiopentone anaesthsia (Den Boer *et al*., 1993).

Catheters were placed in: (i) the inferior vena cava via the left femoral vein for the administration of drugs; and (ii) the aortic arch via the left femoral artery for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) 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. For the infusion of 5-HT another needle was inserted into the same artery. 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 (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by electrocardiogram signals.

Arterial blood pressure, heart rate and carotid blood flow were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). During the experiments 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 15.5±0.1 (mean±s.d.) μm diameter microspheres labelled with either ^{141}Ce, ^{113}Sn, ^{95}Nb, ^{103}Ru or ^{46}Sc (NEN Dupont, Boston, U.S.A.). For each measurement, a suspension of about 200,000 microspheres, labelled with one of the above isotopes was mixed and injected into the carotid artery. At the end of the experiment the animals were killed and the heart, kidneys, lungs and all ipsilateral 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), with suitable windows for discriminating the different isotopes. All data were processed by a set of specifically designed programmes (Saxena *et al*., 1980), with a personal computer.

The fraction of carotid blood flow distributed to the different nutrient tissues (capillary blood flow) 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 AVAs. Therefore, the amount of radioactivity in the lungs was used as an *index* of the arteriovenous anastomotic (AVA) fraction of carotid blood flow (Saxena & Verdouw, 1982).

Experimental protocols

After a stabilization period of about 1 h, the animals were divided into two groups. The first group ($n=12$) was systematically pretreated with ketanserin (0.5 mg kg^{-1}, i.v.) and subsequently subdivided into two subgroups. The first subgroup ($n=5$), which will be referred to as the control group, received an intracarotid infusion of 5-HT (2 μg kg^{-1} min^{-1}), lasting 10 min, after measurement baseline values of heart rate, mean arterial blood pressure, carotid blood flow and its distribution and arterial and jugular venous blood gases. All variables were reassessed at the end of this infusion and, again, 30 min after the infusion had been stopped (recovery). Then, the animals received an i.v. infusion of physiological saline (1 ml min^{-1} for 5 min). Ten minutes after the end of this infusion and at the end of a second 10 min 5-HT infusion, all variables were collated again. In the second subgroup of animals ($n=7$) the same protocol was used, but instead of physiological saline, GR127935 (0.5 mg kg^{-1}, i.v.; 1 ml min^{-1} for 5 min) was administered. This dose of GR127935 completely blocks the carotid vascular effects of sumatriptan (De Vries *et al*., 1996).

The second group of animals ($n=24$; untreated with ketanserin) was divided into four subgroups ($n=6$ each). The first two groups received an i.v. infusion of physiological saline (5 ml), whereas the last two groups received an i.v. infusion of GR127935 (0.5 mg kg^{-1}); both were administered over a period of 4–5 min. Ten minutes after the end of these infusions, baseline values of heart rate, mean arterial blood pressure, carotid blood flow and its distribution, as well as arterial and jugular venous blood gases were measured. Then, the first and third group received sequential i.v. bolus injections of ergotamine (2.5, 5, 10 and 20 μg kg^{-1}), every 20 min, whereas the second and fourth group received sequential i.v. bolus injections of dihydroergotamine (3, 10, 30 and 100 μg kg^{-1}). Fifteen minutes after each dose of ergotamine or dihydroergotamine all haemodynamic variables were reassessed.

Data presentation and statistical analysis

All data have been expressed as the mean±s.e.mean. The significance of the difference between the variables within one group was elevated with Duncan's new multiple range test, once an analysis of variance (random block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). Differences between variables of two groups were evaluated by use of Student's unpaired 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: 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.; courtesy Dr H.E. Connor), 5-HT creatinine sulphate (Sigma Chemical Company, St. Louis, MO, U.S.A.), ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium),

ergotamine tartrate and dihydroergotamine mesylate (both from Sandoz Pharma Ltd., Basel, Switzerland) and heparin sodium (Loe Pharmaceutical Products, Weesp, The Netherlands) to prevent clotting of the catheters. Ketanserin, ergotamine and dihydroergotamine were dissolved in distilled water; 5-HT was dissolved in physiological saline. 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. All doses refer to the respective salts whereas that of 5-HT refers to the free base.

Results

Effects of intracarotid 5-HT infusions before and after physiological saline or GR127935

Systemic haemodynamics The effects of 5-HT on the systemic haemodynamics are depicted in Table 1. Intracarotid infusion of 5-HT (2 μg kg^{-1} min^{-1}) produced a slight increase in heart rate ($+7\pm2\%$, $P<0.05$) in control animals; mean arterial blood pressure ($-5\pm4\%$, $P>0.05$) was not affected. In the second subgroup of animals also, only small changes in heart rate ($+7\pm2\%$, $P<0.05$) and blood pressure ($-8\pm2\%$, $P<0.05$) were observed with 5-HT infusion. Heart rate returned to baseline values after a recovery period of 30 min, but blood pressure remained slightly lower in the second subgroup. The subsequent administrations of saline or GR127935 did not cause significant systemic haemodynamic changes. In control animals, the second infusion of 5-HT did not change heart rate, but produced a slight (though significant) hypotensive effect. In the other subgroup, after treatment with GR127935 the hypotension was not affected, but the tachycardiac effect of 5-HT was attenuated.

Arterio-jugular venous oxygen saturation difference (A-V SO_2) No conspicuous changes were observed to be induced by 5-HT in the A-V SO_2 in both groups. However, the response to the second infusion of 5-HT was significantly different compared to that to the first 5-HT infusion (Table 1).

