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Investigation of the role of 5-HT_{1B} and 5-HT_{1D} receptors in the sumatriptan-induced constriction of porcine carotid arteriovenous anastomoses

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1 It has previously been shown that the antimigraine drug sumatriptan constricts porcine carotid arteriovenous anastomoses *via* 5-HT_1-like receptors, identical to 5-$HT_{1B/1D}$ receptors. The recent availability of silent antagonists selective for the 5-HT_{1B} (SB224289) and 5-HT_{1D} (BRL15572) receptor led us to further analyse the nature of receptors involved.

2 In pentobarbitone-anaesthetized, bilaterally vagosympathectomized pigs, sumatriptan (30, 100 and 300 μg kg^{-1}, i.v.) dose-dependently decreased carotid arteriovenous anastomotic conductance by up to $70 \pm 5\%$.

3 The dose-related decreases in carotid arteriovenous anastomotic conductance by sumatriptan (30, 100 and 300 μg kg^{-1}, i.v.) remained unchanged in animals treated (i.v.) with 1 mg kg^{-1} of BRL15572 (maximum decrease: $72 \pm 3\%$), but were significantly attenuated by 1 mg kg^{-1} (maximum decrease: $30 \pm 11\%$) and abolished by 3 mg kg^{-1} (maximum decrease: $3 \pm 7\%$) of SB224289. The highest dose of SB224289 did not attenuate the hypertension, tachycardia or increases in carotid blood flow induced by bolus injections of noradrenaline (0.1–3 μg kg^{-1}, i.v.).

4 The results indicate that sumatriptan constricts porcine carotid arteriovenous anastomoses primarily *via* 5-HT_{1B}, but not *via* 5-HT_{1D} receptors.

Keywords: 5-$HT_{1B/1D}$ receptors; arteriovenous anastomoses; BRL15572; carotid blood flow; migraine; pig; SB224289; sumatriptan

Abbreviations: A-V SO_2, Difference between oxygen saturation of arterial and jugular venous blood; BRL15572, 1-(3-chlorophenyl)-4-[3,3-diphenyl (2-(S,R) hydroxypropanyl) piperazine] hydrochloride; GR127935, (N-[4-Methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′methyl-4′ (5-methyl-1, 2,4- oxadiazol- 3-yl) [1,1,-biphenyl]-4-carboxamide hydrochloride monohydrate); SB224289, 2,3,6,7-tetrahydro-1′-methyl-5-[2′methyl-4′5-(methyl-1,2,4-oxadiazol-3-yl) biphenyl-4-carbonyl] furo [2,3-f] indole-3-spiro-4′-piperidine hydrochloride; SKF-99101H, 3-(2-dimethylaminoethyl)-4-chloro-5-propoxyindole hemifumarate

Introduction

It has previously been shown in several species that 5-HT causes constriction within the carotid vascular bed predominantly *via* 5-HT_1-like receptors and this effect is potently mimicked by the antimigraine drug, sumatriptan (Den Boer *et al*., 1991; Villalón *et al*., 1995). The carotid vasoconstrictor effect of sumatriptan and other acutely acting antimigraine agents is exclusively due to vasoconstriction of carotid arteriovenous anastomoses (see Saxena *et al*., 1997b; De Vries *et al*., 1999), which may open up during migraine headaches (Heyck, 1969; Saxena, 1995). Since sumatriptan displayed high affinity at the 5-HT_{1D} receptor binding sites identified in calf and human caudate membranes (see Hoyer *et al*., 1994; Martin, 1994), it was suggested that '5-HT_{1D}' receptors were responsible for the sumatriptan-induced carotid vasoconstriction. Presently, we know that the experimental conditions in the above experiments allowed the inclusion of 5-HT_{1B}, 5-HT_{1D} as well as 5-ht_{1F} receptors (see Saxena *et al*., 1998). GR127935 (N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′ (5-methyl-1, 2,4-oxadiazol- 3-yl) [1,1,-biphenyl]-4-carboxamide hydrochloride monohydrate), the first potent and selective antagonist at the '5-HT_{1D}' receptor (Clitherow *et al*., 1994; Pauwels, 1996; Skingle *et al*., 1996), was shown to inhibit the sumatriptan-induced carotid vasoconstriction in pigs (De Vries *et al*., 1996) and dogs (Villalón *et al*., 1996). In the mean time, it was demonstrated that the human 5-HT_{1D} receptor was encoded by two structurally distinct genes, named 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ (Weinshank *et al*., 1992). The 5-$HT_{1D\alpha}$ receptor was shown to be the human homologue of the rat 5-HT_{1B} receptor and, consequently, renamed 5-HT_{1B}, while the 5-$HT_{1D\alpha}$ receptor was renamed 5-HT_{1D} receptor (see Hartig *et al*., 1996). Unfortunately, GR127935 was not able to distinguish between these 5-HT_{1B} and 5-HT_{1D} receptors (Table 1). Recently, however, two new potent compounds were developed, SB224289 (2,3,6,7-tetrahydro-1′-methyl-5-[2′-methyl-4′(5-methyl-1,2,4-oxadiazol-3-yl) biphenyl-4-carbonyl] furo [2,3-f] indole-3-spiro-4′-piperidine hydrochloride; Gaster *et al*., 1998) and BRL15572 (1-(3-chlorophenyl)-4-[3,3-diphenyl (2-(S,R) hydroxypropanyl) piperazine] hydrochloride; Price *et al*., 1997), which show a high degree of selectivity for the 5-HT_{1B} and 5-HT_{1D} receptor, respectively (Table 1). Using these compounds, it was shown that sumatriptan-induced canine external carotid vasoconstriction (De Vries *et al*., 1998a) and human isolated temporal artery contraction (Verheggen *et al*., 1998) as well as hypothermia induced by SKF-99101H (3-(2-dimethylaminoethyl)-4-chloro-5-propox-

