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SOME PROPERTIES OF 5-HYDROXYTRYPTAMINE RECEPTORS IN THE HINDQUARTERS OF THE RAT

MARIE COOPER & JOHN H. WYLLIE

Department of Surgery, University College Hospital Medical School, London

1 The rat hindquarter preparation, as described, responds with reproducible vasoconstriction to noradrenaline and tryptamines.

2 The receptors involved in these responses are distinct.

3 Evidence of heterogeneity of tryptamine receptors was not obtained.

4 The 5-hydroxytryptamine (5-HT) antagonists, methysergide and cyproheptadine, although very potent, displayed antagonism of a non-competitive type whereas a series of phenothiazines and phentolamine displayed competitive antagonism against 5-HT.

5 For the phenothiazines the order of increasing potency was promazine < chlorpromazine < triflupromazine.

Introduction

The complex effects of 5-hydroxytryptamine (5-HT) on the circulation of intact animals are largely explained by 5-HT acting on different organs in the body to produce opposing effects; but this cannot be the whole explanation. Even in strips of isolated blood vessels the actions of 5-HT are incompletely understood. Thus, 5-HT can sometimes be shown to have a vasodilator as well as a vasoconstrictor action; and sometimes its effects are blocked by the 5-HT antagonist, methysergide, in a competitive fashion, sometimes in a non-competitive fashion and sometimes not at all (Apperley, Humphrey & Levy, 1977; Edvinsson, Hardebo & Owman, 1978). The present experiments were carried out in an attempt to define some of the properties of the 5-HT receptors present in the vessels of the hindquarters of rats.

Methods

Wistar rats (mean wt. 300 g, 200 to 400 g) were stunned and decapitated; the abdomen was quickly opened and a polyethylene cannula was inserted into the aorta, distal to the renal and mesenteric vessels. Through it 0.2 to 0.4 ml of heparin (1000 u/ml, Weddel Pharmaceuticals Ltd.) was immediately injected. The inferior vena cava was transected and perfusion of the hindquarters of the rat was started. The perfusate was Krebs solution (composition (mM): NaCl 120, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.18, $CaCl_2$ 2.5, $MgSO_4$ 1.18, and glucose 5.6, gassed at room temperature with 95% O_2 and 5% CO_2). It was delivered at a constant rate of 10 ml/min by a Watson-Marlow pump and was warmed to 36°C by a water jacket before it reached the preparation. The effluent, which became free from obvious blood in about 10 min, was allowed to run to waste. Just proximal to the cannula a side arm led to a pressure transducer (type SEM 4.88, SE Laboratories Ltd.) which operated a Servoscribe 2 chart recorder through a transducer coupler (Z.T. Sabikowski). Although this recording equipment was very stable a mercury manometer was used to calibrate it at the end of each experiment. A bubble trap prevented gas emboli from reaching the preparation.

Agonists, such as 5-HT and noradrenaline, were added to the Krebs solution by infusion at rates of up to 0.9 ml/min with a Watson-Marlow delta pump. The point of infusion was before the Krebs solution reached the main perfusion pump so the perfusion rate was not affected. All agonists were dissolved in Krebs solution with the exception of tryptamine which required the addition of ethanol; the final concentration reaching the rat was up to 0.5% v/v, but this did not obviously interfere with the responses of the blood vessels. Unless otherwise stated, agonist infusions lasted for 60 s. Antagonists, such as phentolamine and chlorpromazine, were added to the reservoir of Krebs solution which was protected from light by a black polythene bag.