Table 1 Values of heart rate, mean arterial blood pressure and difference in arterial and jugular venous oxygen saturations at baseline, during a 10 min intracarotid infusion of 5-HT, after 30 min of recovery, after physiological saline or GR127935 and during a second infusion of 5-HT

	Baseline	*5-HT (1st)*	*Recovery*	*Saline or GR127935*	*5-HT (2nd)*
Heart rate (beats min^{-1})					
Control	92±2	98±4*	93±3	93±3	95±4^{c}
GR127935	93±3	100±3*	93±3	92±2	95±2^{b,c}
Mean arterial blood pressure (mmHg)					
Control	106±3	101±5	102±2	97±2	88±4^{b,c}
GR127935	86±6	80±7*	81±6*	77±5	71±5^{b}
Arterial-jugular venous oxygen saturation difference (%)					
Control	6.1±1.2	4.3±0.7	7.7±1.9	9.0±2.9	12.9±4.1^{c}
GR127935	10.1±2.3	7.9±1.8	11.3±2.2	13.9±3.5	14.5±3.7^{c}

All values have been presented as the mean±s.e.mean. 5-HT was infused at 2 μg kg^{-1} min^{-1}; after 30 min of recovery 5 ml saline ($n=5$) or 0.5 mg kg^{-1} GR127935 ($n=7$) was administered. *$P<0.05$ vs baseline; $^{a}P<0.05$ vs recovery value; $^{b}P<0.05$ vs values after saline or GR127935; $^{c}P<0.05$ vs first response to 5-HT

Carotid haemodynamics As shown in Figure 1A, in control animals a 10 min intracarotid infusion of 5-HT (2 μg kg^{-1} min^{-1}) produced an increase in the total carotid blood flow (31±6%), a decrease in the AVA blood flow (79±4%) and a marked increase in nutrient (capillary) blood flow (482±71%). These variables returned to baseline values after a 30 min recovery period and remained unaltered after the subsequent administration of saline. During a second infusion of 5-HT no changes in the total carotid blood flow were observed ($P<0.05$ vs first response to 5-HT), while the decrease in the AVA blood flow was more pronounced ($-93\pm3\%$; $P<0.05$ vs first response to 5-HT) and the increase in nutrient blood flow was less marked (173±71%; $P<0.05$ vs first response to 5-HT).

In the second subgroup of animals (Figure 1B) no changes in the total carotid blood flow were observed during the initial infusion of 5-HT ($P<0.05$ vs response to 5-HT in control animals), whereas 5-HT produced a decrease in AVA blood flow (84±6%) and an increase in nutrient blood flow (238±74%), of which the latter response was significantly less as compared to control animals. These variables returned to baseline values after a 30 min recovery period. The subsequent administration of GR127935 (0.5 mg kg^{-1}, i.v.) produced a non-significant decrease in the total carotid blood flow

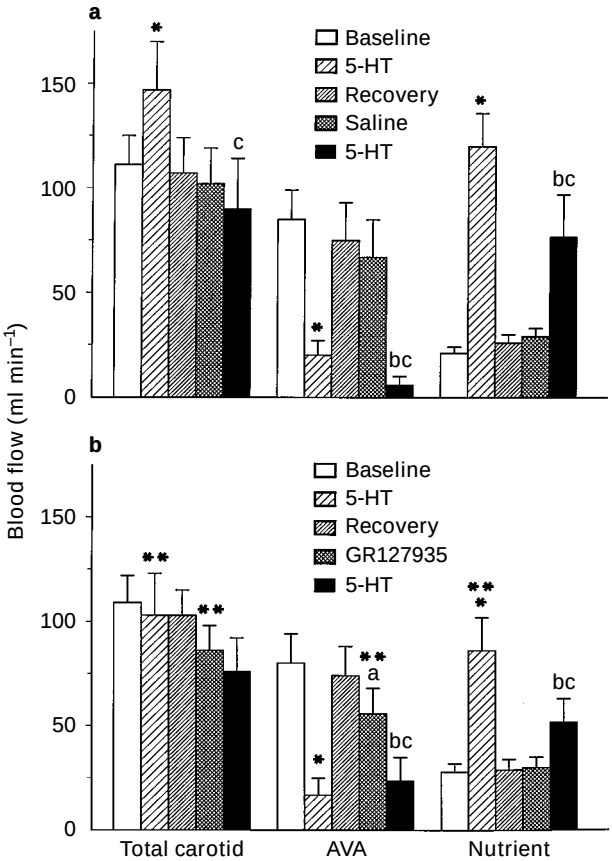

Figure 1 Distribution of total carotid blood flow into its AVA and nutrient fractions in ketanserin (0.5 mg kg^{-1}, i.v.)-pretreated pigs. Effects of 10 min intracarotid infusions of 5-HT (2 μg kg^{-1} min^{-1}) before and after saline (A; $n=5$) or GR127935 (B; 0.5 mg kg^{-1}, i.v.; $n=7$). From left to right the columns signify values at baseline, after an initial infusion of 5-HT, after a recovery period of 30 min after saline (A) or GR127935 (B) and after a second infusion of 5-HT. Data are expressed as the mean±s.e.mean. *$P<0.05$ vs baseline; $^{a}P<0.05$ vs recovery value; $^{b}P<0.05$ vs values after saline or GR127935; $^{c}P<0.05$ vs first response to 5-HT; **$P<0.05$ vs response in control animals.

(−17±3%); this change was significantly different from that observed in control animals (−6±2%) infused with saline. GR127935 caused a decrease in AVA blood flow (−27±3%; $P<0.05$ vs recovery and $P<0.05$ vs response to saline in control animals), without changing nutrient blood flow. In the presence of GR127935, a second infusion of 5-HT did not change the total carotid blood flow, but decreased AVA (−65±12%; $P<0.05$ vs values after GR127935) and increased nutrient (76±26%; $P<0.05$ vs values after GR127935) blood flows. Both these 5-HT-induced changes were significantly less compared to the changes induced by the initial infusion with 5-HT. However, compared to control animals (Figure 1A) the decrease in AVA, as well as the increase in nutrient blood flow, induced by the second 5-HT infusion, was not significantly different from GR127935 treatment (Figure 1B).

The distribution of carotid blood flow to the different head tissues is depicted in Figure 2. In both groups of animals, 5-HT increased blood flow significantly towards the skin, ears, fat and muscles and decreased blood flow to the brain; exclusively in the control group (Figure 2A) significant increases were observed in bones, eye and dural blood flow. In keeping with the above, a marked, sharply defined, redness of the skin and ear, confined to the ipsilateral side of the head, was observed in all animals during the infusion with 5-HT. These variables, including the redness of the skin, returned to baseline values after a 30 min recovery period and remained unaltered after the subsequent administration of saline or GR127935. Overall, the 5-HT-induced increases in blood flow towards the different head tissues were slightly, but significantly, attenuated to the same extent by treatment with saline, as well as by GR127935. However, the increase in ear blood flow was abolished by GR127935, while it was only partly attenuated by saline. The decrease in blood flow to the brain was not affected by either saline or GR127935. The degree of redness caused by 5-HT was not visibly different before and after GR127935 or saline.