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yindole hemifumarate) in the guinea-pig (Hagan *et al*., 1997) are mediated by SB224289-sensitive 5-HT$_{1B}$ receptors, whereas the human atrium heteroreceptor resembles BRL15572-sensitive 5-HT$_{1D}$ receptors (Schlicker *et al*., 1997). In the light of the availability of the above selective ligands, we decided to verify whether 5-HT$_{1B}$ or 5-HT$_{1D}$ receptors are involved in the constriction of carotid arteriovenous anastomoses in anaesthetized pigs by the antimigraine drug, sumatriptan.

Methods

General

After an overnight fast, 21 pigs (Yorkshire x Landrace; 10–15 kg) were anaesthetized with azaperone (160 mg, i.m.), midazolan hydrochloride (5 mg, i.m.) and pentobarbitone sodium (600 mg, i.v.), intubated and connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35–7.48; pCO_2: 35–48 mmHg; pO_2:100–120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of pentobarbitone sodium at 20 mg kg^{-1} min^{-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), thereby producing one of the main putative features of migraine, i.e. vasodilatation of carotid arteriovenous anastomoses (see Heyck, 1969; Saxena, 1995).

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 (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 and both vagi and the accompanying cervical sympathetic nerves were cut between two ligatures, in order to avoid reflex-mediated changes in the carotid vasculature. Another catheter was placed in the right external jugular vein for the withdrawal of venous blood samples, while the right common carotid artery was dissected free and a needle was inserted against the direction of blood flow for the administration and uniform

Table 1 pK$_i$ values of sumatriptan, SB24289, BRL15572 and GR127935 at human cloned 5-HT$_1$ receptor subtypes

	5-HT$_{1A}$	*5-HT$_{1B}$*	*5-HT$_{1D}$*	*5-ht$_{1E}$*	*5-ht$_{1F}$*
Sumatriptan[a]	6.4	7.8	8.5	5.8	7.9
GR127935[c]	7.2	9.0	8.6	5.4	6.4
SB224289[b]	<5.5	8.2	6.3	<5.0	<5.0
BRL15572[c]	7.7	6.1	7.9	5.2	6.0

[a]Leysen *et al*. (1996); [b]Gaster *et al*. (1998); [c]Price *et al*. (1997).

Table 2 The effects of (i.v.) vehicle, SB224289 and BRL15572 on systemic and carotid haemodynamics in vagosympathectomized pigs

	Vehicle (n=6)		*SB224289* (1 mg kg^{-1}; n=6)		*SB224289* (3 mg kg^{-1}; n=3)		*BRL15572 (1 mg kg^{-1}*; n=6)	
	Before	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
HR	93±3	93±2	100±2	101±2	101±6	103±7	100±3	99±3
MAP	105±1	103±3	94±2	105±4*	89±2	96±6	90±2	92±3
A-V SO_2	5.6±0.8	51.±0.7	8.5±1.8	6.9±1.4	10.8±3.8	10.0±4.7	9.1±3.5	9.4±3.7
Total carotid VC	116±6	118±6	138±10	122±13	201±44	174±29	124±7	123±8
AVA VC	92±7	89±7	109±10	88±13*	149±35	119±20	87±8	86±8
Nutrient VC	24±4	29±3	29±3	35±3	51±10	55±10	37±5	37±4

All values have been presented as mean±s.e.mean. *$P<0.05$ after vs before. HR, heart rate (beats min^{-1}); MAP, mean arterial blood pressure (mmHg); A-V SO_2 arteriolar-jugular venous oxygen saturation difference (%); VC, vascular conductance (10^{-2} ml min^{-1} mmHg^{-1}); AVA, anteriovenous anastomotic. The effects of vehicle or the antagonists were measured 15 min after administration.

