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Effects of methysergide and 5-hydroxytryptamine on carotid blood flow distribution in pigs: further evidence for the presence of atypical 5-HT receptors

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1 The effects of acute (50–350 $\mu g\,kg^{-1}$, i.v.) and subacute (350 $\mu g\,kg^{-1}$ orally per day for six days) administration of methysergide, and of intra-arterial infusions of 0.5 and 2.0 $\mu g\,kg^{-1}\,min^{-1}$ 5-hydroxytryptamine (5-HT) on the distribution of carotid blood flow into the capillary (nutrient) and arterio-venous anastomotic (AVA) fractions were studied in anaesthetized pigs.

2 The acute, but not the subacute, administration of methysergide caused a moderate reduction of carotid blood flow. This reduction, noticed only in the AVA fraction, was due to a constriction of the arterio-venous anastomoses (AVAs).

3 Both doses of 5-HT reduced total carotid blood flow but its nutrient fraction – particularly that distributed to the skin and ears – increased substantially. The AVA fraction was greatly diminished.

4 After treatment with methysergide, 5-HT no longer reduced the total carotid blood flow, but increased it. Despite this reversal the constriction of AVAs by the amine was only slightly diminished. On the other hand, the vasodilatation of the nutrient channels was enhanced.

5 The results of the interaction between methysergide and 5-HT provide further evidence for the presence of 'atypical' 5-HT receptors (probably corresponding to 5-HT_1 binding sites) mediating AVA contraction and nutrient vasodilatation. The 5-HT_2 receptors mediate vasoconstriction and are located in the large conducting arteries and possibly, in smaller numbers, in the AVAs and arterioles.

Introduction

The effectiveness of methysergide, with its well known antagonism against 5-hydroxytryptamine (5-HT) (Müller-Schweinitzer & Weidmann, 1978), in the prophylactic treatment of migraine (Sicuteri, 1959; Curran *et al.*, 1967) seems paradoxical in view of decreased blood concentrations of 5-HT during migraine attacks (Anthony *et al.*, 1969). However, in several publications it has been obvious that methysergide also possesses agonistic actions. For example, the drug contracts isolated blood vessels (Fozard, 1975; Müller-Schweinitzer & Weidmann, 1978; Apperley *et al.*, 1980; Müller-Schweinitzer, 1983), and it selectively increases resistance in the carotid vascular bed (Saxena, 1974), probably via 5-HT receptors. Moreover, methysergide and the related anti-5-HT drugs, cyproheptadine and mianserin, are not very effective in antagonizing the carotid vasoconstrictor effects of 5-HT (Saxena *et al.*, 1971; Saxena, 1972; Spira *et al.*, 1976) indicating that the 5-HT receptors in this vascular region do not resemble the musculotropic 'D' type (Gaddum & Picarelli, 1957) which, in present day terminology, is usually denoted as the 5-HT_2 type (Peroutka & Snyder, 1979). Recently, we reported that 5-HT redistributes the carotid blood flow in favour of the nutrient (capillary) fraction, particularly that apportioned to the skin and ears, at the expense of the non-nutrient fraction passing through arterio-venous (A-V) anastomoses (AVAs) (Saxena & Verdouw, 1982). Based on the modification by cyproheptadine, it was suggested that the 5-HT receptors in the carotid vascular bed are at least of two types: the 5-HT_2 type involved in the constriction of large arteries and partly of AVAs; and an 'atypical' type mediating AVA constriction and nutrient vasodilatation in the tissues (Saxena & Verdouw, 1982).

In the present investigation in anaesthetized young Yorkshire pigs, we asked ourselves two main questions. Firstly, in view of a selective carotid vasocon-

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striction by methysergide (Saxena, 1974) and the possible opening of AVAs in migraine (Heyck, 1969; Saxena, 1978), it is of interest to know if this drug constricts AVAs in the head. Secondly, in order to delineate further the nature of 5-HT receptors in the carotid vascular bed, we have also investigated the effect of methysergide on the 5-HT-induced redistribution of the carotid blood flow. Since methysergide is mainly effective as a prophylactic agent in migraine, the above two concepts were studied in animals that were subjected to both acute and subacute (for six days) treatments with the drug.

Methods

Three series of experiments were performed. The first series dealt with the effects of three cumulative doses of methysergide on the distribution of the common carotid artery blood flow. In the second and third series, the effects of acute and subacute administration of methysergide, respectively, on the redistribution of common carotid artery blood flow by 5-HT were evaluated. In this last group, 350 $\mu g\,kg^{-1}$ of the drug was administered orally every day for a period of six days. The total amount of the drug was given in two equal doses at about 09 h 00 min and 18 h 00 min. The results were compared with those in an untreated group. In all experiments, the same experimental set-up was used.

