Carotid haemodynamics in pigs during infusions of 8-OH-DPAT: reduction in arteriovenous shunting is mediated by 5-HT₁-like receptors

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1 The effects of intracarotid infusions of 8-hydroxy-2-[di-n-propyl-amino]-tetralin (8-OH-DPAT) on heart rate, blood pressure and carotid blood flow and its distribution were studied in anaesthetized pigs by use of radioactive microspheres of 15 μ m diameter.

2 Control experiments with physiological saline showed that systemic and carotid haemodynamics remain essentially unchanged during the experimental period. In contrast to results obtained in rat, cat and dog experiments, 8-OH-DPAT did not decrease arterial blood pressure.

3 8-OH-DPAT, which has a high affinity and is selective for the 5-HT_{1A} recognition site, caused a dose-related decrease in arteriovenous anastomotic (non-nutrient) blood flow, resulting in a decrease in carotid blood flow. At the highest dose used, a small increase in arteriolar (nutrient) blood flow was observed.

4 The decrease in arteriovenous anastomotic and carotid blood flow induced by 8-OH-DPAT was not significantly modified by pretreatment with the 5-HT₂ receptor antagonist ketanserin (0.5 mg kg⁻¹), but was markedly reduced by pretreatment with methiothepin (1 mg kg⁻¹), which blocks both the 5-HT₁-like and 5-HT₂ receptors.

5 It is concluded that the effects of 8-OH-DPAT on arteriovenous anastomotic blood flow are mediated by 5-HT₁-like receptors. These receptors, however, cannot yet be classified as belonging to 5-HT_{1A} receptor subtype. Since a number of antimigraine drugs reduce arteriovenous shunting, it is tempting to suggest that 8-OH-DPAT may have similar clinical efficacy.

Introduction

Intracarotid administration of 5-hydroxytryptamine (5-HT) in the anaesthetized pig decreases arteriovenous anastomotic (non-nutrient) blood flow but increases arteriolar (nutrient) blood flow. The increase in arteriolar blood flow is especially marked in the ears and skin which change to bright red colour (Saxena & Verdouw, 1982). These effects of 5-HT are antagonized by methiothepin, which blocks both 5-HT₁-like and 5-HT₂ receptors, but not by the 5-HT₂ receptor antagonist ketanserin or the 5-HT₃ receptor antagonist MDL 72222 (Verdouw *et al.*, 1984). Furthermore, the effects of 5-HT are mimicked by 5-carboxamidotryptamine (Saxena & Verdouw, 1985). Therefore, according to the recent criteria for the classification of functional

5-HT receptors (Bradley *et al.*, 1986), 5-HT₁-like receptors mediate the constriction of arteriovenous anastomoses as well as the dilatation of arterioles (Saxena *et al.*, 1986).

The 5-HT₁-like receptors in the carotid circulation appear to be heterogeneous. Methysergide (Saxena & Verdouw, 1984) and BEA 1654 (Verdouw *et al.*, 1985), which have affinity for 5-HT₁-like binding sites, reduce arteriovenous shunting but, unlike 5-HT (Saxena & Verdouw, 1982) or 5-carboxamidotryptamine (Saxena & Verdouw, 1985), only slightly increase arteriolar blood flow. More recently, another 5-HT₁-like receptor agonist, GR 43175, has been described. This compound lacks 5-HT₁-like receptor-mediated vascular and intestinal smooth muscle relaxant actions, but contracts dog saphenous vein and inhibits noradrenaline release

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from sympathetic nerve terminals (Humphrey *et al.*, 1988). GR 43175 also reduces arteriovenous anastomotic blood flow without affecting arteriolar blood flow in the carotid circulation of the cat (Feniuk *et al.*, 1987).