Table 3 Systematic haemodynamic effects of sequential doses of sumatriptan in pigs treated with vehicle ($n=6$), SB224289 (1 or 3 mg kg^{-1}; $n=6$ or 3, resepectively) or BRL15572 ($n=6$)

		Sumatriptan (μg kg^{-1}, i.v.)		
Treatment	*Baseline*	*30*	*100*	*300*
Heart rate (beat min^{-1})				
Vehicle	93±2	91±2*	90±2*	89±2*
SB224289 (1 mg kg^{-1})	101±2	100±2	98±2*	96±2*
SB224289 (3 mg kg^{-1})	103±7	102±6	100±5*	99±5*
BRL15572 (1 mg kg^{-1})	99±3	98±3	96±3*	94±4*
Mean arterial blood pressure (mmHg)				
Vehicle	103±3	103±4	103±4	97±5*
SB224289 (1 mg kg^{-1})	105±4	94±6*,+	90±5*,+	86±5*,+
SB224289 (3 mg kg^{-1})	96±6	88±7+	81±3*,+	81±2*
BRL15572 (1 mg kg^{-1})	92±3	92±2	90±3	85±4*
Arterial-jugular venous oxygen saturation difference (%)				
Vehicle	5.1±0.8	7.0±0.8	11.4±2.6*	17.7±2.8*
SB224289 (1 mg kg^{-1})	6.9±1.4	12.1±2.8*	12.1±3.3*	14.9±2.2*
SB224289 (3 mg kg^{-1})	10.0±4.7	10.3±2.7	8.4±3.7	10.0±4.2+
BRL15572 (1 mg kg^{-1})	9.4±3.7	10.8±3.4	14.8±5.0*	18.6±4.8*

All values have been presented as mean±s.e.mean. *$P<0.05$ vs baseline. +$P<0.05$ vs response by corresponding dose of sumatriptan in animals treated with vehicle.

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 (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by electrocardiographic signals.

Arterial blood pressure, heart rate and carotid blood flow were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). Body temperature was kept at about 37°C and the animals were continuously infused with saline to compensate for fluid losses during the experiment. The Ethics Committee of the Erasmus University Rotterdam dealing with the use of animals in scientific experiments approved the protocol for this investigation.

Distribution of carotid blood flow

The distribution of common carotid blood flow was determined with 15.5±0.1 (s.d.) μm diameter microspheres labelled with either ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, U.S.A.). 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, using an overdose of pentobarbitone sodium, 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 min in a γ-scintillation counter (Packard, Minaxi autogamma 5000), using suitable windows for discriminating the different isotopes (^{141}Ce: 120–167, KeV, ^{113}Sn: 355–435 KeV, ^{103}Ru: 450–548 KeV, ^{95}Nb: 706–829 KeV and ^{46}Sc: 830–965 KeV). All data were processed by a set of specially designed programs (Saxena *et al*., 1980), using 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 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). Vascular conductance was calculated by dividing blood flow (ml min^{-1}) by blood pressure (mmHg), multiplied by hundred and expressed as 10^{-2} ml min^{-1} mmHg^{-1}.

Experimental protocol

After a stabilization period of about 1 h, 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 determined. At this point the animals were divided into four groups, receiving an i.v. infusion (1 ml min^{-1} over a period of 5 min) of either vehicle (distilled water, 20% propylene glycol, v v^{-1}; $n=6$), SB224289 (1 mg kg^{-1}; $n=6$), SB224289 (3 mg kg^{-1}; $n=3$) or BRL15572 (1 mg kg^{-1}; $n=6$). After a waiting period of 15 min, all parameters were reassessed. Subsequently, sequential i.v. doses of sumatriptan (30, 100 and 300 μg kg^{-1}) were given to all animals every 20 min. Fifteen minutes after each dose of sumatriptan, all haemodynamic variables were assessed again. In the group treated with 3 mg kg^{-1} of SB224289, i.v. bolus injections of noradrenaline (0.1, 0.3, 1 and 3 μg kg^{-1}) were given at the start of the experiment (before SB224289) and at the end of the experiment (after SB224289 and the three doses of sumatriptan).

Data presentation and statistical analysis

All data have been expressed as mean±s.e.mean. The significance of the difference 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). The per cent changes caused by sumatriptan in animals treated with SB224289 or BRL15572 were compared to the corresponding responses in animals treated with vehicle by using Student's unpaired *t*-test. The peak changes induced by noradrenaline before and after 3 mg kg^{-1} of SB224289 were compared by Student's paired *t*-test. Statistical significance was accepted at $P<0.05$ (two-tailed).