Drugs

The following substances were used; all doses are given in terms of the active moiety: 5-hydroxytryptamine creatinine sulphate (mol. wt. 387.4), Sigma;

0007-1188/79/090079-07 $01.00

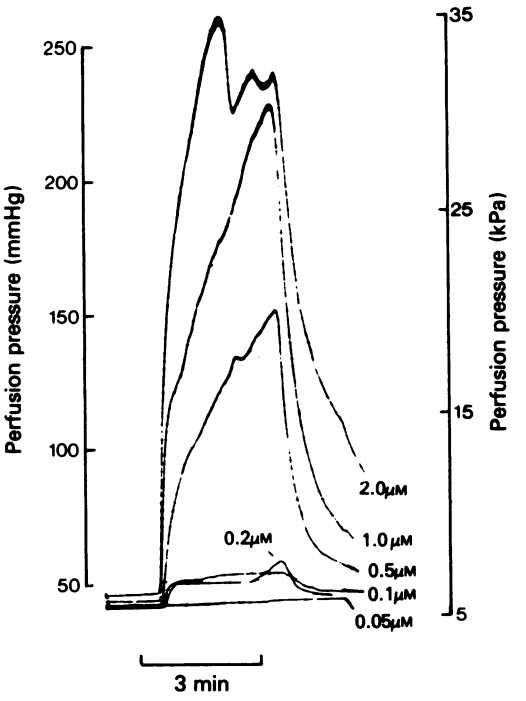

Figure 1 Response of perfused rat hindquarters to infusions of 5-hydroxytryptamine (5-HT). The hindquarters were perfused at a constant rate of 10 ml/min, and during the indicated 3 min period 5-HT was added to the perfusate in the concentrations shown.

tryptamine hydrochloride (mol. wt. 196.7), Sigma; 5-methoxytryptamine hydrochloride (mol. wt. 226.8), Aldrich Chemical Co.; (−)-noradrenaline hydrochloride (mol. wt. 205.7), Sigma; histamine acid phosphate (mol. wt. 307.4), BDH; bradykinin synthetic (mol. wt. 1060.25), Sandoz Chemicals; isoprenaline hydrochloride (mol. wt. 247.7), Sigma; acetylcholine perchlorate (mol. wt. 245.64), BDH; nialamide (mol. wt. 298.6), Sigma; chlorpromazine hydrochloride (mol. wt. 355.44), May & Baker; promazine hydrochloride (mol. wt. 320.9), Wyeth Labs; trifluprazine hydrochloride (mol. wt. 389.0), a gift from Dr Lee Green, Smith Kline & French; phentolamine mesylate (mol. wt. 377.5), Ciba; cyproheptadine hydrochloride (mol. wt. 323.9), a gift from Merck, Sharp & Dohme Research Laboratories; methysergide hydrogen maleate (mol. wt. 469.5), a gift from Sandoz.

Results

At the start of experiments, perfusion of the rat hindquarters at 10 ml/min occurred with a pressure of 43.4 (±2.2 s.e.mean) mmHg. This base-line gradually rose to 62.3 ± 2.5 s.e.mean at 2 h when 5-HT was the agonist, and to an identical figure when tryptamine was the agonist. The base-line rose significantly more rapidly when noradrenaline was the agonist; starting at 44.1 ± 3.0 it rose to 59.4 ± 3.8 at 1 h and to 69.2 ± 5.3 at 2 h. Addition of 5-HT or other agonists to the perfusion fluid produced a rapid, reproducible, dose-related rise in perfusion pressure. In the experiment shown in Figure 1 a fast chart speed was used to show the time-course of the response to infusions of 5-HT. The concentrations of 5-HT were from 0.05 μM, which produced no response, to 2 μM, which caused the perfusion pressure to rise to about 250 mmHg. The rate of rise of perfusion pressure was a function of the concentration of 5-HT and good discrimination was seen after infusions lasting 1 min, so the technique was standardized by always using 1 min infusions of agonists. Figure 2 shows a record of a typical experiment. Contrary to our expectations, 5-HT (and the other agonists tested) never produced vasodilatation. When infusions of 5-HT were stopped the perfusion pressure fell promptly and it was often possible to test successive doses at 5 min intervals. However, it was necessary to lengthen the cycle when using agonists which penetrate cells (Vane, 1959; Handschumacher & Vane, 1967) and when washing out the high concentrations of agonists which were needed in the presence of antagonists. Failure to observe vasodilatation may have been due to absence of vasomotor tone in the preparation. Attempts were made to produce vasodilator responses by 1 min infusions of the following substances at up to the stated concentrations: acetylcholine (2 expts; 2.56 mM), bradykinin (10 μM), histamine (2 expts; 2.56 mM) and isoprenaline (2 expts; 2.56 mM). Vasodilatation was never observed; instead small vasoconstrictor responses were seen with bradykinin (above 2 μM), histamine (above 1.28 mM) and isoprenaline (above 160 μM).