Experimental set-up

After an overnight fast young Yorkshire pigs (body weight: 26.9 ± 0.5 kg; age: 12–16 weeks) were initially sedated with 120 mg (i.m.) azaperone (Stresnil) and 120–150 mg (i.v.) metomidate (Hypnodil). After the animals were intubated, they were connected to a respirator for intermittent positive pressure ventilation with a mixture (1:2) of oxygen and nitrous oxide. A continuous intravenous infusion of pentobarbitone sodium (9–12 $mg\,kg^{-1}\,h^{-1}$) and occasional administration of pancuronium bromide (Pavulon) during the surgical procedure completed the anaesthesia. Aortic blood pressure was recorded with a Statham pressure transducer via a cannula inserted into the left femoral artery. In all animals, respiratory rate and tidal volume were adjusted or sodium bicarbonate (8.4%) infused to keep arterial blood gases, measured with an ABL-3 (Radiometer, Copenhagen), within normal ranges (i.e. pH 7.35–7.45; P_{O_2} 90–150 mm Hg; P_{CO_2} 35–45 mm Hg). The animals were maintained at a body temperature of around 37°C using an electric blanket. A small cannula was inserted in the jugular vein for the withdrawal of 0.5 ml blood, used for the determination of O_2 saturation (OSM hemoxymeter, Radiometer, Copenhagen).

Distribution of common carotid blood flow in the pig

Methods Both common carotid arteries were carefully dissected free in the neck. After a bilateral cervical sympathectomy, to avoid reflex influences on the carotid circulation, the superior thyroid branch was cannulated for intracarotid infusions of 5-HT. After removing its hub, a 0.5 mm (external diameter) needle, connected to suitable polyethylene tubing, was directly inserted into the main artery for microsphere injections. Common carotid artery blood flow was measured with a pre-calibrated flow probe connected to a sine-wave electromagnetic blood flowmeter (Skalar, Delft). Zero-flow values were obtained by a temporary occlusion of the carotid artery, while probe calibration was also checked *in situ* at the end of each experiment. In the first series of experiments, a thermodilution catheter was inserted into the pulmonary artery for the measurement of cardiac output. The distribution of carotid blood flow into nutrient and non-nutrient fractions was determined with the radioactive microsphere method (Saxena *et al.*, 1980; Saxena & Verdouw, 1982) using spheres (15 ± 1 μm) labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Chemicals GmbH, Dreieich, West Germany). For each measurement, a suspension of microspheres labelled with one of the nuclides, was ultrasonicated and injected in 0.5 ml 0.9% w/v NaCl solution (saline) over a 15–20 s period. In order to facilitate uniform mixing, the spheres were injected against the flow of blood. At the end of each experiment the animal was killed with an overdose of pentobarbitione sodium. The various tissues of the right and left half of the head, neck muscles, heart, kidneys and lungs were dissected out, weighed and placed in vials.

Counting of radioactivity The radioactivity in the vials was counted for 5–10 min in a gamma-scintillation counter (Packard, model 5986) equipped with a multichannel pulse height analyser (Conrac) using suitable windows for discriminating the different isotopes (Saxena *et al.*, 1980).

Calculations The microsphere and other data were processed by a PDP-11/70 computer using a set of specially developed programmes (for details, see Saxena *et al.*, 1980). The amount of carotid blood distributed to the individual tissue ($\dot{Q}_{tis[car]}$) of the head was calculated by: $\dot{Q}_{tis[car]}\,(\%) = (I_{tis}/I_{tot}) \times 100$ $\dot{Q}_{tis[car]}\,(ml\,min^{-1}) = (I_{tis}/I_{tot}) \times \dot{Q}_{car}$ where I_{tis} and I_{tot} are, respectively, the radioactivity (c.p.m.) in a particular tissue and that detected in all tissues collectively, and $\dot{Q}_{car}$ is carotid blood flow ($ml\,min^{-1}$). Tissue vascular resistance was calculated by dividing mean arterial blood pressure by tissue blood flow. The values determined for lungs represent the AVA

part of the carotid circulation (Johnston & Saxena, 1978; Spierings & Saxena, 1979; Saxena *et al.*, 1980; Saxena & Verdouw, 1982).