Effects of ergot alkaloids in animals pretreated with physiological saline or GR127935

Systemic haemodynamics As shown in Table 2, ergotamine (2.5–20 μg kg^{-1}) and dihydroergotamine (3–100 μg kg^{-1}) did not cause major changes in heart rate or mean arterial blood pressure; only the highest dose of dihydroergotamine produced small, but significant, tachycardic and hypertensive effects in control and GR127935-pretreated animals, respectively.

Arterio-jugular venous oxygen saturation difference (A-V SO_2) In saline-treated animals, both ergot compounds produced dose-dependent increases in the A-V SO_2. In animals pre-treated with GR127935, the ergotamine-induced increases in the A-V SO_2 seemed to be attenuated, although only at 5 μg kg^{-1} was significance reached. The dihydroergotamine-induced increases in the A-V SO_2 were abolished after pretreatment with GR127935, although, compared to saline-

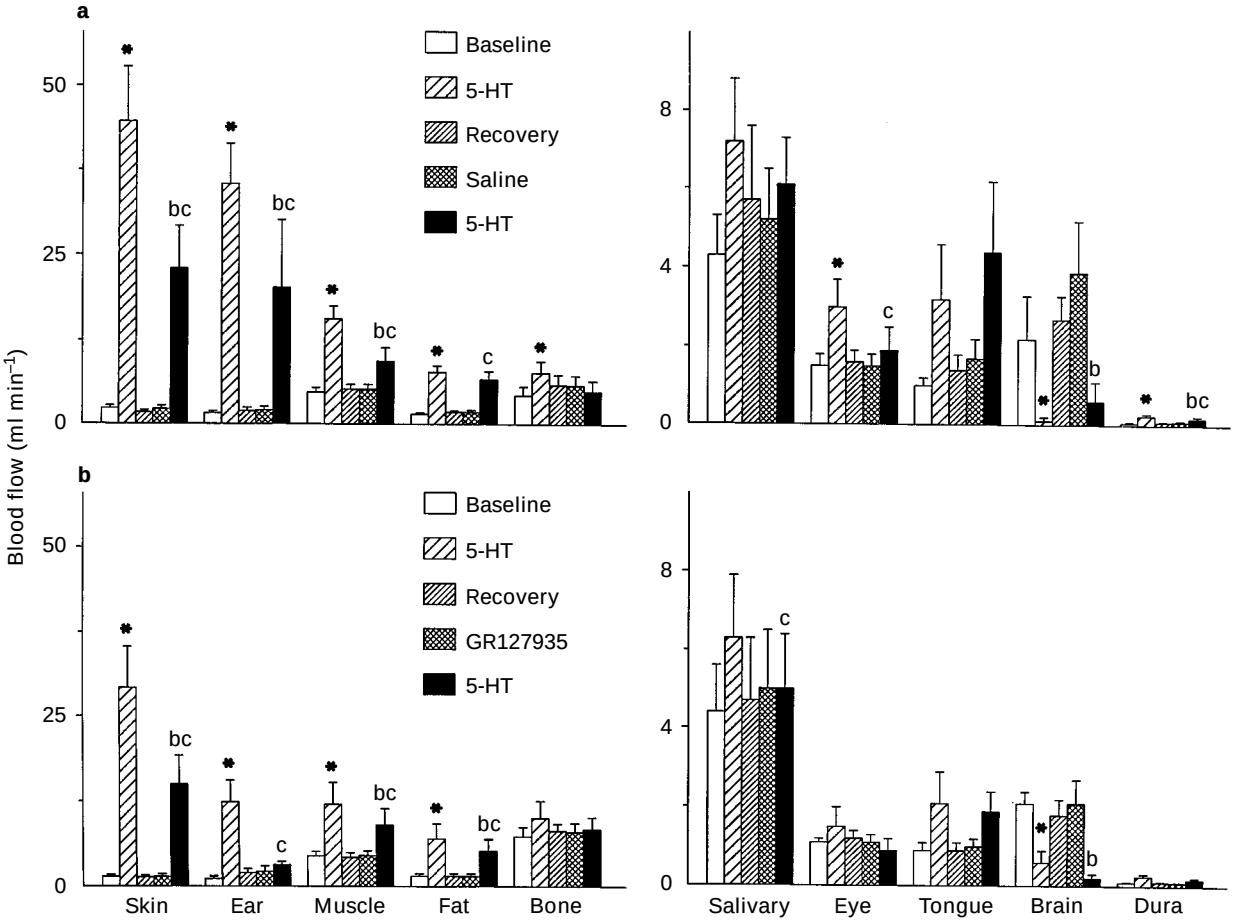

Figure 2 Distribution of total carotid blood flow to the different cranial tissues in ketanserin (0.5 mg kg^{-1}; i.v.)-pretreated pigs. Effects of 10 min intracarotid infusions of 5-HT (2 μg kg^{-1} min^{-1}) before and after saline (A; $n=5$) or GR127935 (B; 0.5 mg kg^{-1}, i.v.; $n=7$). From left to right the columns signify values at baseline, after an initial infusion of 5-HT, after a recovery period of 30 min, after saline (A) or GR127935 (B) and after a second infusion of 5-HT. Data are expressed as the mean±s.e.mean. *$P<0.05$ vs baseline; [a]$P<0.05$ vs recovery value; [b]$P<0.05$ vs values after saline or GR127935; [c]$P<0.05$ vs first response to 5-HT; **$P<0.05$ vs response in control animals.

