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A BEHAVIOURAL AND BIOCHEMICAL STUDY IN RATS OF 5-HYDROXYTRYPTAMINE RECEPTOR AGONISTS AND ANTAGONISTS, WITH OBSERVATIONS ON STRUCTURE-ACTIVITY REQUIREMENTS FOR THE AGONISTS

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1 The effect of the putative 5-hydroxytryptamine (5-HT) receptor antagonists, methysergide, methergoline, mianserin, cyproheptadine, cinanserin (all at 10 mg/kg), methiothepin (5 mg/kg) and (−)-propranolol (20 mg/kg) on the behavioural responses to tranylcypromine (10 mg/kg) followed 30 min later by L-tryptophan (100 mg/kg) was examined.

2 Methysergide, methergoline, methiothepin and (−)-propranolol inhibited head weaving, forepaw treading and hind-limb abduction. Methysergide and methergoline increased reactivity. In contrast, cyproheptadine, cinanserin and mianserin had no effects on the behaviour.

3 Similar findings were obtained when the behaviours were elicited by administration of tranylcypromine (10 mg/kg) followed by the putative 5-HT receptor agonist, 5-methoxy-*N,N*-dimethyltryptamine (5-MeODMT) (2 mg/kg).

4 When the behaviours were elicited by the putative 5-HT receptor agonist, quipazine (50 mg/kg), all the drugs effectively inhibited head weaving and forepaw treading.

5 When the dose of cyproheptadine was doubled to 20 mg/kg an inhibition of the tranylcypromine/L-tryptophan induced behaviours was seen.

6 Methiothepin produced a marked inhibition of apomorphine-induced locomotor activity whilst all the others enhanced this response, suggesting that only methiothepin inhibits the 5-HT behaviours by dopamine antagonism and that the increased reactivity seen following tranylcypromine/L-tryptophan after pretreatment with methysergide or methergoline might be due to enhanced dopamine function.

7 Pretreatment with *p*-chlorophenylalanine resulted in enhanced behavioural responses to both 5-MeODMT and quipazine.

8 Both methergoline and methiothepin decreased the rate of 5-HT synthesis in whole brain but not spinal cord and methergoline decreased spinal cord 5-HIAA concentration. None of the other drugs had any significant effects on the concentration of 5-HT, 5-HIAA or 5-HT synthesis rate in brain or spinal cord.

9 Experiments with compounds structurally related to quipazine and with molecular models suggested that quipazine produces behavioural changes probably by stimulating the 5-HT receptor in a similar way to 5-HT but that it would bind weakly, in agreement with ligand-receptor binding studies.

10 It is suggested, therefore, that cyproheptadine, cinanserin and mianserin fail to inhibit 5-HT and 5-MeODMT-induced behaviours because they are weak antagonists whilst they are able to inhibit the same behaviours induced by quipazine because it is a weak agonist.

11 These data indicate that extreme care should be taken in accepting or rejecting 5-HT as a mediator of behaviours or of other responses unless several antagonists or agonists have been examined.

Introduction

A major problem in the study of the biochemistry and function of brain 5-hydroxytryptamine (5-HT) is the use of suitable antagonists. Compounds known to act as potent 5-HT antagonists in the periphery have been shown in iontophoretic studies to have little effect in blocking the inhibitory responses to 5-HT in

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areas with a heavy 5-hydroxytryptaminergic input (Roberts & Straughan, 1969; Haigler & Aghajanian, 1974, 1977). Various behavioural studies have also reported conflicting data on the actions of putative antagonists (see Discussion).

Several workers have postulated the presence of more than one type of 5-HT receptor in the rat brain (e.g. Peroutka & Snyder, 1979); not only pre- and postsynaptic receptors but also either separate postsynaptic receptor populations or the presence of a single receptor in different conformational states.

In an attempt to clarify the actions of various antagonists and investigate the possibility of demonstrating different 5-HT receptor populations, we have studied the effects of several 5-HT antagonists on the behaviour produced by various putative 5-HT agonists.