Experimental protocols

In all experiments the base-line values were determined after the preparation had been in a stable haemodynamic condition for at least 30–45 min after completion of the surgical procedures. The measurements consisted of recording the heart rate, mean arterial blood pressure and both common carotid blood flows while a batch of microspheres (100,000–150,000) was injected for the determination of tissue and AVA blood flow. Subsequently all measurements were repeated after: intravenous boluses of 50, 100 and 200 $\mu g\,kg^{-1}$ (cumulative dose: 50, 150 and 350 $\mu g\,kg^{-1}$) methysergide ($n=7$); a 10 min intracarotid infusion of 2.0 $\mu g\,kg^{-1}$ 5-HT, a recovery period of 20 min, 350 $\mu g\,kg^{-1}$ methysergide, and again after an identical infusion of 5-HT ($n=7$); consecutive 10 min intracarotid infusions of 0.5 and 2.0 $\mu g\,kg^{-1}\,min^{-1}$ 5-HT and a recovery period of 20 min in an untreated ($n=6$) and the methysergide pretreated group ($n=6$).

Statistical analysis

Due to non-homogeneity of variance, we used non-parametric tests (Siegel, 1956). Initially, the Kruskal-Wallis one-way analysis of variance by ranks was used to establish whether the samples represented different populations. The changes in the haemodynamic variables from the base-line values were calculated separately in each experiment and the significance of these changes was determined by using the Wilcoxon matched-pairs signed-ranks test. Furthermore, in the methysergide pretreated animals the changes obtained after each dose of 5-HT were compared with those in the untreated animals using the Mann-Whitney U test. Statistical significance was accepted at $P<0.05$ (two-tailed). All data have been expressed as mean ± s.e. mean.

Drugs

The drugs used in this study were 5-HT creatinine sulphate and methysergide hydrogen maleate. The dose of 5-HT is mentioned in terms of the base, while that of methysergide in terms of the salt used.

Results

Distribution of common carotid blood flow during base-line

During base-line conditions blood flow in each common carotid artery amounted to 242 ± 17 ml min^{-1}, which is about 7–10% of the total cardiac output. A

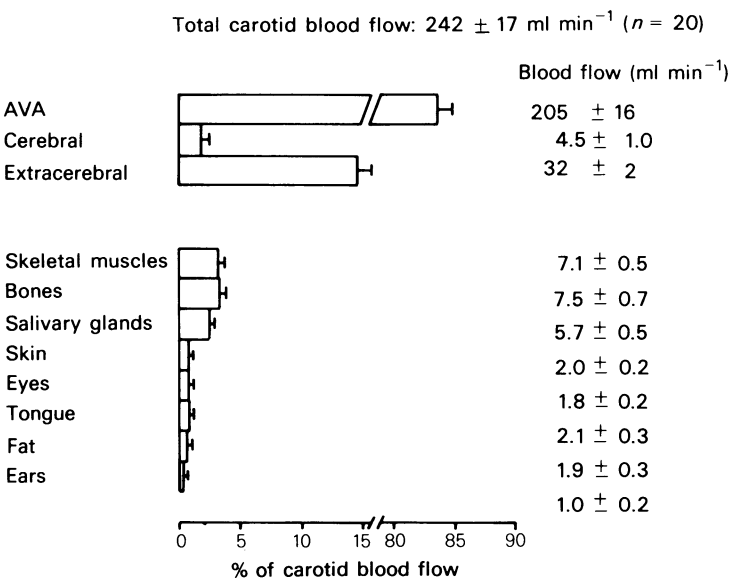

Figure 1 Distribution of common carotid artery blood flow in 20 anaesthetized domestic pigs. AVA = arteriovenous anastomotic flow. The bottom part of the figure depicts the flow to the various extracerebral tissues.

major fraction of this flow was shunted via AVAs, as 80–85% of the intracarotid dose of microspheres was detected in the lungs, whereas only a very minor fraction (about 2%) was found in the brain (Figure 1). In the same figure, the flow to the most important extracerebral tissues has been depicted. Finally, less than 1% of the carotid blood flow was distributed to the contralateral half of the head and less than 0.01% of the microspheres were detected in the heart and kidneys. The latter establishes that the microspheres did not reach these organs retrogradely or after escaping entrapment in the lungs in any significant number.

Effects of intravenous administration of methysergide

Methysergide caused a dose-dependent lowering of the heart rate and a slight increase in mean arterial blood pressure (Table 1). Since cardiac output (12%) and the blood flow in both common carotid arteries (18 and 20% respectively) decreased, there were dose-dependent increases in the resistances of the total systemic circulation and those of the two common carotid vascular beds. Total carotid blood flow diminished due to dose-dependent decreases in the AVA flow as cerebral and extracerebral flows tended to increase (Figure 2). Some of the extracerebral tissues (skin and ears) showed minor (compared to 5-HT; see later), but consistent increases in perfusion after the highest dose of methysergide; the blood flow to skin increased from 1.2 ± 0.3 to 2.4 ± 0.9 ml min^{-1} and that to the ears from 0.3 ± 0.1 to 2.0 ± 0.9 ml min^{-1}. Finally, there was a slight but significant decrease in the O_2 saturation of the ipsilateral jugular venous blood (Table 1).