Table 2 Values of heart rate, mean arterial blood pressure and difference in arterial and jugular venous oxygen saturations at baseline and after cumulative doses of ergotamine or dihydroergotamine in animals pretreated with either saline (control) or GR127935 (0.5 mg kg^{-1})

	Ergotamine (μg kg^{-1}; i.v.)					*Dihydroergotamine* (μg kg^{-1}; i.v.)				
Pretreatment	*Baseline*	*2.5*	*5*	*10*	*20*	*Baseline*	*3*	*10*	*30*	*100*
					Heart rate (beats min^{-1})					
Control	97±4	96±4	96±4	95±4	95±4	91±3	91±3	90±3	91±2	94±3*
GR127935	93±4	90±4	88±4	88±4	88±4	94±4	94±4	93±3	94±3	98±3
					Mean arterial blood pressure (mmHg)					
Control	95±2	104±4	101±6	98±7	101±6	106±4	118±5	116±6	116±7	117±7
GR127935	93±6	97±6	98±7	97±8	102±7	100±2	99±1[a]	98±1	103±2	107±2*
					Arterial-jugular venous oxygen saturation difference (%)					
Control	4.1±0.7	10.0±2.9*	15.5±3.3*	17.6±2.5*	21.2±2.6*	6.2±1.5	9.4±1.6*	12.5±1.6*	14.7±1.7*	16.6±2.1*
GR127935	11.9±5.6	14.6±6.5	16.7±6.9*[a]	19.9±7.0*	22.6±6.2*	15.5±4.9	14.8±4.3	15.4±4.0[a]	18.5±3.8	20.4±3.0

All values have been presented as the mean±s.e.mean ($n=6$). *$P<0.05$ vs basline; [a]$P<0.05$ vs % change from baseline values by the corresponding dose in saline pretreated (control) group.

treated animals, this was only significant at a dose of 10 μg kg^{-1}.

Carotid haemodynamics As shown in Figures 3 (absolute values) and 4 (% changes from baseline), ergotamine (2.5–20 μg kg^{-1}), as well as dihydroergotamine (3–100 μg kg^{-1}) elicited dose-dependent decreases in the total carotid blood flow (maximal decreases: 45±3% and 31±2%, respectively) and in its conductance (maximal decreases: 47±4% and 37±2%, respectively; Table 3). These decreases in the total carotid blood flow were exclusively attributable to marked decreases in its AVA fraction; the highest dose of ergotamine (20 μg kg^{-1}) decreased AVA blood flow and its conductance by 88±3% and 88±4%, respectively, whereas dihydroergotamine (100 μg kg^{-1}) similarly decreased these variables by 86±3% and 87±2%, respectively. Additionally, ergotamine and dihydroergotamine increased nutrient blood flow by up to 118±17% and 152±27%, respectively, accompanied by increases in nutrient vascular conductance (Table 3). Treatment with GR127935 (0.5 mg kg^{-1}) significantly attenuated the above ergot-induced responses. Maximal changes in the total carotid, AVA and nutrient blood flow induced by ergotamine were −31±4% −70±6% and +74±18%, respectively, while dihydroergotamine produced changes of −30±2%, −66±3% and +89±15%, respectively. GR127935 (0.5 mg kg^{-1}) produced 5.5 fold (from 0.8±0.2 to 4.4±0.8 μg kg^{-1}) and 12.2 fold (from 1.1±0.5 to 13.4±2.3 μg kg^{-1}) increases in the ED_{30} (dose eliciting a 30% decrease in AVA conductance, calculated by linear regression analysis) for ergotamine and dihydroergotamine, respectively.

The ergotamine- and dihydroergotamine-induced changes in the distribution of carotid blood flow to the different head tissues are depicted in Figure 5. Both ergot derivatives produced significant, dose-dependent increases in blood flow to skin, ear, fat, bone, brain and dura mater, of which only bone and brain blood flow values were slightly, though significantly, attenuated by GR127935; exclusively in muscle and salivary gland ergotamine-induced blood flow increases were observed, which were slightly less in animals pretreated with GR127935.

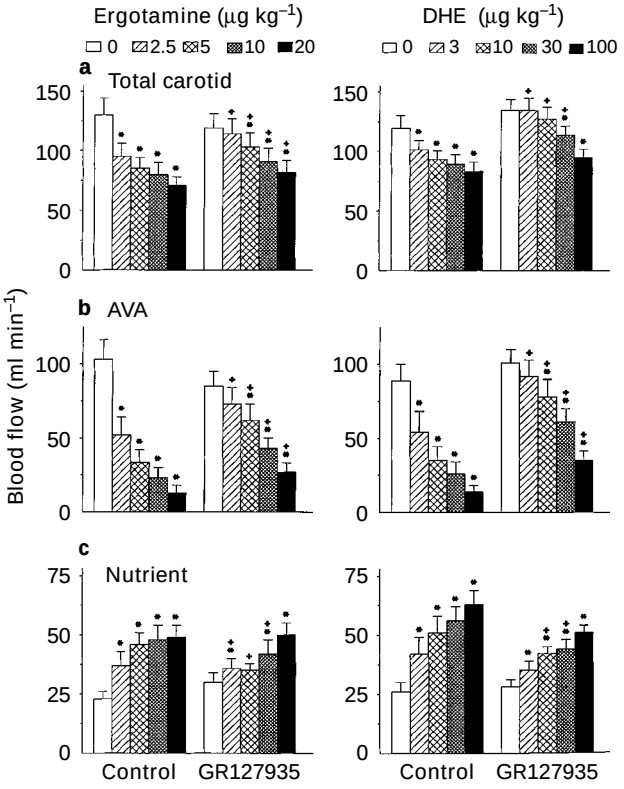

Figure 3 Effects of ergotamine (left panels) and dihydroergotamine (DHE; right panels) on the distribution of (a) total carotid blood flow into its (b) AVA and (c) nutrient fractions in pigs pretreated with either saline (control; $n=6$ each) or GR127935 (0.5 mg kg^{-1}; $n=6$ each). From left to right the columns signify values at baseline (after pretreatment with saline or GR127935) and after ergotamine (2.5, 5, 10 and 20 μg kg^{-1}, i.v.) or DHE (3, 10, 30 and 100 μg kg^{-1}, i.v.). All values are presented as the mean±s.e.mean. *$P<0.05$ vs baseline. +$P<0.05$ vs response to corresponding dose in control animals.

