

# Pharmacological Analysis of 5-Hydroxytryptamine Receptors in Isolated Intracranial and Extracranial Vessels of Cat and Man

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**SUMMARY** In intra- and extracranial arteries from cat and man (under conditions in which dilation was blocked), 5-hydroxytryptamine (5-HT) and D-lysergic acid diethylamide (LSD) produced a contraction at lower doses than that produced by tryptamine. Methysergide inhibited the 5-HT-induced contraction of the extracranial arteries in a competitive manner. In intracranial arteries the inhibition comprised a reduction in slope and maximum of the log dose-response curves. The contractile effects of 5-HT on both types of arteries appear to be mediated by 5-HT receptors, but the receptors have different characteristics in the sense that the mode of antagonism by methysergide was different. The dissociation constant ( $K_B$ ) and the corresponding  $pA_2$  value ( $8.61 \times 10^{-9}$  M and 8.21, respectively) for the interaction between methysergide and 5-HT in extracranial arteries conform with that reported for other smooth muscle tissues. The dilator response was tested in arteries given an active tonic contraction with prostaglandin  $F_{2\alpha}$ . Both 5-HT and tryptamine produced a dose-dependent dilation, 5-HT being the more potent. Neither methysergide nor LSD antagonized the response in a competitive manner whereas the two  $\beta$ -receptor antagonists, propranolol and 1-*N*-isopropyl-*p*-nitrophenyl-ethanolamine (INPEA), caused a parallel shift of the log dose-response curves (tested for 5-HT). The mean values for  $K_B$  ( $pA_2$ ) in the presence of propranolol were  $1.93 \times 10^{-8}$  M (8.29) for the intracranial and  $1.83 \times 10^{-8}$  M (8.50) for the extracranial vessels. Calculated  $pA_2$  values with INPEA were 8.47 and 7.93, respectively. The mode of inhibition by the  $\beta$ -antagonists suggests that the dilator effect of 5-HT is mediated by a receptor that may be the same as, or closely related to, the adrenergic  $\beta$ -receptor.

THERE IS MUCH evidence to suggest that 5-hydroxytryptamine (5-HT) is involved both in the etiology of migraine<sup>1</sup> and in the vasospasm occurring in brain vessels after subarachnoid hemorrhage.<sup>2</sup> In the periphery, 5-HT is stored in high concentrations in blood platelets, in mast cells of certain species, and in enterochromaffin cells.<sup>3</sup> In the central nervous system, the raphe complex of the brain stem contains 5-HT neurons which are involved in, for example, the slow-wave sleep phenomenon.<sup>4</sup> It is postulated that, in association with a subarachnoid hemorrhage or a migraine attack, 5-HT is released from one or several of these storage sites and may then affect the cranial vascular bed. The main effect of 5-HT on blood vessels is constrictor in large arteries and veins, whereas a dilator action has been claimed to occur in smaller vessels, such as arterioles and capillaries; 5-HT also increases capillary permeability.<sup>5</sup> It has been suggested that the vasomotor response is dependent on the preexisting tone of the vessel.<sup>6</sup>

There are conflicting reports on the effect of 5-HT on the intra- and extracranial circulation. Thus, a reduction

of the cerebral blood flow and a large increment of the extracranial blood flow have been measured.<sup>7</sup> On the other hand, some authors have observed a reduction in both intra- and extracranial blood flow.<sup>7</sup> As the above-mentioned experiments all have used complicated in vivo models, it is not possible to decide whether the responses have been specific in the sense that they have involved specific 5-HT receptors in the vascular wall. Moreover, for practical reasons, the in vivo models usually do not allow a detailed characterization of such receptors.

Particularly since there is increasing evidence of involvement of 5-HT in the clinical disorders mentioned above, a detailed knowledge of the receptors mediating the action of this amine is required to interpret its pathophysiological role and to design appropriate antagonistic drugs. A quantitative pharmacological characterization of receptors is more readily carried out on isolated vascular segments in vitro. Further, the suggested differences between the intra- and extracranial vascular beds can be investigated by comparison of responses in representative vascular segments from the two regions.

## Methods

### Material

Fifty cats of both sexes weighing 2.0–5.2 kg, were used. They were anesthetized with sodium pentobarbital (30 mg/kg, ip) and killed by exsanguination and decapitation. The skull was opened and the brain was removed, placed in a Petri dish, and soaked in a Krebs-Ringer

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buffer solution at room temperature. The middle cerebral arteries (300–500  $\mu\text{m}$  in diameter) on both sides were dissected out. Branches of similar caliber were taken from the external maxillary or lingual arteries and placed in the same buffer. Arteries also were removed from some cats in which the superior cervical ganglia had been removed 1 week prior to the tests. The denervation eliminates any possible interference caused by uptake of the agonist into the perivascular adrenergic nerve plexus followed by release of norepinephrine from the nerves.

Material from 15 patients of either sex, aged 20–60 years, was obtained during neurosurgical operations. (Institutional rules governing the protection of human subjects were followed in obtaining and studying these tissues.) Small pial arteries were removed from macroscopically intact parts of resected temporal and frontal lobes, and branches of the superficial temporal artery were taken in connection with the craniotomy. The preparations were transported to the laboratory (less than 30 minutes) in ice-cold buffer solution.