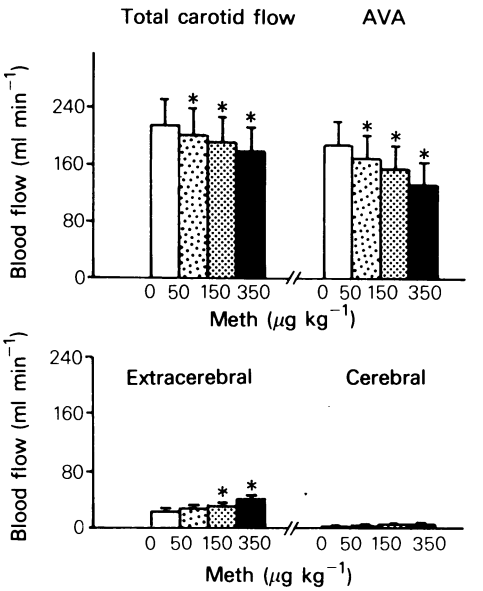

Figure 2 The effect of cumulative doses of methysergide (meth) on the common carotid artery blood flow and its distribution. The decrease in total carotid flow was at the expense of the arteriovenous anastomotic (AVA) flow. The extracerebral flow increased only slightly. * $P < 0.05$ vs. base-line (0).

Effect of acute administration of methysergide on the redistribution of common carotid artery blood flow induced by 5-hydroxytryptamine

The effects of intracarotid infusion of 5-HT ($2.0\ \mu g\ kg^{-1}\ min^{-1}$) on the systemic haemodynamics

Table 1 Haemodynamic effects of cumulative doses of intravenous methysergide in anaesthetized pigs ($n = 7$)

	Base-line	*Methysergide* (μg kg^{-1}) *50*	*150*	*350*
HR	111 ± 11	100 ± 10*	99 ± 10*	92 ± 8*
MAP	11.9 ± 0.7	11.8 ± 0.6	13.1 ± 0.6	13.8 ± 0.6*
CO ($n = 6$)	3.14 ± 0.29	2.90 ± 0.32	2.95 ± 0.28	2.75 ± 0.31
SVR ($n = 6$)	3.98 ± 0.42	4.34 ± 0.50	4.67 ± 0.44*	5.18 ± 0.39*
ILCF	214 ± 37	195 ± 39*	189 ± 37*	176 ± 35*
ILCR	64 ± 8	73 ± 12	86 ± 15*	97 ± 17*
CLCF	243 ± 29	216 ± 30*	210 ± 27*	194 ± 23*
CLCR	57 ± 11	62 ± 12	70 ± 11*	80 ± 12*
O_2 sat ($n = 6$)				
art	97.5 ± 0.3	97.8 ± 0.2	98.1 ± 0.2	98.3 ± 0.2
vein	89.6 ± 1.4	86.0 ± 1.7*	84.9 ± 1.4*	84.8 ± 1.5*

Abbreviations: HR = heart rate (beats min^{-1}); MAP = mean arterial blood pressure (kPa); CO = cardiac output (l min^{-1}); SVR = systemic vascular resistance (kPa l^{-1} min^{-1}); I(C)LCF = ipsilateral (contralateral) common carotid artery flow (ml min^{-1}); I(C)LCR = ipsilateral (contralateral) common carotid artery resistance (kPa l^{-1} min); O_2 sat = oxygen saturation (%); art = arterial; vein = jugular vein (ipsilateral). The terms ipsilateral and contralateral were defined on the basis of the side where the microspheres were infused. * $P < 0.05$ vs. base-line.

Table 2 Haemodynamic effects of 2.0 μg kg^{-1} intracarotid 5-hydroxytryptamine before and after 350 μg kg^{-1} intravenous methysergide in anaesthetized pigs ($n = 7$)

	Base-line	*5-HT*	*Recovery*	*Meth.*	*Meth. + 5-HT*
HR	114 ± 7	120 ± 6	99 ± 4	95 ± 4	114 ± 7[a,b]
MAP	13.3 ± 0.8	14.0 ± 0.8	13.0 ± 0.5	14.8 ± 0.4[a]	14.8 ± 0.4
CO ($n = 5$)	3.51 ± 0.17	3.05 ± 0.23	2.94 ± 0.25	2.75 ± 0.13	2.97 ± 0.19
SVR ($n = 5$)	3.75 ± 0.36	4.51 ± 0.47[a]	4.37 ± 0.72	5.43 ± 0.31[a]	4.99 ± 0.33[a,b]
O_2 sat					
art	98.0 ± 0.2	97.9 ± 0.2	97.9 ± 0.4	97.6 ± 0.2	97.3 ± 0.5
vein ($n = 5$)	94.0 ± 1.4	80.1 ± 6.8[a]	92.5 ± 3.3	90.3 ± 2.6	91.7 ± 2.2[b]