The subdivision of functional 5-HT receptors into 5-HT₁-like, 5-HT₂ and 5-HT₃ receptors has been partially based on results from radioligand binding studies. Peroutka & Snyder (1979) demonstrated that two distinct 5-HT recognition sites exist in the central nervous system. The sites labelled by [³H]-5-HT were designated as 5-HT₁-‘receptors’ and those labelled by [³H]-spiperone were called 5-HT₂-‘receptors’; the former have been further subdivided into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} recognition sites (Pedigo *et al.*, 1981; Pazos & Palacios, 1985; Heuring & Peroutka, 1987). In contrast to the good agreement between 5-HT₂ recognition sites and functional 5-HT₂ receptors, it is still unclear whether the subtypes of the 5-HT₁ recognition site correspond to 5-HT₁-like receptors, which undoubtedly, are heterogeneous (Bradley *et al.*, 1986; Humphrey & Richardson, 1988). The barrier here is the lack of sufficiently selective compounds to discriminate unequivocally between the different 5-HT₁ recognition site subtypes. One possible exception appears to be the 5-HT_{1A} recognition site where 8-OH-DPAT displays a high affinity and about 1000 fold selectivity compared to the other subtypes of 5-HT₁ recognition sites (Middlemiss & Fozard, 1983; Hoyer *et al.*, 1985; Heuring & Peroutka, 1987). Therefore, in an attempt to characterize further the 5-HT₁-like receptors in the porcine carotid circulation we studied the effects of 8-OH-DPAT on carotid blood flow and its distribution in the anaesthetized pig.

Methods

General

After an overnight fast young Yorkshire pigs (body weight: 19–29 kg) were sedated with 120 mg (i.m.) azaperone and 120–150 mg (i.v.) metomidate, intubated and connected to a respirator for intermittent positive pressure ventilation with a mixture (1:2) of oxygen and nitrous oxide. A continuous infusion of pentobarbitone sodium (20–26 mg kg⁻¹ h⁻¹, i.v.) completed the anaesthesia. Respiratory rate and tidal volume were adjusted to keep arterial blood gases and pH within normal limits (pH, 7.35–7.45; P_{O₂}, 90–150 mmHg; P_{CO₂}, 35–45 mmHg). Aortic blood pressure was recorded with a Statham pressure transducer (P23 ID) connected to a cannula inserted into the right femoral artery. The common carotid arteries were dissected free and a bilateral

cervical vagosympathectomy was performed to reduce reflex influences on the carotid circulation. Blood flow in one of the common carotid arteries was measured with a precalibrated flow probe (2.5 or 3 mm, i.d.) connected to a sine-wave electromagnetic blood flow meter (Transflow 601-system, Skalar, Delft, The Netherlands). After removing its hub, a 0.5 mm (external diameter) needle, connected to suitable polyethylene tubing, was inserted into this common carotid artery for the administration of microspheres and drugs. The animals were insulated with a blanket to maintain body temperature at 37–38°C.

Distribution of common carotid blood flow

The distribution of common carotid blood flow was determined with the radioactive microsphere method using 15 ± 1 (s.d.) μ m diameter spheres labelled with either ¹⁴¹Ce, ¹¹³Sn, ¹⁰³Ru, ⁹⁵Nb or ⁴⁶Sc (NEN Company, Dreieich, Federal Republic of Germany). For each measurement a suspension of microspheres, labelled with one of the nuclides, was mechanically agitated and injected against the direction of the carotid blood flow to facilitate uniform mixing with the blood. At the end of the experiment the animal was killed and the heart, kidneys, lungs and the various tissues of the head were dissected out, weighed and placed in vials. The radioactivity in these vials was counted for 5–10 min in a γ -scintillation counter (Packard, Minaxi Autogamma 5000) equipped with a multichannel analyzer using suitable windows for discriminating the different isotopes. The amount of carotid blood flow distributed to the individual tissues ($Q_{tis[car]}$) was calculated as described earlier (Saxena *et al.*, 1980; Saxena & Verdouw, 1982) by: $Q_{tis[car]} \text{ (ml min}^{-1}\text{)} = (I_{tis}/I_{tot}) \times Q_{[car]}$, where I_{tis} and I_{tot} are, respectively, the radioactivity (c.p.m.) in a particular tissue and that detected in all tissues, and $Q_{[car]}$ is carotid blood flow (ml min⁻¹). Because there was complete trapping of microspheres passing through the cranial circulation in the capillaries of the lungs (no significant amounts of radioactivity were detected in the heart or kidneys), the values determined for the lungs provide an index of the arteriovenous anastomotic fraction of the common carotid arterial blood flow (see Johnston & Saxena, 1978; Saxena & Verdouw, 1982).