### Vascular Preparation

A 5-mm long piece of each intra- and extracranial artery was used immediately for testing; the remainder was kept in a refrigerator at  $+4^{\circ}\text{C}$  for later use (up to 24 hours). The two types of arteries were mounted in a 50-ml temperature-controlled organ bath with two separate systems of L-formed metal holders for recording circular contractions with Endevco model 8107-2 force-displacement transducers.<sup>8</sup> The isometric contractions were amplified and recorded on a Grass model 7B polygraph. The bath contained a solution of the following composition (mM): NaCl, 118; KCl, 4.5;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 2.5;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.0;  $\text{NaHCO}_3$ , 25;  $\text{KH}_2\text{PO}_4$ , 1.0; and glucose, 6.0. The bath and the stock solution were maintained at  $37.5 \pm 0.5^{\circ}\text{C}$  (range); the temperature was checked continuously by a needle thermocouple close to the preparations. A mixture of 95%  $\text{O}_2$  and 5%  $\text{CO}_2$  was used for continuous aeration, and the flow of the gas was set to give a pH of  $7.393 \pm 0.005$  (SE). The pH was determined in 50  $\mu\text{l}$  samples removed from the organ bath immediately before and after injection of each dose of test compound, using a Radiometer pH meter 27 with a type E5021 electrode unit. Further details about the experimental setup have been published elsewhere.<sup>8</sup>

Shortly after the two arterial preparations had been mounted in the organ bath, each was subjected to a load of 400 dynes and allowed to stabilize; the tension decreased approximately 50–100 dynes. The test drugs were administered after 2 hours of equilibration. The structure and dimension of the vessels and related considerations on the amount of initial tension given to the vessels have been presented previously.<sup>8,9</sup>

### Drugs

The following compounds were used: 5-hydroxytryptamine creatinine sulfate (Sigma), tryptamine hydrochloride (Sigma), prostaglandin  $\text{F}_{2\alpha}$  (Astra), *d*-LSD (*d*-lysergic acid diethylamide; Sandoz), methysergide (Sandoz), phentolamine hydrochloride (Ciba), phenoxybenzamine

hydrochloride (Dibenzyl; Smith, Kline and French), propranolol chloride (Inderal; Scanmeda), 1-*N*-isopropyl-*p*-nitrophenyl-ethanolamine (INPEA; Selvi). All substances were dissolved in 0.9% saline, and the concentrations throughout this paper are given as the salt and expressed as the final molar concentration in the organ bath.

### Methodological Considerations

The pharmacological differentiation of receptors in isolated tissues requires certain experimental precautions if the results are to be interpreted quantitatively. These problems have been particularly well analyzed in studies on adrenergic receptors<sup>10</sup> but they are, to a certain extent, applicable also to experiments on other receptors. Preliminary work mentioned in the Introduction has shown that 5-HT causes both dilator and constrictor vascular responses. In pilot experiments, the dilator response was blocked by propranolol and, therefore, all constrictor experiments were done in the presence of propranolol. A dilator response was achieved only in vessels having an induced tone<sup>9</sup> and, hence it was tested for after the vessels had been actively constricted by prostaglandin  $\text{F}_{2\alpha}$ . It was found that the contractile action of the agonists (5-HT, tryptamine, and LSD) could be blocked totally by phenoxybenzamine. Thus, the contractile component of the response was eliminated by pretreatment of the vessels by phenoxybenzamine when dilator effects were studied.

After equilibration of the vessels in the organ bath, test doses of the agonists were given cumulatively<sup>11</sup> at 60- to 90-second intervals. Full dose-response curves were run as control up to three times before antagonists were applied to the vessels for 20 minutes before agonist doses. The antagonists, except phenoxybenzamine, remained in the bath during the tests.

Control responses were plotted in terms of maximal response ( $E_{\text{Am}}$ ) against log dose. The  $E_{\text{Am}}$  (expressed in dynes) and the  $\text{ED}_{50}$  (molar concentration of agonist at which half-maximum response occurs) were used as measures of agonist potency. The dose-response curves in the presence of antagonists were of two general types: those in which the  $E_{\text{Am}}$  and the slope of the curve were unchanged by antagonists and those in which the  $E_{\text{Am}}$  and the slope were reduced by the antagonists. The former type fulfills the criteria for competitive antagonism, and such results were further analyzed by the method of Arunlakshana and Schild.<sup>12</sup>

The occupation theory of drug-receptor interaction states that the formation of the complex between the receptor and the agent producing the response is governed by the law of mass action, so that

$$K_B = \frac{[B]}{[A']/[A] - 1}$$

where  $[B]$  is the concentration of antagonist in the region of the receptors and  $[A']$  and  $[A]$ , respectively, are the concentrations of agonist which, in the absence and presence of  $B$ , give equal responses. This requires that a plot of  $\log [A']/[A] - 1$  against  $\log [B]$  gives a straight line with a slope of unity. The intercepts on the abscissa ( $pA_2$ ) and

$K_B$  (dissociation constant for the receptor-antagonist complex) were calculated<sup>10</sup> to provide parameters of the receptor-antagonist interaction.

### Statistical Treatment of Data

Calculations for the regression analysis and of differences between mean values (Student's *t*-test) were performed with a Hewlett-Packard desk computer.

## Results

The majority of tests were performed on feline arteries and the results from these experiments are reported in detail. Experiments were also carried out on human material for the purpose of comparison; these results appear in the last section.

### Experiments on Resting Arteries from Cats

#### Contractile Response

The contractile effects of 5-HT, tryptamine, and LSD were studied in resting vessels, i.e., vessels given a passive load of 400 dynes followed by stabilization to a steady level of tension. Addition of each agonist to the organ bath caused a contraction which fairly rapidly (60–90 seconds) reached a plateau and which was maintained long enough to allow cumulative application of increasing doses. Pilot experiments had revealed that 5-HT produced a dilation that could be inhibited by propranolol (see further below). Therefore, quantitative data on the contractile effect of 5-HT, tryptamine, and LSD were obtained from tests with vessels (from normal and sympathetically denervated animals) in the presence of  $3 \times 10^{-7}$  M propranolol. The mean values for the maximum contractions ( $E_{Am}$ ) of intra- and extracranial vessels induced by these agonists appear in Table 1. Both 5-HT and tryptamine induced a strong contraction of the isolated vessels, whereas the contractile effect of LSD was only about half of the 5-HT response. When successive cumulative dose-response curves were run at appropriate intervals, no significant changes were noted in  $ED_{50}$  or  $E_{Am}$ .