To examine 5-hydroxytryptamine-mediated function, the behaviour following administration of a monoamine oxidase inhibitor (tranylcypromine) plus L-tryptophan was examined. Several of the behavioural changes which follow administration of this drug combination appear to be 5-HT-mediated. The behaviours are blocked by prior treatment with *p*-chlorophenylalanine (Grahame-Smith, 1971a); produced by other putative 5-HT receptor agonists such as 5-methoxy,*N*,*N*-dimethyltryptamine (Grahame-Smith, 1971b), 5-methoxytryptamine (Green, Hughes & Tordoff, 1975), tryptamine (Foldes & Costa, 1975; Marsden & Curzon, 1978), quipazine (Green, Youdim & Grahame-Smith, 1976) and pretreatment with 5-hydroxytryptophan (5-HTP) (Grahame-Smith, 1971a; Modigh, 1972). The same behaviours are elicited by the 5-HT releasing drugs, fenfluramine and *p*-chloramphetamine and these effects are abolished by initial *p*-chlorophenylalanine pretreatment (Trulson & Jacobs, 1976; Green & Kelly, 1976). Chlorimipramine potentiates the changes produced by 5-HTP or L-tryptophan (Modigh, 1973; Green & Grahame-Smith, 1975). These data have been reviewed by Green & Grahame-Smith (1976a), Jacobs (1976), and Trulson, Ross & Jacobs (1976a). Recently Marsden & Curzon (1978) have indicated that tryptamine plays a role in the behavioural syndrome; however, it seems probable that this effect is either via tryptaminergic stimulation of the 5-HT receptor or via 5-HT release or both (Marsden & Curzon, 1978). It therefore seems reasonable to propose that certain tranylcypromine/L-tryptophan induced behaviours are due to increased 5-hydroxytryptaminergic function.

The effects of antagonists on the behaviour produced by the putative 5-HT receptor agonists 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT) and quipazine were also examined. 5-MeODMT has an indole structure and produces the same behavioural changes as seen after tranylcypromine/L-tryptophan (Grahame-Smith, 1971b). Quipazine also produces the same behavioural changes but has no indole structure (see Green *et al.*, 1976).

Finally a study of possible structure-activity relationships of 5-HT receptor agonists has been made in an attempt to clarify some of the behavioural observations.

Methods

Adult male Sprague-Dawley rats (Charles River Ltd) weighing 140–220 g were used in all experiments. They were kept housed in groups on a 08 h 00 min–20 h 00 min light-dark cycle at constant temperature (20 ± 1°C) and fed an *ad libitum* diet of modified 41B pellets and tap water. All behavioural and biochemical studies were performed between 09 h 30 min and 16 h 30 min.

Behaviour and biochemistry

The following changes were scored (the observer being 'blind' where possible), forepaw padding, head weaving, hindlimb abduction, Straub tail and reactivity to a sound stimulus. The scoring was that previously employed (Deakin & Green, 1978): 0 = absent; 1 = equivocal; 2 = present; 3 = severe. Activity was also measured on Automex activity meters (PMS Instruments, Slough).

Brain and spinal cord 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were measured fluorimetrically by the method of Curzon & Green (1970). 5-HT synthesis was measured by examining the rate of 5-HT accumulation following monoamine oxidase inhibition as described by Neff & Tozer (1968).

Statistical evaluation of the biochemical changes was by use of Student's unpaired *t* test and of the behaviour by use of the Mann-Whitney test for non-parametric data.

Rat fundic strip preparation

The preparation was that described by Vane (1957). Contractions in a gut-bath were detected by a Harvard transducer and recorded on a Tekman chart recorder.

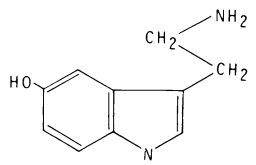
Drugs

The doses of all drugs are quoted as the salt where applicable. All drugs were given intraperitoneally either dissolved in 0.9% w/v NaCl solution (saline) or suspended in saline containing 1% carboxymethyl cellulose. All antagonists were given 60 min before

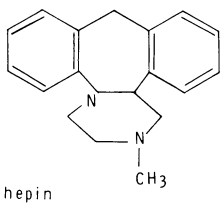
the behavioural test protocols except for cyproheptadine (120 min) and (−)-propranolol (45 min). Details of individual protocols are given in the results section.