Drugs

Apart from the anaesthetics, azaperone (Janssen Pharmaceuticals, Beerse, Belgium), midazolan hydrochloride (Hoffmann La Roche b.v., Mijdrecht, The Netherlands) and pentobarbitone sodium (Apharmo, Arnhem, The Netherlands), the compounds used in this study were: sumatriptan succinate (gift from Dr H.E. Connor, Glaxo Group Research, Stevenage, Hertfordshire, U.K.), SB224289 and BRL15572

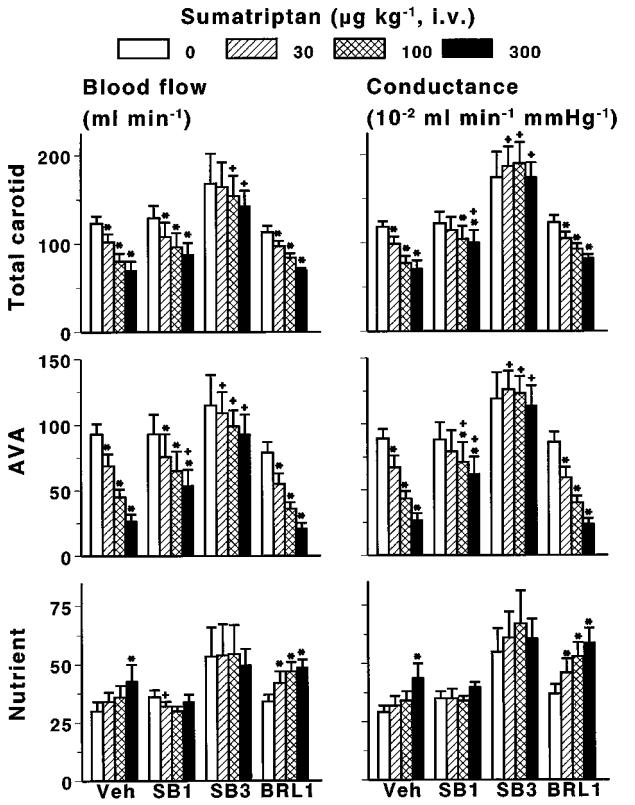

Figure 1 Values of total carotid, arteriovenous anastomotic (AVA) and nutrient blood flows (left panels) and conductances (right panels) at baseline and after sumatriptan (30, 100 and 300 μg kg^{-1}, i.v.) in animals treated i.v. with vehicle (Veh; $n=6$), 1 mg kg^{-1} of SB224289 (SB1; $n=6$), 3 mg kg^{-1} of SB224289 (SB3; $n=3$) or BRL15572 (BRL; 1 mg kg^{-1}; $n=6$). All values are presented as mean± s.e.mean. *$P<0.05$ vs baseline. $^{+}P<0.05$ vs response by corresponding dose in vehicle-treated animals.

(both gifts from Dr A.A. Parsons, SmithKline Beecham Pharmaceuticals, Harlow, Essex, U.K.) and noradrenaline bitatrate (Sigma Chemical Co., St. Louis, Mo., U.S.A.). All compounds were dissolved in distilled water; when needed 20% (v v^{-1}) propylene glycol (SB224289 and BRL15572) was added. Heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) was used to prevent clotting of the catheters. All doses refer to the respective salts.

Results

Systemic and carotid haemodynamic effects by vehicle, SB224289 or BRL15572

The effects of the two antagonists and vehicle on systemic (mean arterial blood pressure and heart rate) and carotid (total, arteriovenous anastomotic and nutrient vascular conductances and the difference between oxygen saturation of arterial and jugular venous blood; A-V SO_2) are shown in Table 2. Except for a moderate, but significant increase in mean arterial blood pressure (12±2%) and decrease in carotid arteriovenous anastomotic conductance (21±5%) after the 1 mg kg^{-1}, i.v. dose of SB224289, there were no changes in these variables.

Systemic haemodynamic effects of sumatriptan in pigs treated with vehicle, SB224289 or BRL15572

Systemic haemodynamic changes induced by sumatriptan in the four different treatment groups are depicted in Table 3. In vehicle-treated animals, sumatriptan slightly decreased heart rate (maximum decrease: 4±1%) and blood pressure (maximum decrease: 7±3%). The sumatriptan-induced bradycardia was not affected by treatment with either SB224289 (maximum decreases after 1 or 3 mg kg^{-1}: 5±1% or 4±1%, respectively) or BRL15572 (maximum decrease: 5±2%). On the other hand, after treatment with 1 mg kg^{-1} of SB224289, sumatriptan produced a significantly more pronounced hypotension (maximum decrease: 19±3%). Similarly, 3 mg kg^{-1} of SB224289 significantly potentiated the hypotension induced by 30 and 100 μg kg^{-1} of sumatriptan. Treatment with BRL15572 (1 mg kg^{-1}) did not affect the sumatriptan-induced decrease in blood pressure (maximum decrease: 7±5%).