Discussion

We have previously shown that sumatriptan-induced carotid haemodynamic responses in pigs (De Vries *et al*., 1996), as well as rabbits (De Vries *et al*., 1997a) are mediated by 5-$HT_{1B/1D}$ receptors, as these responses are completely antagonized by a single i.v. dose of 0.3–0.5 mg kg^{-1} of GR127935. The main aim of the present investigation in the anaesthetized pig was to establish whether the carotid vascular responses to the endogenous ligand (5-HT) and the antimigraine drugs, ergotamine and dihydroergotamine, are also mediated by 5-$HT_{1B/1D}$ receptors. Due to the different pharmacological

properties of 5-HT and the ergot compounds, separate protocols were used. Firstly, as 5-HT_2 receptors play a role in the carotid vascular effects of 5-HT (Saxena & Verdouw, 1982; Verdouw *et al*., 1984b), but not those of the ergot alkaloids (Saxena *et al*., 1983; Bom *et al*., 1989), the animals receiving 5-HT were pretreated with ketanserin. Ketanserin is a potent 5-HT_{2A} receptor antagonist (Hoyer *et al*., 1994) and has recently been shown to possess a moderate blocking property at the h5-HT_{1D} receptor (Zgombick *et al*., 1995). However, one should keep in mind that in the present experiments we cannot provide evidence that the dose employed also blocked the porcine 5-HT_{1D} receptor. Secondly, due to the short duration of action of 5-HT, the compound was infused directly into the carotid artery, whereas the long-acting compounds, ergotamine and dihydroergotamine, were administered i.v. Moreover, the effect of GR127935

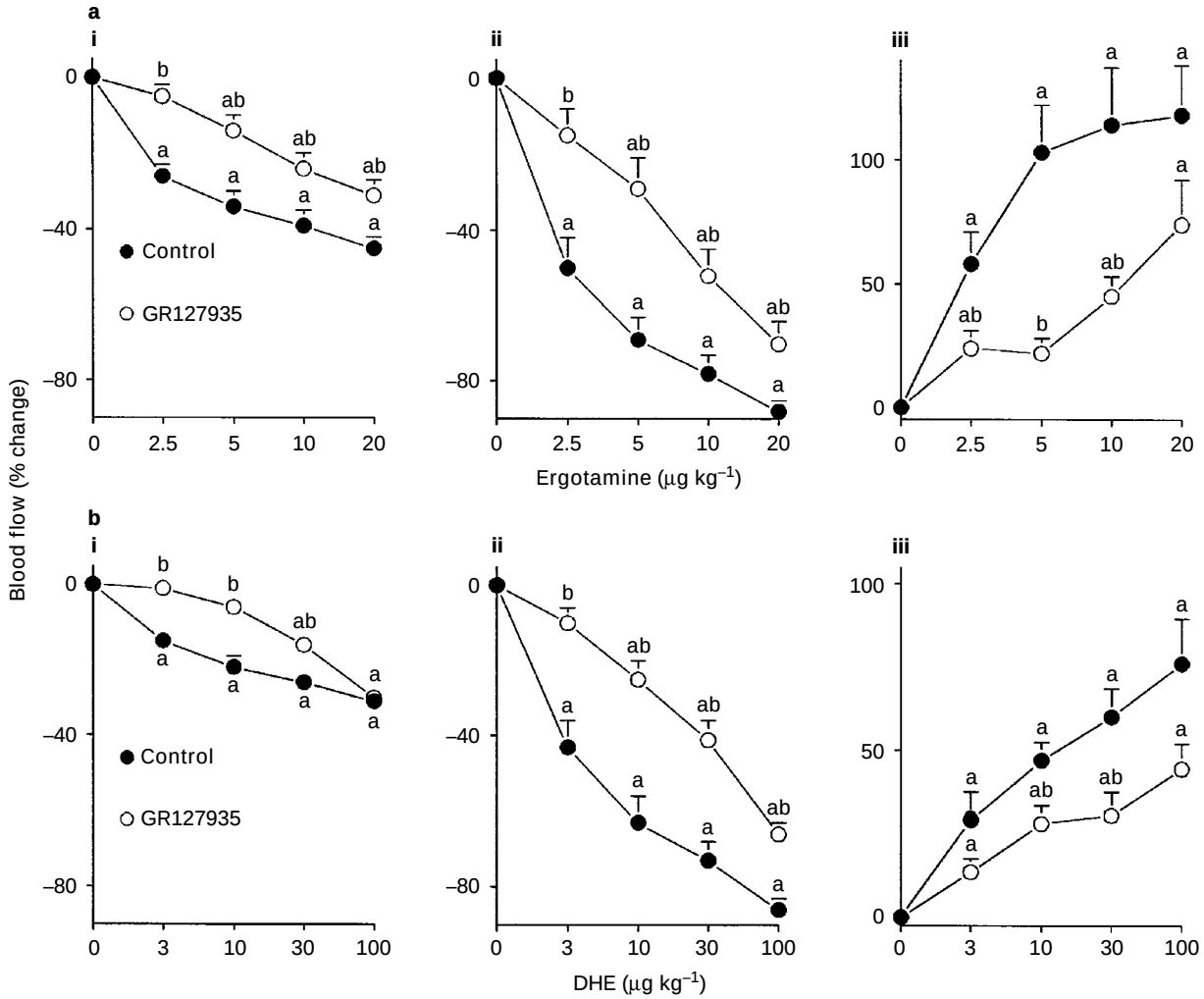

Figure 4 Percent changes from baseline values ($n=6$, each) of (i) total carotid, (ii) AVA and (iii) nutrient blood flow by ergotamine (A; i.v.) and dihydroergotamine (DHE; B; i.v.) in pigs pretreated with either saline (control) or GR127935 (0.5 mg kg^{-1}). All values are presented as the mean ± s.e.mean. [a]$P<0.05$ vs baseline; [b]$P<0.05$ vs the corresponding dose in control animals.