Experimental protocol

Four series of experiments ($n = 6$ each) were performed. In the first series the effects of 4 consecutive intracarotid infusions of physiological saline on carotid blood flow and its distribution were determined. In the following series, the effects of intracarotid infusions of 8-OH-DPAT on carotid blood

flow distribution were assessed in untreated animals (Series 2) and in animals that were pretreated with either 0.5 mg kg^{-1} ketanserin (Series 3) or 1 mg kg^{-1} methiothepin (Series 4) administered at a rate of 1 ml min^{-1} during a period of 10 min, followed by a 10 min period of intracarotid infusion of physiological saline.

In all experiments the baseline values were determined after the preparation had been stable for at least 45 min after completion of the surgical procedure. The measurements consisted of the recording of the electrocardiogram, arterial blood pressure and common carotid blood flow, while a batch of microspheres (100,000–150,000) was injected for the determination of tissue (arteriolar) and arteriovenous anastomotic blood flows. Subsequently, in Series 2–4 the measurements were repeated after consecutive 10 min intracarotid infusions of 8-OH-DPAT, 0.3, 1.0, 3.0 and $10.0 \mu\text{g kg}^{-1} \text{ min}^{-1}$ at the rate of 1 ml min^{-1} , always given in this order. In the first series, in which only physiological saline was infused, an identical protocol was followed.

Statistical analysis

The significance of the differences between variables was evaluated by 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). Statistical significance was accepted at $P < 0.05$ (two-tailed). All data are expressed as means $\pm$ s.e.mean.

Drugs

Apart from the anaesthetics, the drugs used in this study were: 8-OH-DPAT (8-hydroxy-2-[di-n-propyl-amino]-tetralin (courtesy of Dr. J. Traber, Troponwerke, Cologne, Federal Republic of Germany), ketanserin tartrate (courtesy of Dr J.M. Van Nueten,

Janssen Pharmaceutica, Beerse, Belgium) and methiothepin maleate (courtesy of Dr P.J. Pijper, Hoffman La Roche B.V., Mijdrecht, The Netherlands). The doses of 8-OH-DPAT are in terms of the base, whereas those of ketanserin and methiothepin refer to the salt.

Results

Physiological saline experiments

The effects of four consecutive infusions of physiological saline on heart rate, mean arterial blood pressure, carotid blood flow and its distribution are summarized in Table 1. Heart rate and blood pressure showed a tendency to decrease, but a statistically significant change was only observed in heart rate after the last saline infusion. Total carotid blood flow and arteriovenous anastomotic blood flow tended to increase, but the changes were not statistically significant. Nutrient (arteriolar) blood flow remained unaffected.

8-OH-DPAT experiments

Systemic haemodynamics The values for heart rate, mean arterial blood pressure and carotid blood flow before and after the infusion of either ketanserin or methiothepin are shown in Table 2. Both mean arterial blood pressure and heart rate decreased after intracarotid infusion of ketanserin (0.5 mg kg^{-1}), whereas after methiothepin infusion (1 mg kg^{-1}) only mean arterial blood pressure was lowered. Carotid blood flow was not changed significantly by infusion of either antagonist. In Table 3 are summarized the values of mean arterial blood pressure and heart rate recorded during the intracarotid infusions of 8-OH-DPAT. 8-OH-DPAT did not affect mean arterial blood pressure, but decreased heart rate in both

Table 1 Mean arterial blood pressure, heart rate and carotid blood flow and its distribution during four consecutive infusions of physiological saline

	Baseline	First	Saline infusions		
			Second	Third	Fourth
MAP	97 ± 12	92 ± 11	91 ± 11	88 ± 11	87 ± 11
HR	109 ± 13	109 ± 12	105 ± 12	102 ± 11	$99 \pm 10^*$
CBF	108 ± 17	108 ± 20	115 ± 20	123 ± 22	124 ± 23
AVA	87 ± 18	89 ± 20	95 ± 21	100 ± 23	102 ± 24
Nutrient	20 ± 3	19 ± 2	20 ± 3	22 ± 3	22 ± 3

Saline was infused for 10 min periods at a rate of 1 ml min^{-1} ($n = 6$). Abbreviations and units: MAP, mean arterial blood pressure (mmHg); HR, heart rate (beats min^{-1}); CBF, total carotid blood flow (ml min^{-1}); AVA, arteriovenous anastomotic blood flow (ml min^{-1}); Nutrient, arteriolar blood flow (ml min^{-1}).