Drugs were obtained from the following sources: L-tryptophan, 5-methoxy,*N*,*N*-dimethyltryptamine, methamphetamine, *p*-chlorophenylalanine methyl ester HCl (Sigma, Poole), apomorphine (MacFarlan-Smith), quipazine maleate (Miles Laboratories, Elkhart, Indiana, U.S.A.), methysergide tartrate, lysergic acid diethylamide tartrate (Sandoz, Feltham), methiothepin (Sandoz, Basle; gift from Dr P. Jenner), cinanserin (Squibb, Twickenham), cyproheptadine (Merck, Sharp & Dohme, Hoddesdon),

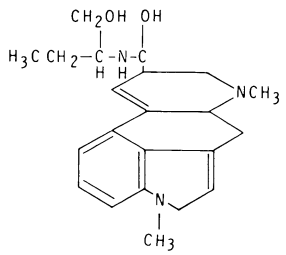
5-HT



Mianserin



Methysergide



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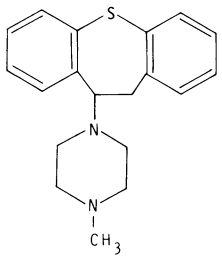
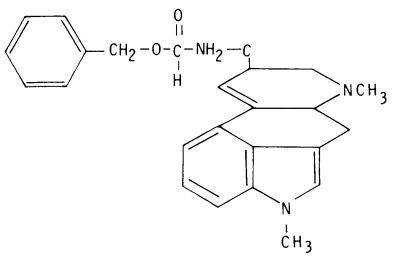
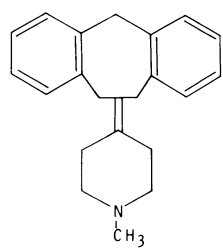


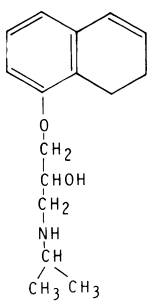
Methergoline



Cyproheptadine



Propranolol



Cinanserin

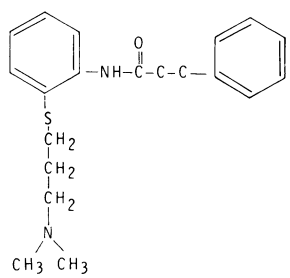


Figure 1 Structure of 5-hydroxytryptamine (5-HT) and the putative 5-HT antagonists used in the study.

methergoline (Farmitalia, Milan), mianserin (Organon, London), (−)-propranolol (ICI Pharmaceuticals, Macclesfield), 2-piperidino-3-methylquinoxaline HCl (W3428A) and 2-pipendinopyrazine HCl (W3368A) both from Warner-Lambert, Morris Plains, N.J., U.S.A., 1-(2-pyridyl)piperazine and N-phenylpiperazine both from Aldrich Chem. Co. (London). The molecular structures of the 5-HT and the antagonists examined are shown in Figure 1.

Molecular models

Space filling models (CPK) were used.

Results

The effects of 5-hydroxytryptamine antagonists on the behaviour produced by tranylcypromine and L-tryptophan

When the rats were given tranylcypromine (10 mg/kg) followed 30 min later by L-tryptophan (100 mg/kg) they developed a series of behavioural changes including head weaving, reciprocal forepaw treading, hindlimb abduction, Straub tail, increased reactivity and hyperactivity as described in detail by Grahame-Smith (1971a), Jacobs (1976) and Deakin & Green (1978).