#### Relative Potency

To evaluate the relative potency of the amines with regard to their contractile effects, full log dose-response

curves were constructed and the mean concentrations required for half-maximum contraction ( $ED_{50}$ ) were compared. As shown in Table 1, the potency ratio was in the order 5-HT = LSD > tryptamine for both intracranial and extracranial arteries. There was no difference in the reactivity of sympathectomized and intact arteries.

#### Antagonism of Response

The 5-HT receptor antagonist, methysergide<sup>13</sup> was applied to test whether the contractile response to 5-HT was mediated by typical 5-HT receptors. Methysergide alone did not contract either of the arteries in the concentrations tested, nor did it affect the response to norepinephrine or prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ). However, in both types of vessels, low concentrations of methysergide ( $3 \times 10^{-12}$  M) consistently caused potentiation of the 5-HT-induced contraction, i.e., the dose-response relationship was shifted toward the left (Fig. 1B). At concentrations of methysergide above  $3 \times 10^{-8}$  M, the dose-response curve of the 5-HT-induced contraction in the extracranial arteries showed a parallel shift to the right (see below) (Fig. 1B). In contrast, the effect of methysergide (and LSD) on the dose-response relationship for the intracranial vessels comprised not only some increase in  $ED_{50}$ , but also a reduction in slope and in the maximum effect (Fig. 1A). The antagonism of methysergide and LSD to the 5-HT response in the intracranial (as well as extracranial) vessels was easily reversed by repeated washing during 30 minutes, after which the sensitivity to the agonist was fully restored.

In addition to its antagonistic action on the contractile response to 5-HT, LSD has itself a vasoconstrictor activity.<sup>14, 15</sup> Thus, although administration of LSD increased the tone in the two types of vessels tested, the maximum contraction with 5-HT and the slopes of the dose-response curves clearly were reduced with increasing LSD concentrations. This combination of agonistic and antagonistic effects of LSD is illustrated in Figure 2.

If the contractile response to the agonists is mediated by  $\alpha$ -adrenergic receptors, a reversible competitive  $\alpha$ -antagonist should decrease the sensitivity of the test system to the various agonists without decreasing the maximum or the slope of the log dose-response curve. To

TABLE 1 Contractile Effects of Various Agonists on Intracranial Arteries\* and Extracranial Arteries†

Compound (species)	Intracranial artery			Extracranial artery		
	$ED_{50}$ (M)	Relative potency	$E_{Am}$ (dynes)	$ED_{50}$ (M)	Relative potency	$E_{Am}$ (dynes)
5-HT (cat)	$3.45 \pm 1.01 \times 10^{-8}$ ( <i>n</i> = 22)	1	$304 \pm 46$	$1.36 \pm 0.29 \times 10^{-7}$ ( <i>n</i> = 21)	1	$879 \pm 157$
5-HT (man)	$0.70 \pm 0.22 \times 10^{-8}$ ( <i>n</i> = 15)		$118 \pm 25$	$1.27 \pm 0.65 \times 10^{-7}$ ( <i>n</i> = 12)		$905 \pm 190$
LSD (cat)	$4.25 \pm 1.30 \times 10^{-8}$ ( <i>n</i> = 4)	0.81	$126 \pm 28$	$1.22 \pm 0.39 \times 10^{-7}$ ( <i>n</i> = 4)	1.11	$323 \pm 134$
Tryptamine (cat)	$5.47 \pm 4.39 \times 10^{-6}$ ( <i>n</i> = 6)	0.006	$303 \pm 63$	$1.03 \pm 0.29 \times 10^{-5}$ ( <i>n</i> = 6)	0.013	$613 \pm 108$

Values are mean  $\pm$  SE.  $ED_{50}$  = molar concentration of agonist at which half-maximum response occurs; 5-HT = 5-hydroxytryptamine; LSD = D-lysergic acid diethylamide.

\* Pial vessels from cat and man.

† Branches from the lingual and maxillary arteries of the cat and branches from the superficial temporal artery of man.

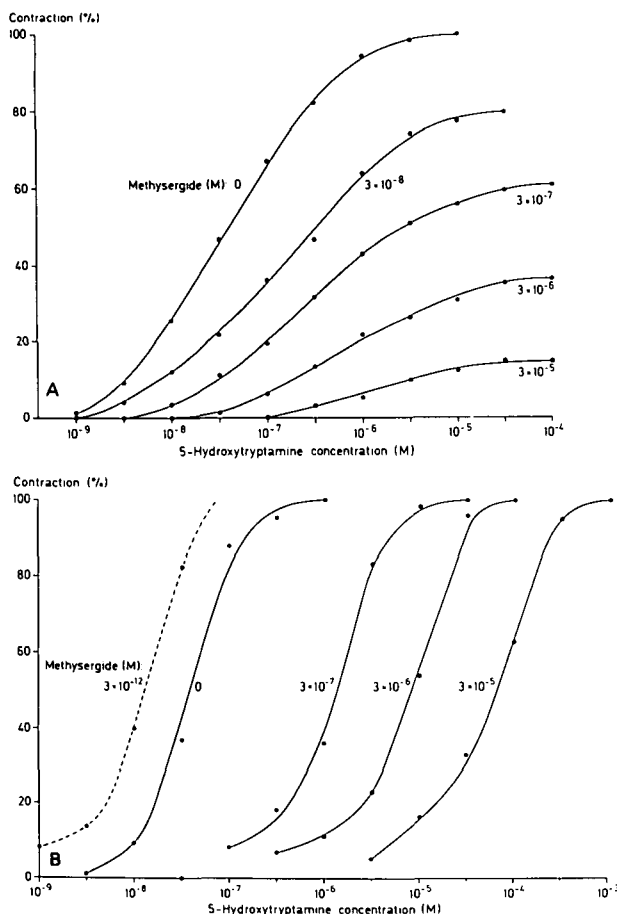


FIGURE 1 Dose-response curves for single experiments with 5-hydroxytryptamine (5-HT) before and after addition of increasing doses of the 5-HT antagonist, methysergide. The antagonist was administered 20 minutes before the experiment and was present during the test. Following washout and after the vessel had returned to its original tension, a new dose of methysergide was added. The organ bath contained  $3 \times 10^{-7}$  M propranolol. The vessels were sympathectomized. A: Intracranial artery, in which both maximum response and slope were reduced; B: extracranial artery, in which the antagonist produced a parallel shift in the log dose-response curves. Note the potentiating effect with the lowest methysergide concentration (hatched curve).