* Significantly different ($P < 0.05$) from the baseline value.

Table 2 Mean arterial blood pressure, heart rate and carotid blood flow before and after ketanserin and methiothepin

	Antagonist pretreatment	Baseline	Immediately after antagonist infusion	10 min after antagonist infusion
MAP	Ketanserin	94 ± 7	85 ± 7*	84 ± 7*
	Methiothepin	98 ± 10	86 ± 10*	80 ± 7*
HR	Ketanserin	114 ± 8	107 ± 6	100 ± 5*
	Methiothepin	100 ± 8	97 ± 8	96 ± 8
CBF	Ketanserin	125 ± 14	111 ± 14	112 ± 13
	Methiothepin	102 ± 8	105 ± 11	97 ± 9

Ketanserin (0.5 mg kg⁻¹; *n* = 6) or methiothepin (1 mg/kg⁻¹; *n* = 6) was infused into the carotid artery at a rate of 1 ml min⁻¹ during a period of 10 min. Thereafter an infusion of physiological saline (1 ml min⁻¹) was given. Abbreviations and units: MAP, mean arterial blood pressure (mmHg); HR, heart rate (beats min⁻¹); CBF, carotid blood flow (ml min⁻¹).

* Significantly different (*P* < 0.05) from the baseline value.

untreated and ketanserin- or methiothepin-pretreated animals during intracarotid infusions of the three highest doses (1, 3 and 10 µg kg⁻¹).

Carotid blood flow and its distribution The effects of intracarotid infusions of 8-OH-DPAT on carotid blood flow and its fractionation over non-nutrient, arteriovenous anastomotic blood flow and nutrient, arteriolar blood flow are shown in Figure 1. As was the case in the experiments with physiological saline, a large fraction (70%) of the total carotid blood flow was diverted through the arteriovenous anastomoses in untreated as well as in ketanserin- or methiothepin-pretreated animals before the infusions of 8-OH-DPAT were started. In the untreated and ketanserin-pretreated animal, 8-OH-DPAT infusions induced a dose-dependent decrease in total carotid blood flow, which was directly related to a decrease in arteriovenous anastomotic blood flow. Only at

the highest dose tested did arteriolar blood flow show an increase in the untreated animals, whereas in the ketanserin-pretreated animals arteriolar blood flow tended to decrease. In the methiothepin-pretreated animals the effects of 8-OH-DPAT infusions on total carotid and arteriovenous anastomotic blood flow were markedly reduced. Furthermore, a small but dose-related decrease in arteriolar blood flow was observed.

Analysis of regional carotid blood flow distribution showed that 8-OH-DPAT infusion at the highest dose caused an increase in blood flow to the skin, ears, tongue, bones and brain in the untreated animals (Figure 2). No changes in colour of the skin and ears, as seen with 5-HT and 5-carboxamidotryptamine (Saxena & Verdouw, 1982; 1985), were observed during the experiments. In the group pretreated with ketanserin, blood flow to the skin and ears increased at the highest dose of 8-OH-

Table 3 Effect of intracarotid infusions of 8-hydroxy-2[di-n-propyl-amino]tetralin (8-OH-DPAT) on mean arterial blood pressure and heart rate

	Antagonist pretreatment	0	8-OH-DPAT (µg kg ⁻¹ min ⁻¹)			
			0.3	1.0	3.0	10.0
MAP	None	79 ± 7	78 ± 7	76 ± 6	76 ± 7	75 ± 6
	Ketanserin	84 ± 7	84 ± 8	84 ± 8	84 ± 8	85 ± 8
	Methiothepin	80 ± 7	80 ± 7	78 ± 7	74 ± 5	74 ± 6
HR	None	109 ± 7	104 ± 6	99 ± 5*	93 ± 4*	88 ± 3*
	Ketanserin	100 ± 5	96 ± 6	92 ± 5*	90 ± 4*	85 ± 2*
	Methiothepin	96 ± 8	94 ± 8	90 ± 8*	86 ± 6*	82 ± 5*

8-OH-DPAT was infused in 10 min periods at a rate of 1 ml min⁻¹ in untreated (*n* = 6), ketanserin (0.5 mg kg⁻¹; *n* = 6) or methiothepin (1 mg kg⁻¹; *n* = 6) pretreated animals. Abbreviations and units: MAP, mean arterial blood pressure (mmHg); HR, heart rate (beats min⁻¹).