Methiothepin (5 mg/kg), methysergide (10 mg/kg) and methergoline (10 mg/kg; not shown in figure) were very effective in blocking head weaving and forepaw treading with (−)-propranolol (20 mg/kg) also having a significant inhibitory effect (Figure 2a). In addition, methysergide, methergoline and (−)-propranolol were effective in inhibiting the hindlimb abduction but methiothepin was ineffective. Both methiothepin and propranolol inhibited reactivity whilst, in confirmation of the previous finding of Deakin & Green (1978), methysergide and methergoline produced a marked enhancement of the reactivity (for details of the behaviour see Deakin & Green, 1978). The automated recordings thus showed an increase following methysergide and methergoline (Figure 2a and Deakin & Green, 1978).

In marked contrast, it was found that none of the behaviours examined was in any way altered by prior treatment with cyproheptadine, cinanserin or mianserin (Figure 2b) all at a dose of 10 mg/kg.

The effects of 5-hydroxytryptamine antagonists on the behaviours produced by tranylcypromine and 5-methoxy-N,N-dimethyl tryptamine

Following pretreatment with tranylcypromine (10 mg/kg) to prolong the response, rats injected with 5-MeODMT (2 mg/kg) developed all the behavioural changes seen after tranylcypromine/L-tryptophan but with a shorter time-course (Grahame-Smith, 1971b; Figure 3a).

Pretreatment with the various antagonists resulted in essentially similar effects to those seen when the antagonists were given before tranylcypromine/L-tryptophan. That is methysergide, methergoline, methiothepin and propranolol all inhibited head-weaving and forepaw treading. Methiothepin was again ineffective in blocking hind-limb abduction, as was propranolol in this test. Again it was found that propranolol and methiothepin inhibited the hyperactivity whilst methysergide and methergoline (not shown) increased it markedly (Figure 3a). Cyproheptadine, cinanserin and mianserin failed to inhibit any of the behaviours studied, mirroring their failure to act on the tranylcypromine/L-tryptophan syndrome (Figure 3b).

Effects of the 5-hydroxytryptamine antagonists on the behaviour produced by administration of quipazine

Administration of quipazine (50 mg/kg) produced all the behaviours seen following tranylcypromine/L-tryptophan or tranylcypromine/5-MeODMT, confirming previous data (Green *et al.*, 1976). However, injection of the rats with the various antagonists prior to administration of quipazine led to markedly different results. Methiothepin again effectively inhibited head weaving, forepaw treading and reactivity. Methysergide and methergoline (not shown) were reasonable inhibitors of forepaw treading and head weaving whilst again enhancing reactivity, albeit this latter effect was much smaller than seen after tranylcypromine/L-tryptophan. Propranolol produced an effective block of head weaving and forepaw treading after the first 10 min but had little effect on reactivity or hind-limb abduction. Cyproheptadine, cinanserin and mianserin were extremely effective inhibitors of head weaving and forepaw treading produced by quipazine and of these three drugs only mianserin failed to inhibit hind-limb abduction. There were mixed effects on reactivity, cinanserin having no effect, mianserin inhibiting and cyproheptadine producing a slight, but statistically significant, enhancement (Figure 4a and 4b).

Effect of dose of antagonist on the behaviour produced by tranylcypromine/L-tryptophan or quipazine

Increase of the dose of cyproheptadine to 20 mg/kg produced partial inhibition of the behaviour induced by tranylcypromine/L-tryptophan administration (Figure 5). When the dose of methysergide was decreased to 7.5 mg/kg the drug now almost totally