test this possibility, phentolamine was used in increasing concentrations ( $3 \times 10^{-8}$  to  $3 \times 10^{-6}$  M) and was found to cause the expected parallel shift of the log dose-response curve obtained with norepinephrine. On the other hand, the dose-response curves obtained with 5-HT were not influenced by phentolamine until relatively high antagonist concentrations ( $3 \times 10^{-6}$  M) had been reached, and the effect then involved a reduction in the maximum response and slope of the curve.

The  $\beta$ -haloalkylamine, phenoxybenzamine, is an antagonist that is irreversible in the sense that the reaction involves inactivation of the receptor.<sup>16</sup> Increasing concentrations of phenoxybenzamine from  $3 \times 10^{-8}$  M to  $3 \times 10^{-6}$  M caused graded blockade of the 5-HT effect with a decrease in maximum and slope of the dose-response curves for intracranial as well as extracranial vessels (Fig. 3). The contractile response was completely inhibited

after a 20-minute exposure of the artery to  $3 \times 10^{-5}$  M to  $5 \times 10^{-5}$  M phenoxybenzamine. Also with norepinephrine the action of phenoxybenzamine mainly involved a reduction in  $E_A$  and slope, but complete blockade was obtained already at a dose of  $3 \times 10^{-6}$  M of the antagonist.

#### Dissociation Constant for the Receptor-Antagonist Complex

Because methysergide behaved as a competitive reversible antagonist of 5-HT on the extracranial vessels, its interaction with the receptor was analyzed further according to the method of Arunlakshana and Schild<sup>12</sup> (Fig. 4). Regression analysis of the values from the tests showed a good linearity. The slope was  $-0.95$  which, according to Student's *t*-test, is not significantly different from  $-1$  ( $P > 0.05$ ). At the intercept of the straight line with the abscissa (when the slope is unity), the value for  $pA_2$  (negative logarithm of that concentration of antagonist which requires twice as high a concentration of agonist to elicit a given response) can be obtained. The  $pA_2$  value for methysergide with 5-HT as the agonist was 8.21 (Fig. 4). The  $K_B$  calculated from individual experimental values was  $8.61 \times 10^{-9} \pm 0.14 \times 10^{-9}$  M (mean  $\pm$  SE).

#### Experiments on Actively Contracting Arteries from Cats

##### General Considerations

Previous experience<sup>9</sup> has shown that, under our experimental conditions, the vessels are almost completely relaxed and dilator responses are therefore difficult to obtain. When the vessels were given a resting tone in this study by the addition of  $2.5 \times 10^{-6}$  M  $PGF_{2\alpha}$ , dilator responses could easily be observed. The contraction by  $PGF_{2\alpha}$  remained at a steady level for at least 40 minutes, and during this time each of the various amines to be

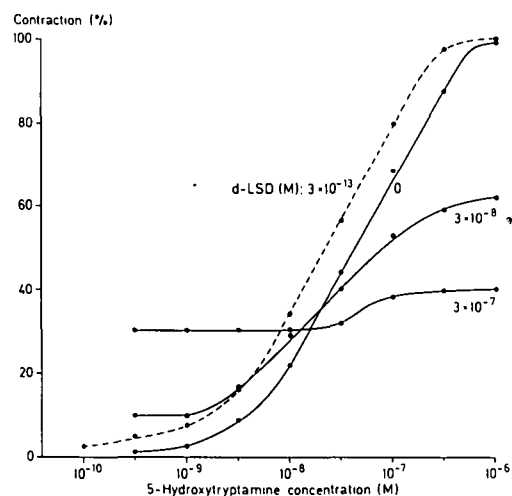


FIGURE 2 Interaction between 5-hydroxytryptamine (5-HT) and D-lysergic acid diethylamide (d-LSD) on a pial artery showing that LSD acts as both an agonist (with contractile effects) and antagonist (reducing  $E_{Am}$  and the slope of the log dose-response curves) and, in addition, potentiates the 5-HT response at low doses (hatched curve). The tests were carried out in the presence of  $3 \times 10^{-7}$  M propranolol (cf. Fig. 1).

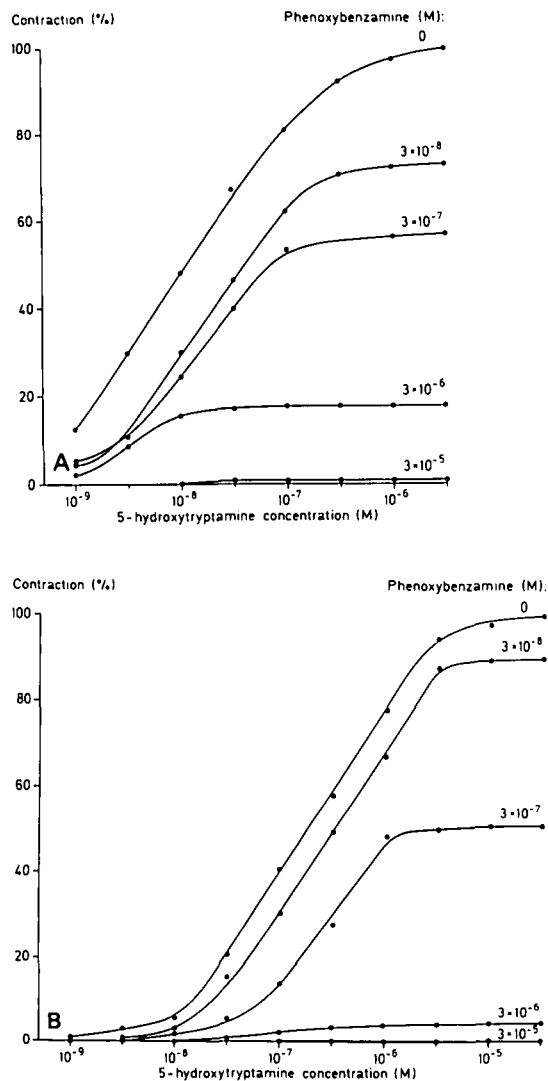


FIGURE 3 Antagonism by phenoxybenzamine under the same conditions as described in Figure 1 (although the antagonist was washed out before each test). In the intracranial (A) as well as the extracranial (B) vessel, the effect on the 5-HT response mainly consisted of a reduction in maximum contraction and in the slope of the log dose-response curves.