To assess the effects of antagonists, a dose-response curve to a particular agonist was first obtained; the antagonist was then added to the Krebs solution and a test dose of agonist was given several times until equilibration of the antagonist appeared to have occurred; a new dose-response curve was then made. This procedure could be repeated on the same preparation, using a higher dose of antagonist, and after washing out the antagonist as is shown for an experiment with 5-HT and chlorpromazine in Figure 2. The rise in perfusion pressure produced by the agonist was the parameter plotted in constructing dose-response curves and despite the slowly rising base-line usable results were obtained for at least 2 h.

During experiments the preparation slowly became more sensitive to agonists. Figure 3 shows this change in an experiment with 5-HT. The dose-response curves a, b, c, d were obtained at the following times

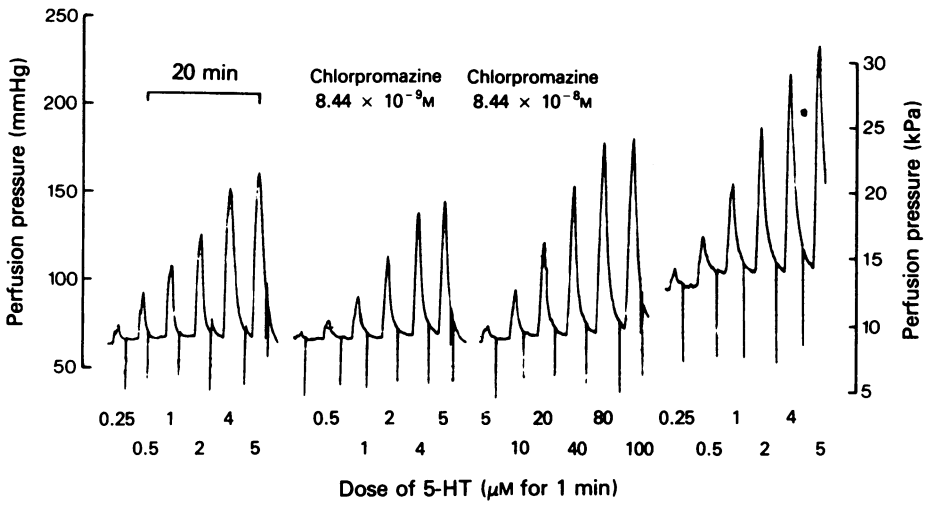

Figure 2 Dose-response curves to 5-hydroxytryptamine (5-HT) before and after equilibration with two concentrations of chlorpromazine. The downward deflections were due to regular purging of the bubble trap. Although the base-line had risen by the end of the experiment the rise in perfusion pressure caused by 5-HT was not greatly altered. Antagonism of 5-HT by 8.44×10^{-8} M chlorpromazine was overcome by increasing the dose about 20 fold.

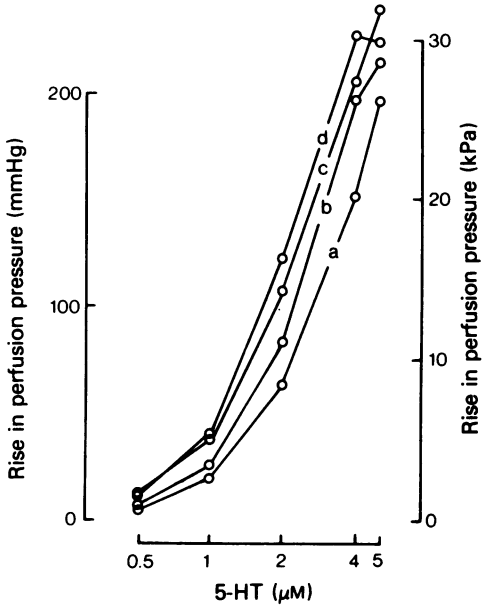

Figure 3 Serial dose-response curves. Four dose-response curves to 5-hydroxytryptamine (5-HT) (labelled a–d) were obtained at 30 min, 1 h, 1 h 40 min, and 2 h 15 min after setting up the preparation. A progressive, but not very great, increase in sensitivity is shown.