Abbreviations: HR = heart rate (beats min^{-1}); MAP = mean arterial blood pressure (kPa); CO = cardiac output (l min^{-1}); SVR = systemic vascular resistance (kPa l^{-1} min^{-1}); Meth. = methysergide; O_s sat = oxygen saturation (%); art = arterial; vein = jugular vein (ipsilateral); [a] $P < 0.05$ vs. values preceding the treatment; [b] $P < 0.05$ vs. changes caused by 5-HT before methysergide treatment.

and the distribution of the ipsilateral common carotid artery blood flow before and immediately after intravenous administration of methysergide (350 μg kg^{-1}) are shown in Table 2 and Figures 3 and 4. 5-HT had little systemic effect other than a moderate elevation of peripheral vascular resistance. Methysergide, which by itself slightly elevated the arterial pressure and systemic vascular resistance, reversed the vasoconstrictor response to 5-HT to a vasodilatation (Table 2). In the carotid vascular bed, the amine caused a decrease in ipsilateral blood flow and, since mean arterial pressure was virtually unaffected, an increase in the ipsilateral resistance. The changes in the flow and resistance of the contralateral side were negligible. Base-line values were resumed within 20 min after the infusion was stopped (Figure 3). The subsequent administration of methysergide resulted in a small but significant decrease in the flow and increase in the resistance of both ipsilateral and contralateral carotid vascular beds. The changes in the contralateral parameters persisted during the subsequent intracarotid 5-HT administration, but the ipsilateral parameters showed a strikingly different pattern as 5-HT now caused an increase rather than a decrease in the total ipsilateral carotid flow (Figure 3). From Figure 4, it can also be concluded that the skin and, to some extent ears and muscles, especially contributed to the increase in extracerebr-

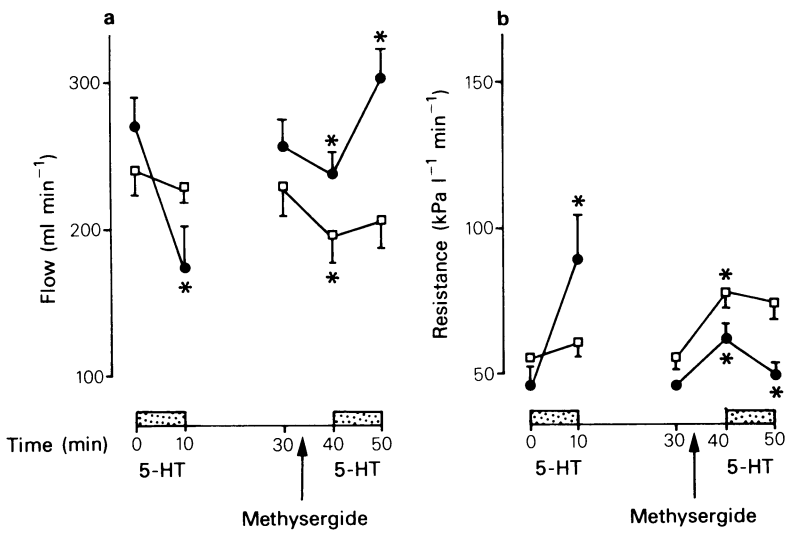

Figure 3 Effect of intravenous administration of methysergide (350 μg kg^{-1}) on the 5-hydroxytryptamine (5-HT) (intracarotid)-induced changes in total ipsilateral (●) and contralateral (□) carotid blood flow (a) and resistance (b). Methysergide moderately reduced both ipsilateral and contralateral carotid blood flow. It is further shown that 5-HT caused only a decrease (increase) in ipsilateral flow (resistance), but that after pretreatment with methysergide the changes were completely reversed. * $P < 0.05$ vs. preceding values.