Sumatriptan dose-dependently increased A-V SO_2 by up to 270±71%. The increases in A-V SO_2 by sumatriptan were not significantly modified by treatment with 1 mg kg^{-1} of SB224289 or BRL15572 (maximum increases: 161±55 and 151±38%, respectively), whereas after 3 mg kg^{-1} of SB224289 this effect was absent.

Carotid haemodynamic effects of sumatriptan in pigs treated with vehicle, SB224289 or BRL15572

Changes in carotid haemodynamics by sumatriptan in the four different groups are depicted in Figures 1 (absolute values) and 2 (per cent changes). In vehicle-treated animals, sumatriptan dose-dependently decreased total carotid blood flow and conductance by up to 44±7% and 40±7%, respectively, accompanied by decreases in arteriovenous anastomotic blood flow and conductance by up to 72±4% and 70±5%, respectively. Nutrient blood flow and conductance increased after sumatriptan by up to 55±25% and

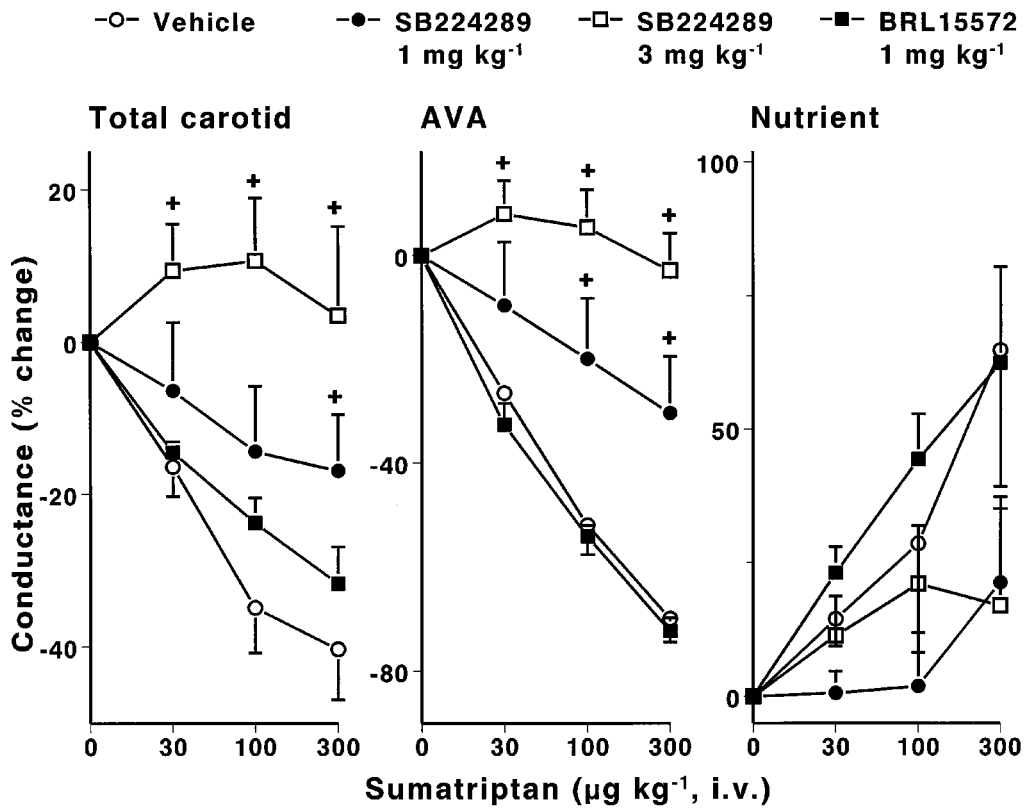

Figure 2 Effect (% change from baseline values) of sumatriptan (30, 100 and 300 μg kg^{-1}, i.v.) on total carotid, arteriovenous anastomotic (AVA) and nutrient conductances in animals treated with vehicle ($n=6$), 1 mg kg^{-1} of SB224289 ($n=6$), 3 mg kg^{-1} of SB224289 ($n=3$) or BRL15572 (1 mg kg^{-1}; $n=6$). All values are presented as mean±s.e.mean. $^{+}P<0.05$ vs response by corresponding dose in vehicle-treated animals.