* Significantly different (*P* < 0.05) from the value before the administration of 8-OH-DPAT.

Figure 1 Total carotid blood flow, arteriovenous anastomotic (AVA) blood flow and arteriolar (nutrient) blood flow in untreated (control) and ketanserin (0.5 mg kg^{-1}) or methiothepin (1 mg kg^{-1}) pretreated pigs ($n = 6$ each) before and after intracarotid infusions of increasing doses of 8-hydroxy-2-[di-n-propyl-amino]tetralin (8-OH-DPAT). * $P < 0.05$ vs baseline.

DPAT, but blood flow to the tongue, bones and brain remained unchanged, while that to the skeletal muscles and eyes was reduced. Pretreatment with methiothepin prevented the 8-OH-DPAT-induced increase in blood flow to the skin, ears, tongue and brain, whereas blood flow to the muscles, bones and eyes decreased.

Discussion

Validity of the experimental model

Our experiments, in which four consecutive intracarotid infusions of physiological saline were given to the anaesthetized pig, clearly show that no appreciable changes occur in either systemic or carotid haemodynamics over a period of about 40–60 min.

Similarly, we have previously shown that within minutes of stopping the infusions of 5-HT, which is quickly metabolised, the distribution of carotid blood flow is restored to that observed during the baseline period (Saxena & Verdouw, 1982; 1984; Verdouw *et al.*, 1984). Therefore, we can conclude that no time-dependent changes or changes due to the injection of microspheres themselves are seen in carotid haemodynamics during the experimental period in the animal model used here.

Effects of 8-OH-DPAT

In contrast to the rat (Gradin *et al.*, 1985; Martin & Lis, 1985; Fozard *et al.*, 1987), cat (Ramage & Fozard, 1987; McCall *et al.*, 1987; Doods *et al.*, 1988) and dog (Di Francesco *et al.*, 1988), 8-OH-DPAT did not lower blood pressure in the anaesthetized pig. Whether this is caused by differences in species, anaesthesia or the level of baseline arterial blood pressure, remains unclear. The decrease in heart rate observed in our experiments cannot be unequivocally attributed to an action of 8-OH-DPAT, since this was also observed in experiments in which only physiological saline was infused. The use of nitrous oxide as an anaesthetic might be an explanation for this phenomenon. Nitrous oxide is reported to reduce heart rate and cardiac output with systemic arterial blood pressure being maintained by increased total peripheral resistance (Eisele & Smith, 1972).

Intracarotid infusion of 8-OH-DPAT, which is a selective agonist at 5-HT_{1A} receptors (Middlemiss & Fozard, 1983; Hoyer *et al.*, 1985), caused a dose-related decrease in carotid blood flow in the anaesthetized pig. This effect on carotid blood flow, unrelated to changes in mean arterial blood pressure, was associated with a selective decrease in the arteriovenous anastomotic fraction. Pretreatment with methiothepin, but not with ketanserin, considerably reduced the effects of 8-OH-DPAT, indicating that 8-OH-DPAT was acting on 5-HT_{1A} -like receptors.

The increase in tissue blood flow to the skin and ears observed during the infusion of the highest dose of 8-OH-DPAT is probably also mediated by 5-HT_{1A} -like receptors since this effect was antagonized by methiothepin but not by ketanserin. However, it should be noted that like methysergide and BEA 1654 (Saxena & Verdouw, 1984; Verdouw *et al.*, 1985) and GR 43175 (Feniuk *et al.*, 1987), but in contrast to 5-HT and 5-carboxamidotryptamine (Saxena & Verdouw, 1982, 1985), the effect of 8-OH-DPAT on total nutrient (arteriolar) blood flow was not marked. Compatible with this difference, intracarotid infusions of 5-HT and 5-carboxamidotryptamine changed the colour of the

Figure 2 The distribution of carotid blood flow to various cranial tissues in untreated (control), ketanserin (0.5 mg kg^{-1}) or methiothepin (1 mg kg^{-1}) pretreated pigs ($n = 6$ each) before and after intracarotid infusions of increasing doses of 8-hydroxy-2-[di-*n*-propyl-amino]tetralin (8-OH-DPAT). * $P < 0.05$ vs baseline.

skin and ears to bright red (Saxena & Verdouw, 1982; 1985), but infusions of 8-OH-DPAT did not. The above findings are suggestive of heterogeneity of the 5-HT₁-like receptors mediating the constriction of arteriovenous anastomoses and the dilatation of arterioles in the carotid vascular bed.