tested was added by cumulative application. To avoid interference with the contractile effects mediated by 5-HT, the vessels were exposed to  $3\text{--}5 \times 10^{-5}$  M phenoxybenzamine during 30 minutes, followed by washout. This totally blocks the contractile effect of 5-HT without affecting the tone elicited by  $\text{PGF}_{2\alpha}$ . When, as a control, several dose-response curves with 5-HT were run after each other, the  $\text{ED}_{50}$  or  $\text{E}_{\text{Am}}$  did not change significantly.

#### Dilator Response

Administration of  $\text{PGF}_{2\alpha}$  produced a tonic contraction in the intra- and extracranial arteries (mean: 335 dynes and 700 dynes, respectively). Both 5-HT and tryptamine dilated the actively contracted vessels in a dose-dependent way in the majority (90%) of the intracranial vessels, but only in approximately one-third of the extracranial ones.

The remainder of the arteries were unreactive or even showed a further constriction. The maximum dilation in response to 5-HT was approximately 200 dynes, whereas the action of tryptamine on the intracranial vessels was much less pronounced. The amount of dilation produced by 5-HT is not directly comparable with the  $\text{E}_{\text{Am}}$  values previously presented for isoproterenol<sup>9</sup> because, in the latter experiments, 5-HT was used to obtain the active tonic contraction of the vessel required to disclose the dilatory response. In terms of the  $\text{ED}_{50}$  values, 5-HT was more potent than tryptamine in dilating both types of vessels. Sympathetic denervation had no effect on the reactivity. The results are summarized in Table 2.

#### Antagonism of Dilator Response

Methysergide alone did not affect the vascular tone induced by  $\text{PGF}_{2\alpha}$  and tested in the presence of phenoxybenzamine. However, both methysergide and LSD in progressively increasing concentrations antagonized the dilator effect of 5-HT and also slightly increased the  $\text{ED}_{50}$  values in the two types of vessels (Fig. 5A). On the other hand, propranolol (Fig. 5B) and INPEA in increasing doses showed competitive antagonism of 5-HT, not changing the slope or  $\text{E}_{\text{Am}}$  of the log dose-response curve. The values for the dose ratio  $-1$  and the concentration of either antagonist, plotted against each other in their logarithmic form according to the method of Arunlakshana and Schild, fitted a straight line for the experiments on intracranial and extracranial vessels with the slopes indicated in Figure 6. Neither of these are different from unity, as shown by Student's *t*-test ( $P > 0.05$ ). The mean  $\text{pA}_2$  values are also shown in Figure 6. The dissociation constants for the receptor-antagonist complex with propranolol, when 5-HT was used as agonist, were calculated as the relation between the antagonist concentration and the dose ratio  $-1$ <sup>10</sup> using the individual values from the

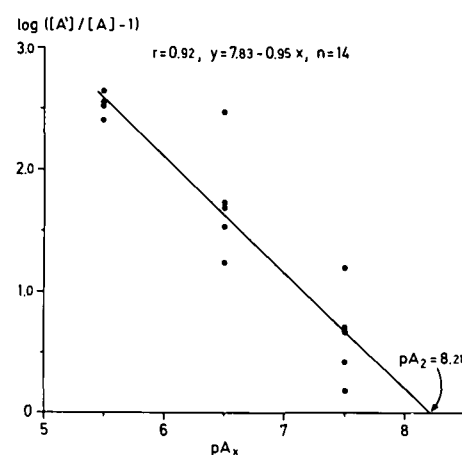


FIGURE 4 Arunlakshana-Schild plot from experiments on extracranial arteries using methysergide as antagonist of the contractile response to 5-HT. One of the experiments is illustrated in Figure 1B. The  $\text{pA}_2$  value (the negative logarithm of that concentration of the antagonist which requires twice as high a concentration of the agonist to elicit a given response) at the intercept of the line with the abscissa is given together with the equation for the line and the correlation coefficient (*r*).

TABLE 2 Dilator Effect of 5-Hydroxytryptamine and Tryptamine on Intracranial and Extracranial Arteries (cf. Table 1) from Cat and Man

Compound (species)	Intracranial artery			Extracranial artery		
	ED <sub>50</sub> (M)	Relative potency	E <sub>Am</sub> (dynes)	ED <sub>50</sub> (M)	Relative potency	E <sub>Am</sub> (dynes)
5-HT (cat)	6.77 ± 3.68 × 10 <sup>-8</sup> (n = 7)	1	200 ± 40	2.13 ± 1.56 × 10 <sup>-7</sup> (n = 7)	1	224 ± 11
5-HT (man)	3.1 × 10 <sup>-8</sup> (n = 2)		173	9 × 10 <sup>-8</sup> (n = 2)		200
Tryptamine (cat)	1.22 ± 0.27 × 10 <sup>-6</sup> (n = 4)	0.55	84 ± 27	1.33 ± 0.31 × 10 <sup>-6</sup> (n = 4)	0.16	163 ± 36

Abbreviations are defined in footnote to Table 1.

experiments illustrated in Figure 5B. The K<sub>B</sub> values were 1.93 × 10<sup>-8</sup> ± 0.99 × 10<sup>-8</sup> M for the intracranial vessels and 1.83 × 10<sup>-8</sup> ± 1.15 × 10<sup>-8</sup> M for the extracranial vessels.