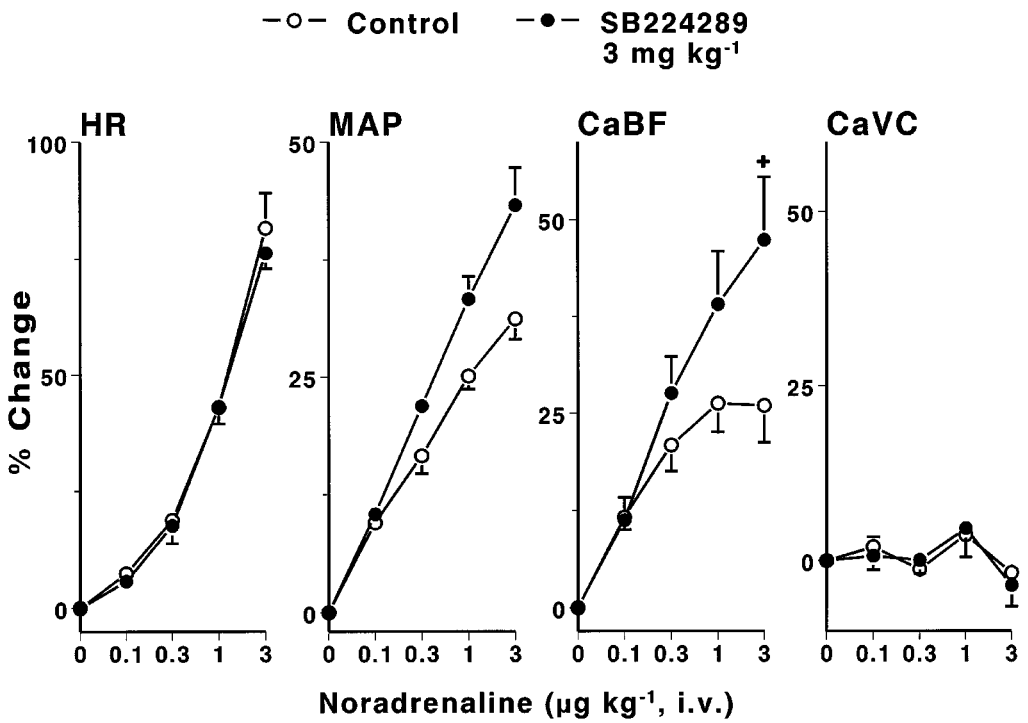

Figure 3 Effect (% change from baseline values) of noradrenaline (0.1, 0.3, 1 and 3 μg kg^{-1}, i.v.) on heart rate, mean arterial blood pressure (MAP), carotid blood flow (CaBF) and carotid vascular conductance (CaVC) before and after SB224289 (3 mg kg^{-1}, i.v.). All values are presented as mean$\pm$s.e.mean. $^{+}P<0.05$ after vs before.

65$\pm$26%, respectively. These effects of sumatriptan were dose-dependently reduced by SB224289. Thus, in animals treated with 1 mg kg^{-1} of SB224289 the sumatriptan-induced decreases in total carotid blood flow and conductance amounted to only 32$\pm$6% and 17$\pm$7%, respectively, while carotid arteriovenous anastomotic blood flow and conductance decreased by only up to 43$\pm$9% and 30$\pm$11%, respectively. After the higher dose (3 mg kg^{-1}) of SB224289, the sumatriptan-induced maximal decreases in total carotid and arteriovenous anastomotic blood flows (12$\pm$7% and 18$\pm$3%, respectively) and conductances (4$\pm$12% and 3$\pm$7%, respectively) were completely blocked. Treatment with BRL15572 (1 mg kg^{-1}) did not affect the sumatriptan-induced decreases in total carotid and arteriovenous anastomotic blood flows (maximum decreases: 37$\pm$3% and 74$\pm$3%, respectively) and conductances (maximum decreases: 32$\pm$5% and 72$\pm$3%, respectively). Neither SB224289 nor BRL15572 significantly affected the increases in nutrient blood flow and conductance.

Effect of SB224289 on noradrenaline-induced changes in heart rate, mean arterial blood pressure and total carotid blood flow and conductance

As shown in Figure 3, bolus injections of noradrenaline (0.1–3 μg kg^{-1}, i.v.) produced dose-dependent increases (with maximum changes) in heart rate (+82$\pm$7%), mean arterial blood pressure (+31$\pm$2%) and total carotid blood flow (+26$\pm$5%) without affecting total carotid vascular conductance (maximum change: +4$\pm$1%). The maximum changes by noradrenaline remained either unchanged (heart rate, 76$\pm$3%; mean arterial blood pressure, 43$\pm$4%; total carotid vascular conductance, 5$\pm$4%) or were even enhanced (total carotid blood flow, 47$\pm$8%) after SB224289 (3 mg kg^{-1}, i.v.).