The selectivity of 8-OH-DPAT for the 5-HT_{1A} recognition sites (Middlemiss & Fozard, 1983; Hoyer *et al.*, 1985) obviously tempts one to suggest that the 5-HT₁-like receptors mediating the reduction of arteriovenous blood flow belong to the 5-HT_{1A} subtype. However, it is still unclear whether this is so, since no selective antagonists for the putative 5-HT_{1A} receptor are as yet available. Moreover, in initial experiments, ipsapirone, which also has a high affinity for the 5-HT_{1A} recognition site (Peroutka, 1986; Traber & Glaser, 1987) and reduces blood pressure in anaesthetized cats by an agonist action on putative 5-HT_{1A} receptors in the brain (Ramage & Fozard, 1987), was found to be inactive in our experimental model (Bom *et al.*, 1988). It remains possible that the inactivity of ipsapirone is

due to a low efficacy of the drug at the putative 5-HT_{1A} receptors or to some differences in 5-HT₁ recognition sites in the brain and in the carotid circulation.

Lastly we would like to draw some attention to the possible clinical relevance of the main finding of this study, i.e. the constriction of arteriovenous anastomoses by 8-OH-DPAT. It should be recalled that dilatation of arteriovenous anastomoses has been suggested to form part of the pathophysiology of migraine headache (Heyck, 1969; Saxena, 1978; 1987). Indeed, a number of proven and effective anti-migraine drugs, including ergotamine, methysergide and isometheptene (Johnston & Saxena, 1978; Spierings & Saxena, 1980a,b; Saxena & Verdouw, 1984; Saxena, 1987), constrict arteriovenous anastomoses. Also, GR 43175, the newly developed agonist at 5-HT₁-like receptors (Humphrey *et al.*, 1988), reduces arteriovenous anastomotic blood flow (Feniuk *et al.*, 1987) and has shown promise in the treatment of acute attacks of migraine (Doenicke *et al.*, 1988). It is therefore possible that 8-OH-DPAT

may also have an antimigraine action. However, we do not know how the behavioural effects of 8-OH-DPAT described in animals (Tricklebank, 1985) would manifest in man.

In conclusion the present study demonstrates that 8-OH-DPAT, which has a high affinity and is selective for 5-HT_{1A} recognition sites, decreases common carotid arterial blood flow in the pig by selectively reducing arteriovenous anastomotic blood flow. Since methiothepin, but not ketanserin, antagonized

the 8-OH-DPAT-induced constriction of arteriovenous anastomoses, this response is mediated by 5-HT₁-like receptors. It is, however, not yet certain whether these 5-HT₁-like receptors correlate with the 5-HT_{1A} recognition sites.