after setting up the preparation; 30 min, 1 h, 1 h 40 min and 2 h 15 min respectively. This increase in sensitivity to 5-HT was measured in 18 experiments and the dose required to raise the perfusion pressure by 100 mmHg was calculated from control dose-response curves at the beginning and end of experiments (as in Figure 2). There was an average increase in sensitivity corresponding to a dose-ratio of 1.49 ($\pm$0.068 s.e. mean), the observed range of dose-ratios being from 0.57 to 6.90.

This effect was partially allowed for in some experiments by obtaining a second control dose-response curve after washing out the antagonist; changing sensitivity of the tissues was then compensated for by relating the first control curve to the first dose of antagonist and the final control curve to a second dose of antagonist. Error due to changing tissue sensitivity was further reduced by working with dose-ratios up to 1000, so making the error relatively insignificant.

It was striking that two 5-HT antagonists in clinical use, methysergide and cyproheptadine both appeared to antagonize the effects of 5-HT in a non-competitive fashion. The contrast between this behaviour and that shown by promazine is shown in Figure 4. Methysergide and cyproheptadine tilted the dose-response curve so that maximal vasoconstriction was no longer produced by any dose of 5-HT that could be tested,

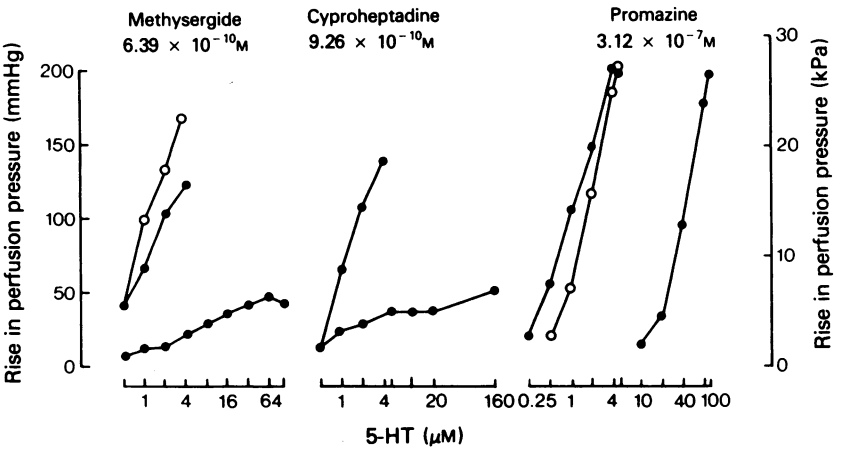

Figure 4 Antagonism of 5-hydroxytryptamine (5-HT) by methysergide, cyproheptadine and promazine. The three experiments were performed on different preparations by making a dose-response curve to 5-HT before adding the antagonist (●), by repeating this after equilibration with the antagonist in the concentration shown, and (in cases of methysergide and promazine) by checking recovery of sensitivity after washing out the antagonist (○). Initially all three preparations responded similarly. Methysergide and cyproheptadine altered the slope of the dose-response curve, whereas promazine displaced it to the right without alteration of slope.

behaviour typical of non-competitive antagonism. However, inhibition was easily reversed by washing the preparation in plain Krebs solution; recovery is shown for the case of methysergide. In contrast, promazine (Figure 4) and its more potent homologues chlorpromazine and trifluprazine, produced parallel displacement of the dose-response curve. Promazine produced a blockade which could be surmounted by increasing the dose of 5-HT by a factor assessed in this experiment at 44.7. In making such