### Experiments on Arteries from Humans

5-HT contracted small pial arteries and branches from the superficial temporal artery in a dose-dependent manner (Table 1). This effect of 5-HT on intracranial vessels was antagonized by methysergide (Fig. 7) in doses above 3 × 10<sup>-8</sup> M in the same manner as shown above for the cat. In extracranial vessels, methysergide in increasing doses shifted the dose-response relation of 5-HT toward higher agonist concentrations without reducing E<sub>Am</sub>. A potentiation of the 5-HT-induced contraction was demonstrated with a low dose of methysergide (3 × 10<sup>-12</sup> M) (Fig. 7). A similar potentiation was seen with 3 × 10<sup>-13</sup> M LSD. At larger doses, LSD was seen to have the same combined agonistic and antagonistic action as described above for the cat (cf. Fig. 2).

Provided the arteries had been exposed to phenoxybenzamine (3 to 5 × 10<sup>-5</sup> M during 30 minutes) and given an active tonic contraction by PGF<sub>2α</sub> (2.5 × 10<sup>-6</sup> M), 5-HT dilated both types of vessels. Increasing doses of propranolol caused a parallel shift of the log dose-response curve for 5-HT.

### Discussion

5-HT is a potent vasoconstrictor compound in most vascular beds.<sup>3</sup> A vasodilator capacity also has been demonstrated, e.g., in the limb.<sup>17, 18</sup> Surprisingly little is known about the mechanisms involved in the vasomotor action of 5-HT despite a considerable amount of research in the field. Previous attempts to characterize smooth muscle 5-HT receptors have not been conclusive.<sup>19</sup> Woolley and Shaw<sup>20</sup> and Allen et al.<sup>21</sup> have approached the problem by comparing the effect of various 5-HT analogues. However, no further studies have been carried out using the more standardized conditions required for a pharmacological characterization<sup>10</sup> of 5-HT receptors in blood vessels. Therefore, the present series of experiments was undertaken with the particular aim of comparing intra- and extracranial vascular beds.

Our knowledge about the mechanisms for the interaction between drugs and receptors as an operational approach to characterize various amine receptor mechanisms

is based mainly on the studies reviewed by Furchgott.<sup>10</sup> A drug-receptor system is composed of the tissue component that interacts with the agonist, and the complete sequence of events that connects the receptor with the response mechanism. The receptor may be characterized either by obtaining responses to various agonists or by the use of specific competitive antagonists. Because the actual receptors have not yet been isolated, we have to rely on the assumption that similarities or differences in the response also reflect similarities or differences in the characteristics of the receptors mediating these responses.

### Contractile Response

Previous studies on isolated cranial vessels have demonstrated only a contractile effect of 5-HT.<sup>2, 22-25</sup> However, the identity of the receptor mediating the response has not been extensively investigated. In the present study, the potency rank for the contractile response to 5-HT, LSD, and tryptamine was equal for both types of arteries, and in the order of 5-HT = LSD > tryptamine. Tryptamine has been shown to be less potent than 5-HT in producing contraction of carotid artery segments<sup>20</sup> and stomach muscle strips,<sup>26</sup> whereas the two agonists are equipotent in pulmonary arteries.<sup>26</sup> LSD was a partial agonist in the sense that it only produced less than half of the maximum contraction obtained by 5-HT.

One difficulty in a pharmacological characterization of vascular responses as being mediated by specific 5-HT receptors is the problem of finding clearly defined 5-HT receptor antagonists. This might partly be due to the ability of available substances to influence not only 5-HT receptors but also adrenergic, cholinergic, and histaminergic receptors, so that they have only a narrow dose range in which the antagonism can be regarded as specific for one or the other of these receptors.<sup>27</sup> Methysergide has been shown to be a specific 5-HT receptor antagonist on smooth muscle (for review, see Gyermek<sup>13</sup>) and its effect on adrenergic receptors is, at least in vitro, negligible.<sup>27-29</sup> Furthermore, methysergide does not interfere with the smooth muscle effects of acetylcholine and histamine<sup>29</sup> and has, like other lysergic acid derivatives, a structural similarity with 5-HT. It has been suggested that methysergide may substitute for 5-HT at the specific receptor sites, the affinity group being the indole nucleus that they have in common.<sup>30</sup> Structurally, LSD resembles methysergide and is commonly used as a 5-HT antago-

nist:<sup>13</sup> it has little or not anticholinergic and adrenergic effects<sup>31</sup>

The potency rank for 5-HT, tryptamine, and LSD appears to be consistent with the presence of 5-HT receptors, although additional experiments on receptor characterization still have to be done to make sure that each of these agonists is producing its response by acting on only one type of receptor. The parallel shift of the dose-response curves to 5-HT caused by increasing doses of methysergide in extracranial vessels offered further evidence for the presence of 5-HT receptors. The  $pA_2$  value for the interaction between 5-HT and methysergide as obtained in the present study is in agreement with similar findings from experiments on methysergide blockade using rabbit aorta<sup>28</sup> and rat fundus strip.<sup>27</sup> It has been assumed that part of the contractile 5-HT effect might involve stimulation of  $\alpha$ -adrenergic receptors. However, moderate concentrations of the  $\alpha$ -antagonist, phentolamine, which in pilot experiments produced a clear shift in the log dose-response curve for norepinephrine toward