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References

- BOM, A.H., SAXENA, P.R. & VERDOUW, P.D. (1988). Further characterization of the 5-HT₁-like receptors in the carotid circulation of the pig. *Br. J. Pharmacol.*, **94**, 327P.
- BRADLEY, P.B., ENGEL, G., FENIUK, W., FOZARD, J.R., HUMPHREY, P.P.A., MIDDLEMISS, D.N., MYLECHARANE, E.J., RICHARDSON, B.P. & SAXENA, P.R. (1986). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacol.*, **25**, 563–576.
- DI FRANCESCO, G.F., PETTY, M.A. & FOZARD, J.R. (1988). Antihypertensive effects of 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) in conscious dogs. *Eur. J. Pharmacol.*, **147**, 287–290.
- DOENICKE, A., BRAND, J. & PERRIN, V.L. (1988). Possible benefit of GR43175, a novel 5-HT₁-like receptor antagonist, for the treatment of severe migraine. *Lancet*, **i**, 1309–1311.
- DOODS, H.N., BODDEKE, H.W.G.M., KALKMAN, H.O., HOYER, D., MATHY, M.-J. & VAN ZWIETEN, P.A. (1988). Central 5-HT_{1A} receptors and the mechanism of the central hypotensive effect of (+)8-OH-DPAT, DP-5CT, R28935, and urapidil. *J. Cardiovasc. Pharmacol.*, **11**, 432–437.
- EISELE, J.H. & SMITH, N.T. (1972). Cardiovascular effects of 40% nitrous oxide in man. *Anesth. Analg.*, **51**, 956–963.
- FENIUK, W., HUMPHREY, P.P.A. & PERREN, M.J. (1987). Selective vasoconstrictor action of GR 43175 on arteriovenous anastomoses (AVAs) in the anaesthetized cat. *Br. J. Pharmacol.*, **92**, 756P.
- FOZARD, J.R., MIR, A.K. & MIDDLEMISS, D.W. (1987). The cardiovascular response to 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) in the rat: site of action and pharmacological analysis. *J. Cardiovasc. Pharmacol.*, **9**, 328–347.
- GRADIN, K., PETERSSON, A., HEDNER, T. & PERSSON, B. (1985). Acute administration of 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), a selective 5-HT₁-receptor agonist, causes a biphasic blood pressure response and bradycardia in the normotensive Sprague-Dawley rat and the spontaneously hypertensive rat. *J. Neural. Transm.*, **62**, 305–319.
- HEURING, R.E. & PEROUTKA, S.J. (1987). Characterization of a novel ³H-5-hydroxytryptamine binding site subtype in bovine brain membranes. *J. Neurosci.*, **7**, 894–903.
- HEYCK, H. (1969). Pathogenesis of migraine. *Res. Clin. Stud. Headache*, **2**, 1–28.
- HOYER, D., ENGEL, E. & KALKMAN, H.O. (1985). Molecular pharmacology of 5-HT₁ and 5-HT₂ recognition sites in rat and pig brain membranes: radioligand binding studies with [³H]5-HT, [³H]8-OH-DPAT, (–)[¹²⁵I]iodocyanopindolol, [³H]mesulergine and [³H]ketanserin. *Eur. J. Pharmacol.*, **118**, 13–23.
- HUMPHREY, P.P.A. & RICHARDSON, B.P. (1989). 5-HT receptor classification: a current view based on a workshop debate. In *Serotonin*, ed. Mylecharane, E.J., Angus, J.A., De La Lande, I. & Humphrey, P.P.A., Basingstoke: Macmillan, (in press).
- HUMPHREY, P.P.A., FENIUK, W., PERREN, M.J., CONNOR, H., OXFORD, A.W., COATES, I.H. & BUTINA, D. (1988). GR 43175, a selective agonist for the 5-HT₁-like receptor in dog isolated saphenous vein. *Br. J. Pharmacol.*, **94**, 1123–1132.
- JOHNSTON, B.M. & SAXENA, P.R. (1978). The effect of ergotamine on tissue blood flow and the arteriovenous shunting of radioactive microspheres in the head. *Br. J. Pharmacol.*, **63**, 541–549.
- MARTIN, G.E. & LIS, E.V. (1985). Hypotensive action of 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) in spontaneously hypertensive rats. *Arch. Int. Pharmacodyn.*, **273**, 251–261.
- MCCALL, R.B., PATEL, B.N. & HARRIS, L.T. (1987). Effects of serotonin₁ and serotonin₂ receptor agonists and antagonists on blood pressure, heart rate and sympathetic nerve activity. *J. Pharmacol. Exp. Ther.*, **242**, 1152–1159.
- MIDDLEMISS, D. & FOZARD, J. (1983). 8-hydroxy-2-(di-n-propylamino)-tetralin discriminates between subtypes of the 5-HT₁ recognition site. *Eur. J. Pharmacol.*, **90**, 151–153.
- PAZOS, A. & PALACIOS, J.M. (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. 1. Serotonin-1 receptors. *Brain Res.*, **346**, 205–230.
- PEDIGO, N.W., YAMAMURA, H.I. & NELSON, D.L. (1981). Discrimination of multiple [³H]5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. *J. Neurochem.*, **36**, 220–226.
- PEROUTKA, S.J. (1986). Pharmacological differentiation and characterization of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} binding sites in rat frontal cortex. *J. Neurochem.*, **47**, 529–540.
- PEROUTKA, S.J. & SNYDER, S.H. (1979). Multiple serotonin receptors. Differential binding of [³H]5-hydroxytryptamine, [³H]lysergic acid diethylamide and [³H]-spiroperidol. *Molec. Pharmacol.*, **16**, 687–699.
- RAMAGE, A.G. & FOZARD, J.R. (1987). Evidence that the putative 5-HT_{1A} receptor agonists, 8-OH-DPAT and