Discussion

General

We have previously shown that sumatriptan decreases porcine carotid blood flow by a selective vasoconstriction of cranial arteriovenous anastomoses (Den Boer *et al*., 1991). The drug seems to exert this response *via* 5-HT$_{1B/1D}$ receptors, since GR127935, a selective antagonist at these 5-HT$_1$ receptor subtypes (Clitherow *et al*., 1994; Pauwels, 1996; Skingle *et al*., 1996), was able to abolish the sumatriptan-induced responses (De Vries *et al*., 1996). The recent availability of silent selective antagonists for the 5-HT$_{1B}$ (SB224289) and 5-HT$_{1D}$ (BRL15572) receptors led us to further analyse the nature of the receptors mediating these responses. The present study in anaesthetized pigs clearly showed that the sumatriptan-induced carotid arteriovenous anastomotic constriction was potently and specifically antagonized in a dose-dependent manner by the selective 5-HT$_{1B}$ receptor antagonist SB224289, but not by the selective 5-HT$_{1D}$ receptor antagonist BRL15572. Apart from the implications discussed below, these data indicate that the vasoconstrictor response to sumatriptan on porcine carotid arteriovenous anastomoses is mainly mediated by 5-HT$_{1B}$ receptors.

Systemic and carotid haemodynamic effects of vehicle, SB224289 and BRL15572

Statistically significant changes were noticed only in the group that received the lower dose (1 mg kg^{-1}) of SB224289. A moderate increase in mean arterial blood pressure and a vasoconstrictor effect in the carotid vasculature, confined to the arteriovenous anastomotic fraction, was observed. We do not have a clear explanation for this, but it is interesting to note that the 5-HT$_{1B/1D}$ receptor antagonist GR127935 also

constricts porcine arteriovenous anastomoses, but, in contrast to SB224289, decreases blood pressure (De Vries *et al*., 1996). Whereas the GR127935-induced carotid vasoconstriction may be related to its intrinsic activity at the h5-HT$_{1B}$ receptor (Pauwels, 1996; Selkirk *et al*., 1998), this seems unlikely in the case of SB224289, because this compound rather behaves as an inverse agonist at this receptor (Selkirk *et al*., 1998).

Systemic haemodynamic effects induced by sumatriptan in animals treated with vehicle, SB224289 or BRL15572

Sumatriptan caused a small bradycardiac and hypotensive effect, similar to that reported earlier (De Vries *et al*., 1996). This seems to be a class effect, since several other triptans also display this property (Saxena *et al*., 1997a; De Vries *et al*., 1998b; Willems *et al*., 1998). The mechanism involved in the hypotensive and bradycardiac action of sumatriptan is not clear, but may involve sympathoinhibition either at the level of ganglia, neurovascular junction (Jones *et al*., 1995; Villalón *et al*., 1998) or perhaps within the central nervous system (see Saxena & Villalón, 1990); the latter mechanism seems unlikely on the basis of poor central penetration of sumatriptan. The hypotensive response to sumatriptan was unaffected by GR127935 (De Vries *et al*., 1996), but potentiated by the 5-HT$_{1B}$ receptor antagonist SB224289 (Table 3), suggesting that sumatriptan simultaneously stimulates a systemic vasodilator and vasoconstrictor mechanism, of which the latter is amenable to blockade by SB224289. The sumatriptan-induced systemic dilatation may involve sympathoinhibition mediated by the 5-ht$_{1F}$ receptor, for which SB224289 displays a very low and GR127935 only a moderate affinity (see Table 1); this possibility requires further investigation. Whatever the mechanism, this effect of sumatriptan is clinically of little relevance. Sumatriptan (MacIntyre *et al*., 1993) as well as other similar drugs such as rizatriptan (Sciberras*et al*., 1997), alniditan (Goldstein *et al*., 1996) and zolmitriptan (Seaber *et al*., 1996) produce increases rather than decreases in blood pressure in humans.

Carotid haemodynamics

Sumatriptan decreased total carotid blood flow, mainly due to a potent vasoconstrictor action on the cephalic arteriovenous anastomoses. The effect was similar to that observed earlier with this antimigraine agent (Den Boer *et al*., 1991; De Vries *et al*., 1996). In animals treated with 1 mg kg^{-1} of the 5-HT$_{1B}$ receptor ligand SB224289, the sumatriptan-induced decreases in total carotid and carotid arteriovenous anastomotic blood flow were not much affected; only at 300 μg kg^{-1} of sumatriptan a significant attenuation was observed (see Figure 1). However, as described above, in the presence of SB224289 an enhancement of the sumatriptan-induced hypotension was observed. The latter will decrease the carotid perfusion pressure and, consequently, may exaggerate decreases in blood flow, thereby masking possible inhibition of the sumatriptan-induced effects by SB224289. Indeed, the decreases in total carotid and carotid arteriovenous anastomotic conductance (where the changes in blood flow are corrected for changes in blood pressure) were potently antagonized, although not completely eliminated, in animals treated with 1 mg kg^{-1} SB224289 (Figure 3). In contrast, 1 mg kg^{-1} of the 5-HT$_1$ receptor antagonist BRL15572 did not affect the carotid vascular effects of sumatriptan in any way. As SB224289 and BRL15572 display similar affinities at their respective receptors (Table 1), the lack of inhibitory effects by BRL15572, combined with the potent blockade by SB224289 at similar doses, it is reasonable to assume that 5-HT$_{1B}$, but not 5-HT$_{1D}$ receptors, are involved in the vasoconstriction of carotid arteriovenous anastomoses. Admittedly, this conclusion is based on the assumption that species differences between the binding of SB224289 and BRL15572 to porcine and human 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors do not play a major role.