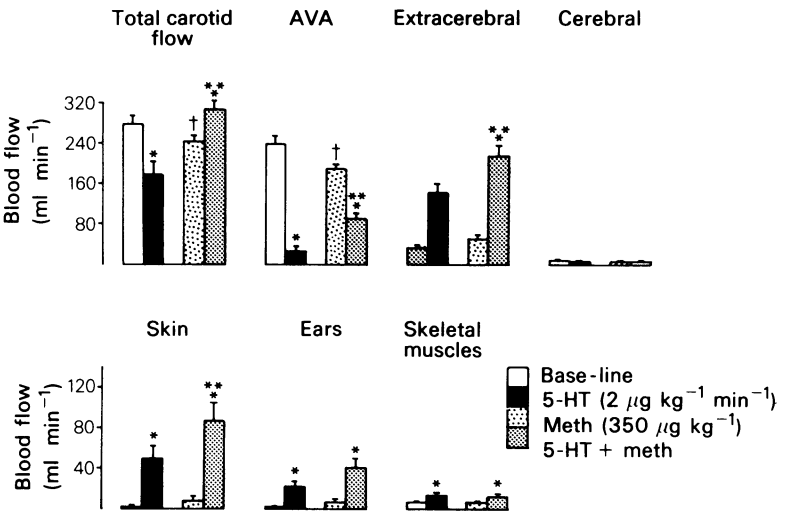

Figure 4 Effect of intravenous administration of methysergide (Meth, 350 $\mu g\,kg^{-1}$) on the 5-hydroxytryptamine (5-HT)-induced redistribution of common carotid blood flow. Methysergide completely reversed the reduction of total carotid blood flow by 5-HT, but it only partially attenuated the response on AVA flow. The dilatation of the extracerebral bed by 5-HT was enhanced by methysergide. This enhanced vasodilatation was especially noticeable in the skin. * $P<0.05$ vs. pre-5-HT value. ** $P<0.05$ vs. 5-HT-induced change before methysergide. † $P<0.05$ vs. base-line values.

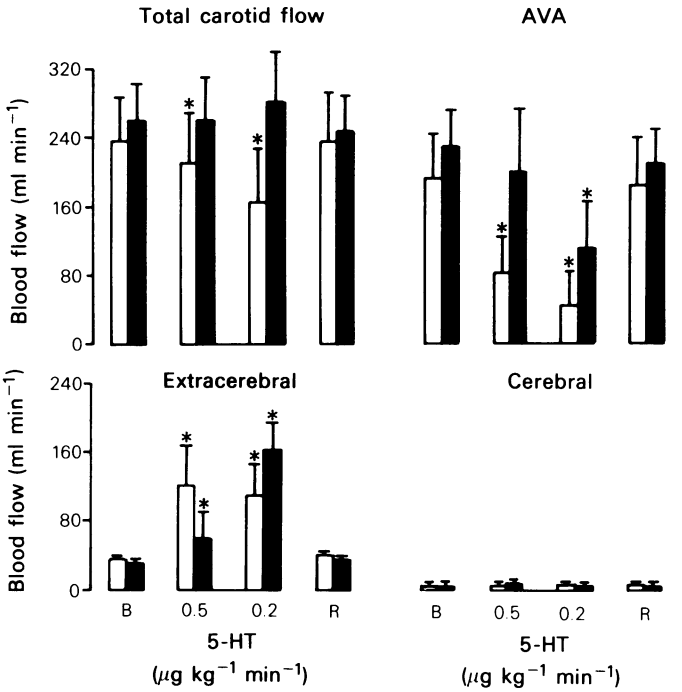

Figure 5 Effect of chronic treatment (350 $\mu g\,kg^{-1}$ daily) with methysergide on the 5-hydroxytryptamine (5-HT)-induced redistribution of the common carotid blood flow. Pretreatment with methysergide had no effect on the distribution of the flow during base-line, but partially antagonized the 5-HT-induced vasoconstriction of the arteriovenous anastomoses (AVA). Open columns, untreated; solid columns, methysergide-treated; B = base-line and R = recovery. * $P<0.05$ vs. base-line.

al flow caused by 5-HT. Changes in the other tissues were negligible (not shown). Methysergide accentuated the vasodilator response to 5-HT in these tissues. 5-HT, which caused a decrease in the O_2 saturation of the jugular venous blood, failed to do so after pretreatment with methysergide (Table 2).

Effect of subacute pretreatment with methysergide on the redistribution of common carotid artery flow caused by 5-hydroxytryptamine