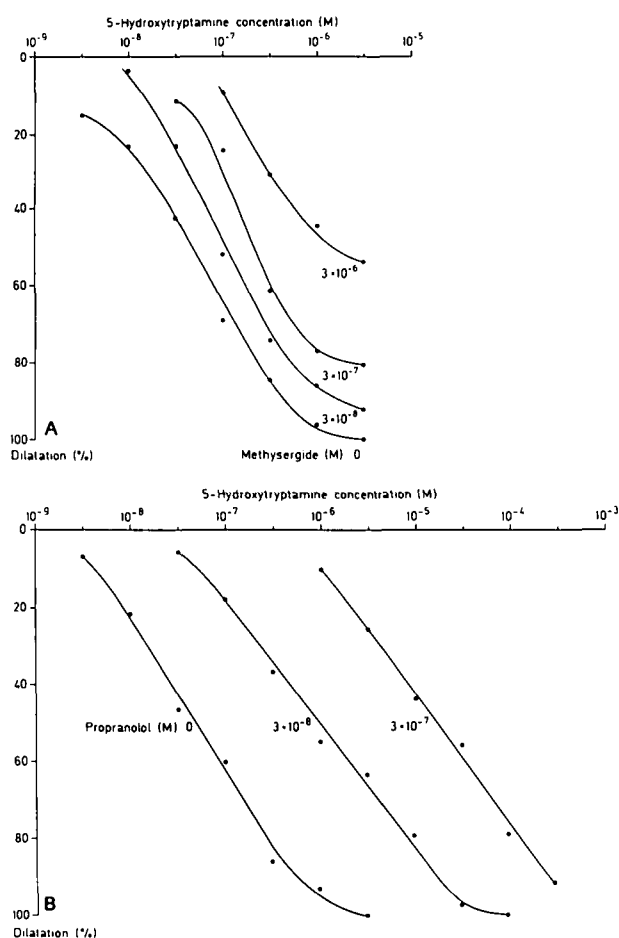


FIGURE 5 Dose-response curves for the dilator effect of 5-HT tested on actively contracted (by  $2.5 \times 10^{-6}$  M  $PGF_{2\alpha}$ ), sympathectomized arteries after pretreatment with  $3 \times 10^{-5}$  M phenoxybenzamine for 30 minutes. The 5-HT antagonist, methysergide (A; intracranial artery) reduced  $E_{Am}$  and only slightly affected  $ED_{50}$ , whereas the  $\beta$ -receptor antagonist, propranolol (B; extracranial artery) produced a clear parallel shift in the log dose-response curves without affecting  $E_{Am}$ .

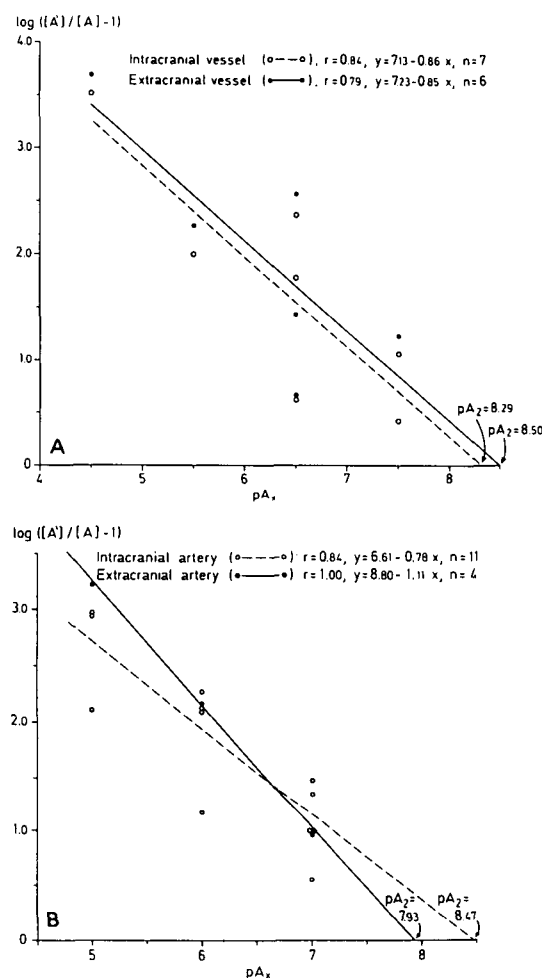


FIGURE 6 Arunlakshana-Schild plots for the dilator response to 5-HT and its antagonism by (A) propranolol (one of the experiments on the extracranial vessels is illustrated in Figure 5B) and (B) 1-N-isopropyl-p-nitrophenyl-ethanolamine (INPEA). The  $pA_2$  values are given together with the equation for the lines, the correlation coefficients ( $r$ ), and the number of experiments ( $n$ ).

higher doses, did not affect the constriction induced by 5-HT. Phenoxybenzamine, an irreversible  $\alpha$ -antagonist, tested in a dose that completely abolished the norepinephrine response, only reduced the 5-HT effect. Taken together, these results favor the assumption that 5-HT mediates its contractile effect on extracranial arteries via specific 5-HT receptors. The possible involvement of adrenergic receptor stimulation in the contractile 5-HT effect in extracranial arteries thus seems to be negligible in analogy with previous findings in other tissues.<sup>27, 28</sup>

In both cat and man, the intracranial vessels were more sensitive than the extracranial ones to 5-HT. The contractile effect of 5-HT on intracranial vessels was inhibited by methysergide and LSD, but neither compound shifted the log dose-response relation in a manner that could be expected for true competitive inhibition of the response. Instead, the maximum contraction and the slope of the dose-response curve were reduced by increasing concentrations of either compound; the antagonistic effect was reversible in the sense that it was easily eliminated by