Since a part of the sumatriptan-induced carotid arteriovenous anastomotic constriction persisted after 1 mg kg^{-1} of SB224289, it may suggest that other receptors/mechanisms may be involved. Notwithstanding, we have previously shown that 0.5 mg kg^{-1}, but not 0.25 mg kg^{-1} of the 5-HT$_{1B/1D}$ receptor antagonist GR127935 was needed for a complete blockade of the sumatriptan-induced porcine carotid vasoconstrictor effects (De Vries *et al*., 1996). In view of the 10 fold higher affinity displayed at the h5-HT$_{1B}$ receptor by GR127935 compared to SB224289 (Table 1), it can be expected that higher doses of SB224289 are needed to bring about a complete blockade. Indeed, 3 mg kg^{-1} of SB224289 completely abolished all sumatriptan-induced carotid vascular effects. In keeping with this, we have previously shown that 3–10 fold higher concentrations of SB224289 (De Vries *et al*., 1998a) than GR127935 (Villalón *et al*., 1996) were required to completely inhibit the canine external carotid vasoconstriction by sumatriptan.

In order to ascertain the specificity of SB224289 (3 mg kg^{-1}), we decided to study cardiovascular responses to noradrenaline before and after administration of the antagonist. As expected, noradrenaline produced short-lasting increases in mean arterial blood pressure, which were accompanied by increased heart rate, as the baroreflex mediated bradycardia was absent due to vagotomy (see Hoffman & Lefkowitz, 1996). Moreover, noradrenaline increased carotid blood flow, which was mainly due to the hypertensive effect, as carotid vascular conductance did not change in response to the drug, as shown earlier (Verdouw *et al*., 1984). SB224289 did not attenuate these effects; in fact, the noradrenaline-induced hypertension was potentiated, probably resulting in an enhancement of the increases in carotid blood flow. Therefore, it is concluded that 3 mg kg^{-1} of SB224289 most likely produced a specific antagonism against the sumatriptan-induced effects. In keeping with this, SB224289 displays low affinities at α- and β-adrenoceptors (Gaster *et al*., 1998).

Taking the above into account, the present results imply that sumatriptan constricts porcine arteriovenous anastomoses primarily *via* the 5-HT$_{1B}$ receptor, which in contrast to the 5-HT$_{1D}$ receptor is abundantly expressed on vascular smooth muscle (Ullmer *et al*., 1995; Bouchelet *et al*., 1996; Longmore *et al*., 1997). Moreover, the results obtained in this study imply that the so-called 5-HT$_1$-like receptor mediating vascular smooth muscle contraction (Saxena *et al*., 1998), including porcine carotid arteriovenous anastomotic constriction (Den Boer *et al*., 1991), is most likely to be identical to the 5-HT$_{1B}$ receptor. This is also shown in the isolated human temporal artery (Verheggen *et al*., 1998) and the canine external carotid vascular bed (De Vries *et al*., 1998a). Additionally, in view of the complete blockade by SB224289 and the highly selective nature of the compound, it seems unlikely that the other known 5-HT$_1$ subtypes (5-HT$_{1A}$, 5-ht$_{1E}$ and 5-ht$_{1F}$) are involved in the sumatriptan-induced carotid vascular effects. Thus, intracarotid infusions of LY344864 (1–3100 μg min^{-1}), a selective 5-ht$_{1F}$ receptor agonist (Phebus *et al*., 1997), does not produce vasoconstriction in the canine external carotid vascular bed (Villalón *et al*., 1999). Also, mRNAs for 5-HT$_{1A}$ and 5-ht$_{1E}$ receptor have not been detected in the vascular smooth muscle (Ullmer *et al*., 1995).

In conclusion, the results of the present experiments show that the constriction of porcine carotid arteriovenous anastomoses by the 5-HT$_1$ receptor agonist sumatriptan, being antagonized by the selective 5-HT$_{1B}$ receptor antagonist SB224289, but not by the 5-HT$_{1D}$ receptor ligand BRL15572, is predominantly mediated by 5-HT$_{1B}$ receptors. In view of the putative pathophysiological role of arteriovenous anastomotic dilatation in migraine (see Heyck, 1969; Saxena, 1995), the constriction of these nonnutrient vessels by sumatriptan *via* a 5-HT$_{1B}$ receptor mechanism may be, at least partly, responsible for the therapeutic effect of the drug in migraine.

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