Table 1 Slope of dose-response curves to 5-hydroxytryptamine (5-HT) and other agonists in absence and presence of antagonists

Agonist	*Antagonist (molar concentration range)*	n	*Mean control slope ± s.e.mean*	*Mean test slope ± s.e.mean*	t	P
5-HT	Promazine (3.0×10^{-8} to 3.0×10^{-6})	12	83.92 ± 6.82	92.55 ± 7.15	2.35	0.05
5-HT	Chlorpromazine (2.8×10^{-9} to 2.8×10^{-7})	13	62.15 ± 5.24	60.23 ± 6.16	0.62	NS
5-HT	Trifluprazine (7.8×10^{-10} to 7.8×10^{-8})	8	56.66 ± 7.04	52.31 ± 6.25	1.25	NS
5-HT	Phentolamine (1.3×10^{-7} to 2.6×10^{-5})	8	86.55 ± 8.93	86.93 ± 6.61	0.053	NS
Noradrenaline	Phentolamine (2.6×10^{-8} to 2.6×10^{-6})	7	84.64 ± 5.98	81.75 ± 5.50	0.41	NS
Tryptamine	Chlorpromazine (2.8×10^{-9} to 2.8×10^{-7})	7	64.24 ± 10.52	66.20 ± 6.18	0.25	NS
Tryptamine (with nialamide)	Chlorpromazine (8.4×10^{-10} to 8.4×10^{-8})	7	69.87 ± 9.61	67.64 ± 5.89	0.29	NS
5-Methyl-tryptamine (with nialamide)	Chlorpromazine (8.4×10^{-10} to 8.4×10^{-8})	4	65.24 ± 9.70	62.77 ± 12.02	0.50	NS
5-Methoxy-tryptamine (with nialamide)	Chlorpromazine (8.4×10^{-10} to 8.4×10^{-8})	6	72.02 ± 4.91	71.34 ± 7.51	0.15	NS
5-HT	Methysergide (2.1×10^{-10} to 2.1×10^{-9})	5	56.80 ± 7.36	7.63 ± 1.79	6.14	0.01
5-HT	Cyproheptadine (6.2×10^{-10} to 9.3×10^{-10})	3	111.04 ± 25.08	16.34 ± 7.74	4.34	0.05

Slopes of 50 and 100 mean that the perfusion pressure increased by 50 or 100 mmHg when the agonist concentration was increased by a factor of 2.718 (this being 1 log unit on the natural scale).

assessments of the dose-ratio we took the value corresponding to a rise in perfusion pressure of 100 mmHg, this being about the middle of the dose-response curve. Checks that control and test dose-response curves were essentially parallel were made in all experiments. Table 1 shows mean values for the slope of the regression of (increase in perfusion pressure in mmHg) on ($\log_e$ molarity of agonist). Although there was some variation in the slope (due probably to differing mean weight of different batches of rats) there were no significant differences between control and test measurements except in the case of promazine in which the difference was small, and in experiments with methysergide and cyproheptadine in which the slope was drastically altered by the antagonist. The values of t given in the table refer to paired-sample t tests.

There was never any difficulty in washing antagonists out of the rat hindquarters and restoring sensitivity to the original level; this is illustrated in Figures 2 and 4.

Another finding was that 5-HT and noradrenaline produced similar vasoconstriction when infused into the rat hindquarters; the dose-response curves were parallel and similar doses were required to produce a given degree of vasoconstriction. However, the idea that these two agonists may act on the same receptors (Innes, 1962) is untenable for this preparation, as was shown by comparing the pA_2 values for antagonism of each agonist by phentolamine. Table 2 shows the results. The pA_2 for noradrenaline and phentolamine was 8.23 with 95% confidence limits of 7.92 to 8.57 whereas the pA_2 for 5-HT and phentolamine was 6.62 with 95% confidence limits of 5.68 to 7.80. In both cases the slope of the regression of log (DR-1) on log [antagonist] was not significantly different from unity so there was no reason to doubt that antagonism was competitive.