Table 3 Values of total carotoid conductance and its fractionation into AVA and nutrient parts at baseline and after cumulative doses of ergotamine and dihydroergotamine in animals pretreated with either saline (control) or GR127935 (0.5 mg kg^{-1})

		Ergotamine (μg kg^{-1}; i.v.)					*Dihydroergotamine* (μg kg^{-1}; i.v.)			
Pretreatment	*Baseline*	*2.5*	*5*	*10*	*20*	*Baseline*	*3*	*10*	*30*	*100*
					Total carotoid conductance					
Control	136±13	92±10*	85±9*	82±10*	71±8*	113±10	86±6*	81±7*	78±8*	72±7*
GR127935	126±8	116±9*,[a]	103±8*,[a]	94±9*	81±10*	136±10	137±11[a]	131±10[a]	109±6*,[a]	89±6*
					AVA conductance					
Control	108±14	50±11*	32±8*	24±7*	13±5*	84±10	45±10*	30±7*	23±6*	12±3*
GR127935	89±6	74±9*,[a]	61±9*,[a]	43±7*,[a]	25±6*,[a]	102±11	94±12[a]	79±12*,[a]	59±8*,[a]	33±6*,[a]
					Nutrient (capillary) conductance					
Control	24±3	36±6*	46±6*	50±6*	49±5*	25±3	36±7*	45±6*	50±6*	54±6*
GR127935	32±3	37±4	36±3[a]	45±6*,[a]	49±5*	28±3	36±4*	43±4*	43±5*	47±3*

All values have been presented as the mean ± s.e.mean ($n=6$) and are shown as 100 × ml min^{-1} $mmHg^{-1}$. *$P<0.05$ vs baseline; [a]$P<0.05$ vs % change from baseline values by the corresponding dose in saline pretreated (control) group.

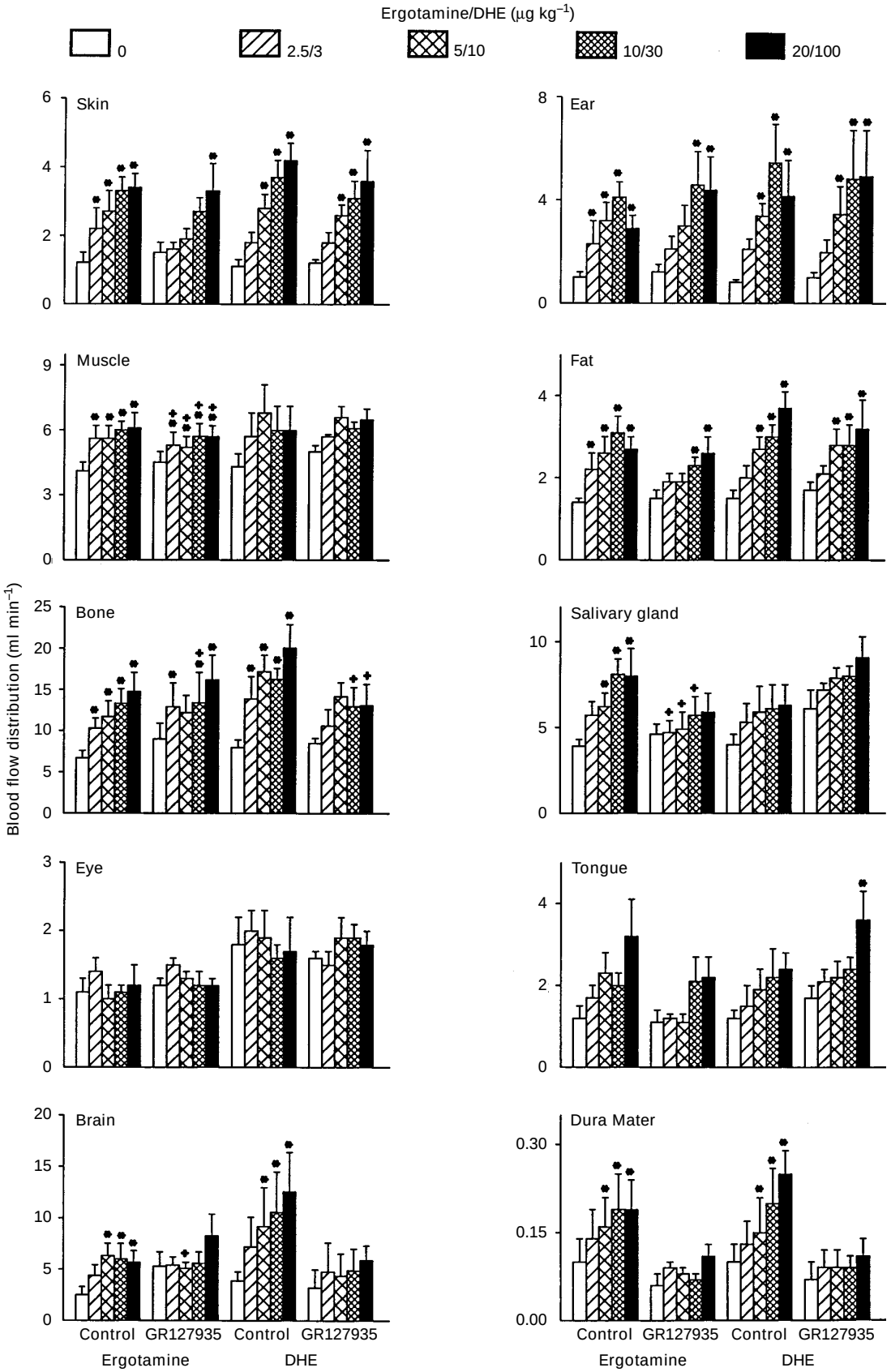

Figure 5 Effects of ergotamine and dihydroergotamine (DHE) on the distribution of total carotid blood flow to the different cranial tissues in pigs pretreated with either saline (control; $n=6$ each) or GR127935 (0.5 mg kg^{-1}; $n=6$ each). From left to right the columns signify values at baseline (after pretreatment with saline or GR127935) and after ergotamine (2.5, 5, 10 and 20 μg kg^{-1}, i.v.) or DHE (3, 10, 30 and 100 μg kg^{-1}; i.v.). All values are presented as the mean $\pm$ s.e.mean. *$P<0.05$ vs baseline. +$P<0.05$ vs response to corresponding dose in control animals.