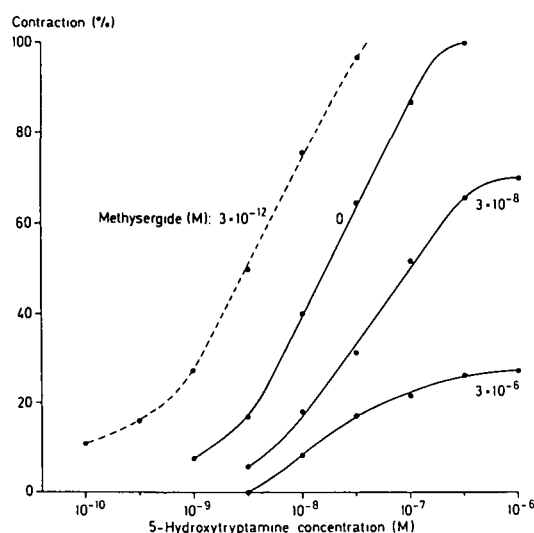


FIGURE 7 Dose-response curves for the effect of 5-HT on a human pial artery and the interaction with methysergide: at low doses methysergide potentiates the 5-HT effect (hatched curve) whereas, at higher doses, it reduces  $E_{Am}$  and slope of the curves. The tests were carried out in the presence of  $3 \times 10^{-7}$  M propranolol administered as described in Figure 1.

washing. Similar findings previously have been obtained in this vascular bed with methysergide<sup>22, 25, 32-34</sup> and LSD.<sup>24</sup> These results indicate a discrepancy between intracranial and extracranial vessels with regard to the nature of the receptor mediating the contractile response to 5-HT and related compounds. The results also emphasize the importance of using full dose-response curves before and after applying the antagonist in varying concentrations—rather than tests with single concentrations—in attempts to characterize a receptor, even when such a “specific” antagonist as methysergide is used. In the same way as for the extracranial arteries, the  $\alpha$ -receptor antagonist, phentolamine, did not affect the 5-HT-induced contraction in low doses. Moreover, phenoxybenzamine reduced the contraction induced by 5-HT under conditions when the norepinephrine-induced contraction was totally inhibited. The results showing that the potency rank for 5-HT and related agonists is the same in both types of vessels, together with the finding that intracranial arteries are more sensitive to 5-HT than the extracranial ones, indicate that a 5-HT receptor mediates contraction also in intracranial arteries. However, the receptor does not conform with that found in the extracranial arteries, i.e., a typical 5-HT receptor which can be blocked competitively by methysergide, and it does not seem to involve  $\alpha$ -receptor properties. The contractile 5-HT receptors would correspond in the older classification of Gaddum and Picarelli<sup>35</sup> to the D-type of tryptamine receptor.

### Dilator Response

The competitive antagonist, phenoxybenzamine, is for practical purpose considered to be irreversible because of the very slow recovery following inactivation of the receptor.<sup>16</sup> It was possible to find a certain minimum dose at which this antagonist completely abolished the 5-HT-induced contraction, a condition required when the dilator

activity of this agonist was under study. The dilator responses are best revealed *in vitro* if the vessels are given an active tone.<sup>9</sup> In this study, prostaglandin  $F_{2a}$  was used, since its vasoconstrictor action is not mediated via 5-HT receptors<sup>32</sup> and it does not potentiate or reduce the activity of 5-HT.<sup>36, 37</sup>

Both 5-HT and tryptamine were able to dilate both types of arteries, and the potency relation was similar to that observed for the contractile effects. Evidence for a dilator effect of 5-HT has been demonstrated also in terms of increased cerebral blood flow,<sup>7</sup> dilation of pial arteries of less than 100  $\mu$ m in diameter<sup>38</sup> and actions on the peripheral vascular bed.<sup>5, 17, 18</sup> Neither of the 5-HT receptor antagonists tested (methysergide and LSD) was able to produce a parallel shift of the dose-response curves of 5-HT. Instead, increasing doses of the antagonists successively reduced the maximum effect of 5-HT without markedly affecting the  $ED_{50}$  value. This indicated that the antagonism of 5-HT did not involve a competitive blockade of a 5-HT receptor. The dilator response to 5-HT could, on the other hand, be blocked in a competitive manner by increasing concentrations of two  $\beta$ -receptor antagonists, propranolol as well as INPEA, which has an unusually high specificity in that it lacks local anesthetic or “quinidine-like” side effects.<sup>39</sup> This shows that the receptor on which 5-HT acts to cause dilation resembles adrenergic  $\beta$ -receptors insofar as propranolol and INPEA are potent antagonists to both 5-HT and isoproterenol as dilating agents. In the extracranial arteries, the  $K_B$  (and corresponding  $pA_2$ ) values for propranolol against 5-HT were not significantly different from the corresponding values against isoproterenol as an agonist.<sup>25</sup> The  $pA_2$  value (7.93) with INPEA as antagonist was somewhat higher than those previously reported<sup>40</sup> for guinea pig trachea (7.12) and atrium (6.81). The  $pA_2$  value (as well as  $K_B$ ) is an important parameter in the characterization of a receptor: it should be the same, irrespective of the agonist used, for a particular antagonist acting on a specific receptor, and values of  $pA_2$  should be equal for various tissues when a given antagonist is acting on the same type of receptor.<sup>41</sup> The results suggest that 5-HT dilates extracranial arteries by an effect on  $\beta$ -adrenergic receptors. The  $pA_2$  value for the dilator action of 5-HT on the intracranial arteries was somewhat lower (and the corresponding  $K_B$  higher) in the presence of propranolol (8.29) and higher in the presence of INPEA (8.47), compared with values for the dilation produced with isoproterenol.<sup>9, 40</sup> Additional competitive antagonism experiments with other antagonists will have to be conducted before it can be concluded firmly that the intracranial vascular receptor on which 5-HT acts to produce dilation is the same as the  $\beta$ -receptor on which isoproterenol acts.

In conclusion, the present experiments have demonstrated that extracranial arteries possess a contractile 5-HT receptor similar to that described for other peripheral tissues. Evidence has also been obtained for a contractile 5-HT receptor in intracranial arteries, with certain features which distinguish it from the usual type. Provided the contractile effect of 5-HT is totally inhibited and the vessels are given an active tone, 5-HT can dilate both types of arteries. On the basis of studies with propranolol



and INPEA antagonism, the dilator response has been attributed to an action of 5-HT on a receptor that may be the same as, or closely related to, the adrenergic  $\beta$ -receptor.

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