To illustrate the effects of pretreatment (350 $\mu g\,kg^{-1}$ daily for six days) with methysergide more clearly, the results of this series of experiments are compared to those of an untreated group which received the same doses of 5-HT. The base-line values in the untreated and methysergide-pretreated pigs of either the heart rate (98 ± 6 and 111 ± 7 beats min^{-1}, respectively), arterial blood pressure (11.7 ± 1.3 and 13.4 ± 0.8 kPa, respectively), or the common carotid blood flow (see Figure 5) did not differ significantly. The systemic haemodynamic (heart rate and arterial blood pressure) changes caused by intracarotid infusions of 5-HT (0.5 and 2.0 $\mu g\,kg^{-1}\,min^{-1}$) were minimal in both groups. However, differences were noted in the carotid vascular responses to 5-HT. In the untreated animals, intracarotid infusions of 0.5 and 2.0 $\mu g\,kg^{-1}\,min^{-1}$ 5-HT produced dose-dependent decreases in the total carotid flow (Figure 5) and consequently an increase in the resistance of the vascular bed. The decrease in total flow was at the expense of the AVA fraction as extracerebral blood flow, especially in the skin and the ears, increased markedly. No change was observed in the fraction distributed to the brain. Within 20 min after the infusion was stopped, base-line values were resumed (Figure 5). In animals pretreated with methysergide, infusions of 5-HT did not cause a reduction of total carotid blood flow which tended, although not significantly, to increase. Hence, instead of an increase (60%) in the untreated animals, we found a minor decrease in the resistance of the carotid bed. The redistribution of the carotid blood flow towards extracerebral tissues at the expense of AVA flow caused by the lower (0.5 $\mu g\,kg^{-1}\,min^{-1}$) but not by the higher (2.0 $\mu g\,kg^{-1}\,min^{-1}$) dose of 5-HT was attenuated by methysergide (Figure 5).

Discussion

Significance of microsphere shunting

The suitability of the radioactive microsphere technique for quantifying the fractionation of arterial blood into nutrient (tissue; capillary) and non-nutrient (AVA) parts has been extensively discussed (Johnston & Saxena, 1978; Saxena & Verdouw, 1982; Saxena, 1984). Since the diameter of capillaries is less than 10 μm (Burton, 1944; Baez, 1977; Laursen & Diemer, 1980; Potter & Groom, 1983) and that of the AVAs usually more than 25 μm (Clark & Clark, 1934; Burton, 1944; Daniel & Prichard, 1956; Sherman, 1963), and since 15 μm spheres get impacted in vessels having a mean diameter of less than 27.7 μm (Dickhoner *et al.*, 1978), it must be concluded that the proportion of microspheres appearing in the lungs after intra-arterial injections represents the AVA fraction. As found earlier (Saxena & Verdouw, 1982), 80–85% of the microspheres injected into the carotid artery escaped via AVAs to lodge in the lungs. A high degree of A-V shunting in the carotid vascular bed is also indicated by a high O_2 saturation in the jugular venous blood (see Tables 1 and 2).

Using microspheres of four different diameters (10, 15, 25 and 35 μm) we have recently found that AVAs in the carotid territory, besides being in the dura mater (Rowbotham & Little, 1965), nasal mucosa (Änggård, 1974) and probably, in the rete mirabile (Gillilan & Markesbery, 1963), are mainly located in the skin and ears and, to a lesser extent, in the tongue and eyes (Saxena & Verdouw, unpublished).

Effects of 5-hydroxytryptamine in the carotid vascular bed

Intracarotid infusions of 5-HT, as we have also found with bolus injections in cervical sympathectomized dogs (Saxena *et al.*, 1971; Saxena, 1972; Saxena & de Vlaam-Schluter, 1974), resulted in a reduction of total carotid blood flow in our pigs. Despite this reduction, tissue blood flow, particularly to the skin and ears (their colour changed to bright red), increased and the AVAs constricted. These findings, which are in agreement with earlier reports (Saxena *et al.*, 1978; Forsyth & Saxena, 1978; Saxena & Verdouw, 1982), show that 5-HT has differential effects on the different vascular segments. The main trunk of the common carotid artery (as evidenced by an elevation of the gradient between the common carotid and vertebral artery wedge pressures: Heistad *et al.*, 1976), constricts, as do the AVAs, but the arterioles dilate. Thus, the overall response to 5-HT in the carotid vascular bed is determined by the relative magnitude of the effects in the three segments, though obviously the large artery responses are the least important *in vivo*. The situation is further complicated as the amine can also reduce the release of noradrenaline by a presynaptic action (Apperley *et al.*, 1980; Engel *et al.*, 1983). Therefore, in the presence of a substantial sympathetic neural tone, total carotid blood flow may increase due to a more marked arteriolar vasodilatation (for references, see Saxena & Verdouw, 1982).