(0.5 mg kg^{-1}) on 5-HT-induced changes was studied within animals, while the ergot-induced changes were studied in different groups pretreated with either saline or GR127935. It should be noted that the intrinsic activity of GR127935 at receptors mediating AVA constriction (De Vries *et al*., 1996), also observed in the present experiments (see Figure 1B), did not allow us to use higher doses of the compound. Interestingly, this AVA constriction by GR127935 is not amenable to blockade by methiothepin (3 mg kg^{-1}, i.v.; $n=5$), suggesting that 5-HT_1 or 5-HT_2 receptors do not mediate this effect (unpublished observation).

Systemic haemodynamics

As observed previously (Saxena & Verdouw, 1982), intracarotid infusion of 5-HT (2 μg kg^{-1} min^{-1}) produced a slight tachycardia and hypotension. The tachycardia could be due to an action at cardiac 5-HT_4 receptors (Villalón *et al*., 1990), but it should be noted that heart rate did not increase during the second 5-HT infusion after treatment with physiological saline. Therefore, the apparent attenuation of the 5-HT-induced tachycardiac effect by GR127935 seems to be a non-specific (tachyphylactic) effect. The small hypotensive response caused by 5-HT may be due to stimulation of 5-HT_7 receptors (De Vries *et al*., 1997b).

Arterio-jugular venous oxygen saturation difference (A-V SO_2)

5-HT did not increase the A-V SO_2. Considering the potent vasoconstriction of cephalic AVAs by 5-HT (see below), one would expect an increase in the A-V SO_2 as observed with the ergot alkaloids (present results; Den Boer *et al*., 1991a) and sumatriptan (Den Boer *et al*., 1991b; De Vries *et al*., 1996). However, in contrast to the latter compounds, 5-HT produces a very marked arteriolar dilatation, leading to a huge increase in nutrient (capillary) blood flow. As a consequence, arterial blood with high O_2 saturation mixes with jugular venous blood ('physiological shunting'), thereby decreasing the A-V SO_2.

The effects of the ergot alkaloids on A-V SO_2 were not potently blocked, whereas those of sumatriptan were completely antagonized by GR127935 (De Vries *et al*., 1996). This is in keeping with the extent of blockade of AVA constriction by GR127935, which abolished the response to sumatriptan (De Vries *et al*., 1996), but only partly affected that to the ergot alkaloids. Moreover, the increase in nutrient blood flow by ergotamine (Figures 3 and 4) was more than that elicited by sumatriptan (De Vries *et al*., 1996).

Effects of GR127935 on carotid vascular responses to 5-HT and ergot alkaloids

In control animals, intracarotid infusion of 5-HT decreased AVA blood flow, but caused a marked arteriolar vasodilatation; as a result the total carotid blood flow increased. This is in keeping with our previous findings, where treatment with 5-HT_2 receptor antagonists, such as ketanserin, cyproheptadine and WAL1307, partly blocked 5-HT-induced AVA constriction and enhanced arteriolar dilatation (Saxena & Verdouw, 1982; Verdouw *et al*., 1984b). Surprisingly, the second 5-HT infusion, as well as the initial 5-HT infusion in the other subgroup, did not produce significant changes in the total carotid blood flow. We have also previously observed that, whereas the magnitude of 5-HT-induced decreases in AVA blood flow appears to be constant, the magnitude of increases in nutrient blood flow is subject to variation (see Saxena & Verdouw, 1982; 1984; Verdouw *et al*., 1984b; Saxena *et al*., 1986; Den Boer *et al*., 1992). Although we have no direct explanation for this variable response in the arteriolar vascular bed, it is worth noticing that differences in the degree of pre-existing sympathetic vascular tone, probably produced by the degree of initial anaesthesia (Den Boer *et al*., 1993), might play a role. Thus, in experimental groups with high values of baseline blood pressure (higher sympathetic tone), the increases in nutrient blood flow seemed to be more pronounced (present results; Den Boer *et al*., 1992) and *vice versa* (present results).

As observed previously in several species (Saxena, 1974; Mylecharane *et al*., 1978; Spierings & Saxena, 1980; Den Boer *et al*., 1991a), both ergotamine and dihydroergotamine dose-dependently decreased the total carotid blood flow, and this response was exclusively caused by a selective constriction of carotid AVAs with concomitant conductance changes.

Our findings show that the AVA constriction by 5-HT was partly attenuated after GR127935. However, it should be noted that the observed attenuation might be, at least in part, a result of the second 5-HT infusion starting at a lower level of blood flow, being related to the intrinsic activity of GR127935 itself (see above). Accordingly, the same minimum level of AVA blood flow was obtained before and after GR127935 and, when compared to the corresponding response by 5-HT in control animals, no significance was reached. These results lead us to conclude that the 5-HT-induced carotid AVA constriction in ketanserin-pretreated pigs was primarily mediated by receptors not identical to the 5-$HT_{1B/1D}$ receptor subtypes. As the AVA constriction by sumatriptan was abolished by 0.5 mg kg^{-1} GR127935 (De Vries *et al*., 1996), these findings imply that 5-HT and sumatriptan constrict porcine AVAs predominantly via distinct receptors. In keeping with the above, Yu *et al*. (1997) have recently found that, although GR127935 was capable of antagonizing sumatriptan-induced inhibition of neurogenic plasma extravasation in the guinea-pig, 5-carboxamidotryptamine (5-CT)-induced effects remained unaffected. On the other hand, the decrease in AVA conductance by both ergotamine and dihydroergotamine was attenuated to a considerable degree (5.5 and 12.2 fold shifts, respectively, in ED_{30} values) by GR217935 (0.5 mg kg^{-1}). Den Boer *et al*. (1991a) have previously shown that methiothepin (3 mg kg^{-1}) caused 3.1 and 5.2 fold increases in ED_{30} values for ergotamine and dihydroergotamine, respectively. The higher shifts in ED_{30} values by GR127935 are in keeping with the higher affinities displayed by GR127935 at h5-$HT_{1B/1D}$ receptors (pK_i 9.9 and 8.9, respectively; Pauwels, 1996) compared to those exhibited by methiothepin (pK_i 7.7 and 7.7., respectively; P.J. Pauwels, personal communication). In view of the high affinities displayed at 5-HT_{1B} and 5-HT_{1D} receptors by ergotamine (pK_i 8.7 and 7.8, respectively; Hoyer *et al*., 1994), dihydroergotamine (pK_i: 9.1 and 9.3, respectively; P.J. Pauwels, personal communication), methiothepin, as well as by GR127935, it is suggested that both ergot compounds partly constrict porcine AVAs via 5-$HT_{1B/1D}$ receptors.