- ipsapirone, have a central hypotensive action that differs from that of clonidine in anaesthetized cats. *Eur. J. Pharm.*, **138**, 179–191.
- SAXENA, P.R. (1978). Arteriovenous shunting and migraine. *Res. Clin. Stud. Headache*, **6**, 89–102.
- SAXENA, P.R. (1987). The arteriovenous anastomoses and veins in migraine research. In *Migraine – Therapeutic, Conceptual and Research Aspects*, ed. Blau, J.N. pp. 581–596. Amsterdam: Elsevier.
- SAXENA, P.R. & VERDOUW, P.D. (1982). Redistribution by 5-hydroxytryptamine of carotid arterial blood at the expense of arteriovenous blood flow. *J. Physiol.*, **332**, 501–520.
- SAXENA, P.R. & VERDOUW, P.D. (1984). Effects of methysergide and 5-hydroxytryptamine on carotid blood flow distribution in pigs: Further evidence for the presence of atypical 5-HT receptors. *Br. J. Pharmacol.*, **82**, 817–826.
- SAXENA, P.R. & VERDOUW, P.D. (1985). 5-Carboxamido tryptamine, a compound with a high affinity for 5-hydroxytryptamine₁ binding sites, dilates arterioles and constricts arteriovenous anastomoses. *Br. J. Pharmacol.*, **84**, 533–544.
- SAXENA, P.R., SCHAMHARDT, H.C., FORSYTH, R.P. & LOEVE, J. (1980). Computer programs for the radioactive microsphere technique. Determination of regional blood flows and other haemodynamic variable in different experimental circumstances. *Comp. Program. Biomed.*, **12**, 63–84.
- SAXENA, P.R., DUNCKER, D.J., BOM, A.H., HEILIGERS, J. & VERDOUW, P.D. (1986). Effects of MDL 72222 and methiothepin on carotid vascular responses to 5-hydroxytryptamine in the pig: Evidence for the presence of “5-hydroxytryptamine₁-like” receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **333**, 198–204.
- SPIERINGS, E.L.H. & SAXENA, P.R. (1980a). Effect of isometheptene on the distribution and shunting of 15 μ m microspheres throughout the cephalic circulation of the cat. *Headache*, **20**, 103–106.
- SPIERINGS, E.L.H. & SAXENA, P.R. (1980b). Antimigraine drugs and cranial arteriovenous shunting in the cat. *Neurology (Minn.)*, **30**, 696–701.
- STEEL, R.G.D. & TORRIE, J.H. (1980). *Principles and Procedures of Statistics, a Biomedical Approach*. 2nd edn., Kogakusha, Ltd, Tokyo: McGraw-Hill.
- TRABER, J. & GLASER, T. (1987). 5-HT_{1A} receptor related anxiolytics. *Trends Pharmacol. Sci.*, **8**, 432–437.
- TRICKLEBANK, M.D. (1985). The behavioural responses to 5-HT receptor agonists and subtypes of central 5-HT receptor. *Trends Pharmacol. Sci.*, **6**, 403–407.
- VERDOUW, P.D., JENNEWEIN, H.M., HEILIGERS, J., DUNCKER, D.J. & SAXENA, P.R. (1984). Redistribution of carotid artery blood flow by 5-HT: effects of the 5-HT₂ receptor antagonist ketanserin and WAL 1307. *Eur. J. Pharmacol.*, **102**, 499–509.
- VERDOUW, P.D., JENNEWEIN, H.M., MIERAU, J. & SAXENA, P.R. (1985). N-(3-acetylaminophenyl)piperazine hydrochloride (BEA 1654), a putative 5-HT₁ agonist, causes constriction of arteriovenous anastomoses and dilatation of arterioles. *Eur. J. Pharmacol.*, **107**, 337–346.

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