Effects of methysergide in the carotid vascular bed

It is known that methysergide has a rather selective vasoconstrictor effect in both dogs (Saxena, 1972; 1974) and monkeys (Spira *et al.*, 1976). In the present experiments in pigs, acute intravenous administration of methysergide ($50-350\ \mu g\ kg^{-1}$) also caused a reduction in the carotid blood flow which was entirely due to a constriction of AVAs. In lower doses ($25-100\ \mu g\ kg^{-1}$), however, methysergide was not able to reduce A-V shunting in cats (Spierings & Saxena, 1980) but in a higher dose ($0.5\ mg\ kg^{-1}$) peripheral AVA flow decreased in the rabbit (Forsyth & Saxena, 1978). It seems that methysergide has qualitatively similar, though weaker, effects in the carotid vascular bed to 5-HT. Isolated segments of large cranial arteries (Müller-Schweinitzer & Weidmann, 1978; Müller-Schweinitzer, 1983) and AVAs constrict and there may be some weak vasodilatation in the arterioles. In addition, like 5-HT, methysergide can also reduce noradrenaline release by a presynaptic action on sympathetic neurones (Feniuk *et al.*, 1981).

In the animals pretreated orally with methysergide, the base-line values of neither carotid blood flow nor its distribution differed from those in the untreated animals. Thus, it seems that the constriction of AVAs observed acutely is probably not of sufficient duration when the drug was given orally in the doses mentioned.

Nature of 5-hydroxytryptamine receptors in carotid vascular bed

Large arteries, including the carotids, can be isolated *in vitro* and a number of elegant studies show that the contractile effect of 5-HT is effectively antagonized by drugs such as cyproheptadine, pizotifen, methysergide and ketanserin (Edvinsson *et al.*, 1978; Cohen *et al.*, 1981; Van Nueten *et al.*, 1981; Müller-Schweinitzer, 1983). The receptors mediating the constriction of the main segment of the carotid artery must, therefore, be classified as the 'D' or 5-HT_2 type. In the carotid vascular bed, the 5-HT_2 receptors are perhaps also located, though in a smaller number, in AVAs and arterioles as both cyproheptadine and methysergide attenuated the reduction of AVA flow, and unmasked or enhanced the arteriolar vasodilatation, by 5-HT (Walsh, 1967; Saxena & Verdouw, 1982; present results). On the 5-HT_2 receptors, methysergide appears to be a partial agonist as this drug contracts isolated cranial arteries and antagonizes the contractile responses to 5-HT (Müller-Schweinitzer, 1983).

The main part of constriction of AVAs, and the arteriolar vasodilatation by 5-HT were not antagonized by either cyproheptadine (Saxena & Verdouw, 1982) or methysergide (present results) in doses which are several times that needed to completely block 5-HT_2 receptors. The receptors mediating these responses also do not resemble the 'M' receptors for 5-HT found on the neural tissues (unpublished observations) and, until further characterization, should be termed 'atypical' in nature. Whether or not the 'atypical' 5-HT receptors on the AVAs (constriction) and arterioles (dilatation) are of the same type is not yet known. However, the nature of these receptors resembles those present on the pre- and postsynaptic sites in the dog saphenous vein (Apperley *et al.*, 1980) as the effects of 5-HT are not blocked by cyproheptadine, pizotifen and methysergide, and are mimicked by methysergide. More recently, evidence has been presented which attempts to link the 5-HT_1 binding sites with the presynaptic 5-HT receptors in the dog saphenous vein (Engel *et al.*, 1983) and the vasodilator response in the pithed rat (Kalkman *et al.*, 1983). Whether the 'atypical' receptors of the AVAs and arterioles of the carotid artery bed described here are associated with the 5-HT_1 binding sites cannot be established now, but it remains an attractive possibility.

The above characterization of the 5-HT receptors in the carotid vascular bed explains the weak antagonistic effects of mianserin (Saxena *et al.*, 1971), cyproheptadine, methysergide (Saxena, 1972) and pizotifen (Spira *et al.*, 1976) against the reduction of total carotid blood flow elicited by bolus injections of 5-HT in dogs. It is now obvious that the antagonism will depend upon the relative recruitment of 5-HT_2 and the 'atypical' receptors in the final response to 5-HT. Our results also emphasize the fact that *in vitro* studies, elegant though they may be, are limited due to technical feasibility to large vessels, and can produce results that may or may not have much bearing on the *in vivo* responses of the whole circulatory unit.

In conclusion, we have shown that methysergide ($50-350\ \mu g\ kg^{-1}$) can cause a selective vasoconstriction of AVAs upon acute intravenous administration. This effect was probably of short duration as it was not discernible upon subacute oral administration ($350\ \mu g\ kg^{-1}$ per day), by which route one apparently needs a higher dose than that used in this study. The drug reversed the vasoconstrictor effect of 5-HT in the total carotid bed. However, it only partially blocked AVA constriction, and did not modify the arteriolar vasodilatation by 5-HT. We confirm that the 5-HT receptors in the AVAs and arterioles are 'atypical' in nature. Whether or not these receptors are associated with 5-HT_1 binding sites remains to be seen.

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