Since the 5-HT receptors in this preparation are pharmacologically distinct from catecholamine α-receptors, we next wondered whether the 5-HT receptors were homogeneous. Three tryptamine analogues were tested against chlorpromazine to see whether they showed the same pA_2 as 5-HT. In the event of any of these compounds differentially stimulating one of a number of different types of 5-HT receptor one might find variations in pA_2 values. Table 2 shows the results. With chlorpromazine as antagonist the estimated pA_2 for tryptamine (in the absence of monoamine oxidase inhibitor) was 9.07. To make this estimate with concentrations of chlorpromazine covering the range 10^{-9} to 10^{-7} M it was necessary to infuse concentrations of tryptamine up to 10 mM. When nialamide (0.1 mM) was added to the system the tryptamine dose-response curve was displaced to the left in parallel with a dose-ratio of about 2. Under these conditions the pA_2 for chlorpromazine and tryptamine was unaltered at 9.15 (7.99 to 11.22). These values do not differ significantly from those obtained with 5-HT. There was thus no evidence in this preparation for the existence of separate receptors for tryptamine and 5-HT. Two other agonists were studied; 5-methyltryptamine and 5-methoxytryptamine. Competitive antagonism was again shown by chlorpromazine, and with the same pA_2 (Table 2). Thus, although the concentration of antagonist varied by a factor of 100 or more, no significant deviation from simple competitive antagonism was found; nor

Table 2 Antagonism of vasoconstriction in rat hindquarters

Agonist	*Antagonist (B)*	*No. of expts.*	*Mean slope of regression of log (DR-1) on log(B)*	*Mean value of pA_2*
5-HT	Promazine	12	1.08 (0.77–1.39)	7.72 (6.99–8.65)
5-HT	Chlorpromazine	19	0.88 (0.65–1.11)	8.97 (7.94–10.23)
5-HT	Triflupromazine	12	0.76 (0.48–1.04)	10.34 (9.19–12.17)
5-HT	Phentolamine	9	0.76 (0.48–1.03)	6.62 (5.68–7.80)
Noradrenaline	Phentolamine	8	0.96 (0.86–1.06)	8.23 (7.92–8.57)
Tryptamine	Chlorpromazine	10	0.89 (0.63–1.15)	9.07 (8.43–9.92)
Tryptamine (with nialamide)	Chlorpromazine	7	1.01 (0.46–1.56)	9.15 (7.99–11.22)
5-Methyltryptamine (with nialamide)	Chlorpromazine	4	1.24 (0.66–1.83)	8.54 (7.98–9.64)
5-Methoxytryptamine (with nialamide)	Chlorpromazine	6	1.04 (0.81–1.27)	8.86 (8.38–9.43)

95% confidence limits are shown in parentheses.
Note: The nos. of experiments in this Table exceed those in Table 1 because more than one concentration of antagonist was sometimes studied in a single rat preparation.

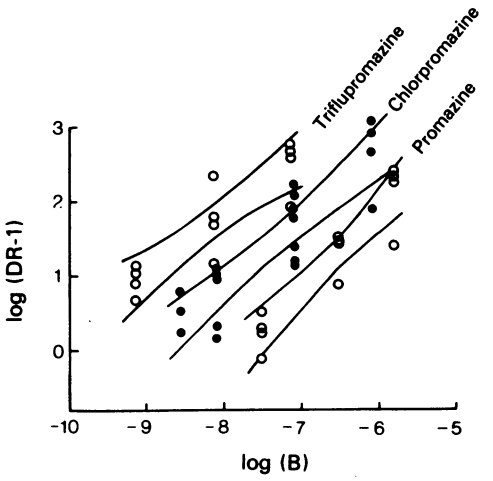

Figure 5 Schild plots. A series of three phenothiazines caused competitive blockade of 5-hydroxytryptamine-induced vasospasm in the rat hindquarter preparation. Envelopes show 95% confidence limits for the regression lines which are distinct.

did estimates of pA_2 differ enough to warrant the conclusion that the 5-HT receptors involved in these experiments were heterogeneous.