Do the GR127935-resistant receptors resemble any of the other 5-HT receptors?

Based on the above, the greater part of the 5-HT-induced, as well as a considerable part of the ergot-induced AVA constriction does not seem to be mediated by 5-$HT_{1B/1D}$ receptors. Furthermore, as the AVA constriction-induced by 5-HT was completely blocked (Saxena *et al*., 1986) and that of the ergots only partly affected (Den Boer *et al*., 1991a) by methiothepin, it is likely that 5-HT and the ergot compounds

act via different (non-5-$HT_{1B/1D}$) receptors. In this context, several subtypes of the 5-HT_1 receptor family (5-HT_{1A}, 5-ht_{1E} and 5-ht_{1F}) may be considered as possible candidates. Whereas the involvement of 5-HT_{1A} receptors has already been excluded (Saxena & Villalón, 1990; Den Boer *et al*., 1992), the participation of 5-ht_{1E} and 5-ht_{1F} receptors is also unlikely because: (i) 5-carboxamidotryptamine (5-CT), a compound with little affinity at 5-ht_{1E} and 5-ht_{1F} receptors (Hoyer *et al*., 1994), is a potent agonist (Saxena & Verdouw, 1985); (ii) the 5-HT-induced AVA constriction was abolished by methiothepin (Saxena *et al*., 1986), but not by GR127935 (present results), despite their similar affinities at the h5-ht_{1F} receptor (pK_i: 7.0 and 7.3, respectively; P.J. Pauwels, personal communication); and (iii) ergotamine and dihydroergotamine display low affinities (pK_i values <6.5) at 5-ht_{1E} and 5-ht_{1F} receptors (P.J. Pauwels, personal communication; McAllister *et al*., 1992).

We have previously shown that the 5-HT- and ergot-induced AVA constriction is not mediated by 5-HT_2 or 5-HT_3 receptors (Saxena *et al*., 1983; 1986; Bom *et al*., 1989). The pharmacological profile of the receptors mediating ergot- or 5-HT-induced carotid vascular effects also seems to be inconsistent with 5-HT_4, 5-ht_5 or 5-ht_6 classification on the basis of: (i) the high potency of 5-CT relative to 5-HT (Saxena & Verdouw, 1985), an order which is reversed for the 5-HT_4 and 5-ht_6 receptors (Hoyer *et al*., 1994); (ii) the potent blockade of 5-HT-induced AVA constriction by methiothepin (Saxena *et al*., 1986), which is inactive at 5-HT_4 receptors (Hoyer *et al*., 1994); (iii) dihydroergotamine displaying low affinity at guinea-pig 5-HT_4 receptors (Leysen *et al*., 1996) and; (iv) the absence of mRNA for 5-ht_5 receptors on blood vessels (Ullmer *et al*., 1995). Similarly, 5-HT_7 receptors are not likely to mediate constrictor responses on the basis of positive coupling to adenylate cyclase and, therefore, leading to an increase in adenosine 3′:5′-cyclic monophosphate (cyclic AMP), which is associated with dilatation (Rand *et al*., 1987; Hoyer *et al*., 1994). Additionally, sumatriptan, which potently constricts porcine AVAs, is inactive at 5-HT_7 receptors (Hoyer *et al*., 1994).

5-HT-induced arteriolar dilatation in cranial tissues

In accordance with earlier observations (Saxena & Verdouw, 1982; Verdouw *et al*., 1984b; Saxena *et al*., 1986; Den Boer *et al*., 1992), 5-HT produced arteriolar dilatation, which was confined to the head skin and ears. As demonstrated in other vascular preparations (Eglen *et al*., 1997; Villalón *et al*., 1997a; De Vries *et al*., 1997b), it is possible that this carotid arteriolar dilatation may also be mediated by the 5-HT_7 receptor. This contention is supported by the fact that (i) 5-CT potently mimics the vasodilator response (Saxena & Verdouw, 1985), whereas sumatriptan is a very weak agonist (Den Boer *et al*., 1991b; De Vries *et al*., 1996) and (ii) methiothepin, a compound with high affinity at cloned 5-HT_7 receptors (Hoyer *et al*., 1994), acts as a potent antagonist (Saxena *et al*., 1986). Furthermore, in contrast to the vasodilator effect of 5-HT (Figure 2), the sumatriptan-induced vasodilatation is abolished by GR127935 (De Vries *et al*., 1996), implying the involvement of 5-$HT_{1B/1D}$ receptors (see Schoeffter & Hoyer, 1990). In view of the attenuation of the ergot-induced (weak) arteriolar dilatation by GR127935 (Figure 5), their vasodilator effect also seems to be mediated, at least in part, via 5-$HT_{1B/1D}$ receptors.

In conclusion, our results show that in pigs pretreated with ketanserin, 5-HT constricts AVAs primarily via a receptor type that cannot be classified by use of guidelines for the current nomenclature. Similarly, although the present results demonstrate a substantial role for 5-$HT_{1B/1D}$ receptors, novel, yet unidentified, receptors mediate the GR127935-resistant part of the ergot-induced AVA constriction. Since previous studies have shown that methiothepin completely blocks 5-HT-induced, but only partly attenuates the ergot-induced AVA constriction, multiple receptors seem to be involved. In view of the putative role of cranial AVA dilatation during migraine headache (Heyck, 1969; Saxena, 1995), as well as the high predictive value of constriction of these structures in antimigraine therapy (Saxena, 1995), these novel receptors could be a target for further antimigraine drug development (Villalón *et al*., 1997b).

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