5-HT antagonism by phenothiazines is well known, chlorpromazine being regarded as particularly potent (Douglas, 1975) so we were interested to see whether homologous compounds would also antagonize 5-HT. As shown in Figure 5 this proved to be the case; the compound with weakest tranquillizing effect, promazine, had a pA_2 against 5-HT of 7.72, for chlorpromazine the pA_2 was 8.97 and for the most potent tranquillizer, trifluprazine, the pA_2 was 10.34.

Discussion

The rat hindquarter preparation used in these experiments suffers from the disadvantage that it becomes oedematous during perfusion with Krebs solution, the baseline perfusion pressure rising steeply if experiments are continued for much more than 2 h. However, if this time limit is not exceeded, the preparation gives results that are reasonably reproducible. There was no tendency for maximal responses to decline, so the contractility of the vascular muscle did not demonstrably deteriorate during experiments; on the contrary there was a tendency for sensitivity to agonists to increase as time passed.

Two types of antagonism of responses were found, either the dose-response curve was displaced in parallel or its slope was reduced. The former behaviour, characteristic of competitive antagonism, was seen in all experiments except those with methysergide and cyproheptadine. As shown in Tables 1 and 2, statistically significant deviation from parallel displacement was seen with methysergide and cyproheptadine (in which cases it could not be doubted) and with promazine (in which case the deviation was slight and only just reached significance at the 5% level). Also, in conformity with competitive antagonism, the Schild plots of log (dose-ratio minus one) against log (antagonist concentration) gave slopes which did not significantly differ from unity in any of the experiments in Table 2. Thus, apart from experiments with methysergide and cyproheptadine, the results met criteria of competitive antagonism.

However, it appears that noradrenaline and 5-HT stimulated the tissue by activating different receptors since distinct pA_2 values were obtained when phentolamine antagonized responses to these two agonists. In contrast, all the experiments with chlorpromazine and different tryptamines gave similar pA_2 values suggesting (but not, of course, proving) that the receptors involved are homogeneous.

Moreover, results in this paper agree well with those in the literature in showing a pA_2 of about 8 for noradrenaline (or other α-adrenoceptor stimulant) versus phentolamine (Furchgott, 1967; Gulati, Parikh & Umar, 1968; Clineschmidt, Geller, Govier & Sjoerdsma, 1970; Patil, Fudge & Jacobowitz, 1972; Fozard, 1976; Aperley *et al.*, 1977). This is confirmation of the reliability of the results obtained with the rat hindquarter preparation.

There is also good agreement between the pA_2 value obtained for 5-HT versus phentolamine in this paper and that obtained by Apperley *et al.* (1977) using dog femoral artery; in both cases the value was about 6.6. However, it is difficult to accept that this pA_2 of 6.6 characterizes a type of 5-HT receptor common to both preparations because this would leave unanswered the question why methysergide should behave as a competitive antagonist on dog artery strips and as a non-competitive antagonist in the rat hindquarter preparation. Moreover, it seems that in the cat (and man) methysergide behaves as a competitive antagonist of 5-HT ($pA_2 = 8.2$) on extracranial arteries but as a non-competitive antagonist on intracranial arteries (Edvinsson *et al.*, 1978). Thus it is difficult to explain results with methysergide on the basis of homogeneous vascular 5-HT receptors.

Although it is apparently well known that chlorpromazine is a powerful 5-HT antagonist we have not been able to find pA_2 values in the literature; data in Figure 5 (summarized in Table 2) show that the series of homologous phenothiazines promazine, chlorpromazine, trifluprazine show increasing competitive antagonism towards 5-HT.

The spectacular pA_2 value of 10.3 found for triflupromazine versus 5-HT far surpasses that shown by methysergide in those experiments in which methysergide acts as a competitive antagonist of 5-HT. It would be interesting to know whether the 5-HT receptors in all tissues show the same susceptibility to blockade by this drug.

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