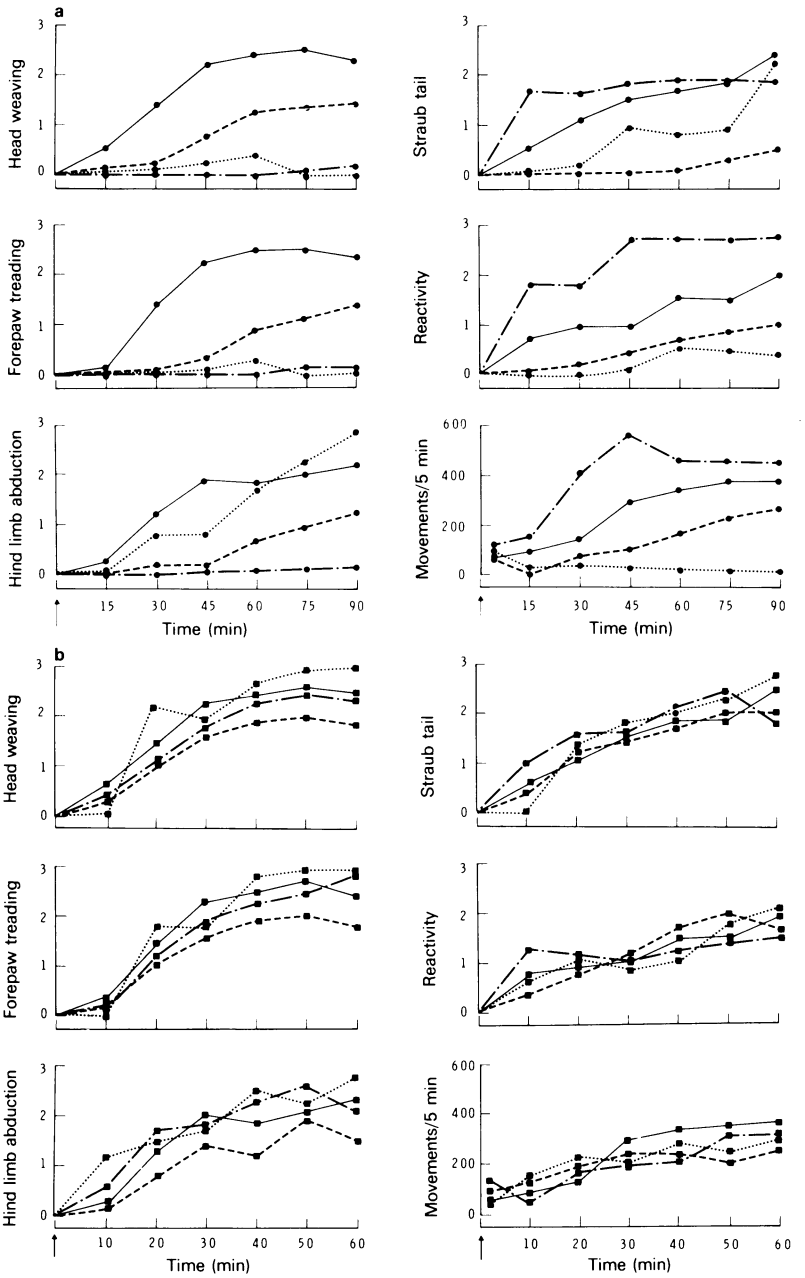


Figure 2(a) Effects of methysergide, methiothepin and propranolol on behaviour elicited by tranylcypromine and L-tryptophan in rats: (–●–) saline pretreated; (–·–●–·–) methysergide (10 mg/kg); (·····●·····) methiothepin (5 mg/kg); (– – –●– – –) (–)-propranolol (20 mg/kg). Tryptophan (100 mg/kg) was injected 30 min after tranylcypromine (10 mg/kg) in each group. Details of activity and behavioural ratings in Methods. Animals were grouped in threes for activity recording. Individual animals were rated at the same time as activity recording. Behavioural ratings show means of 9 or more saline pretreated animals and 6 or more in each of the drug pretreated groups. Differences between saline and drug pretreated animals are significant ($P < 0.05$ or better) for at least 3 time points on all measures except Straub tail. (b) Effects of cyproheptadine, cinanserin and mianserin on behaviour elicited by tranylcypromine and L-tryptophan in rats: (–■–) saline pretreated; (–·–■–·–) cyproheptadine (10 mg/kg); (·····■·····) cinanserin (10 mg/kg); (– – –■– – –) mianserin (10 mg/kg). For experimental details see legend to Figure 2a and Methods. No statistically significant ($P < 0.05$) changes were seen in any behavioural measurement.

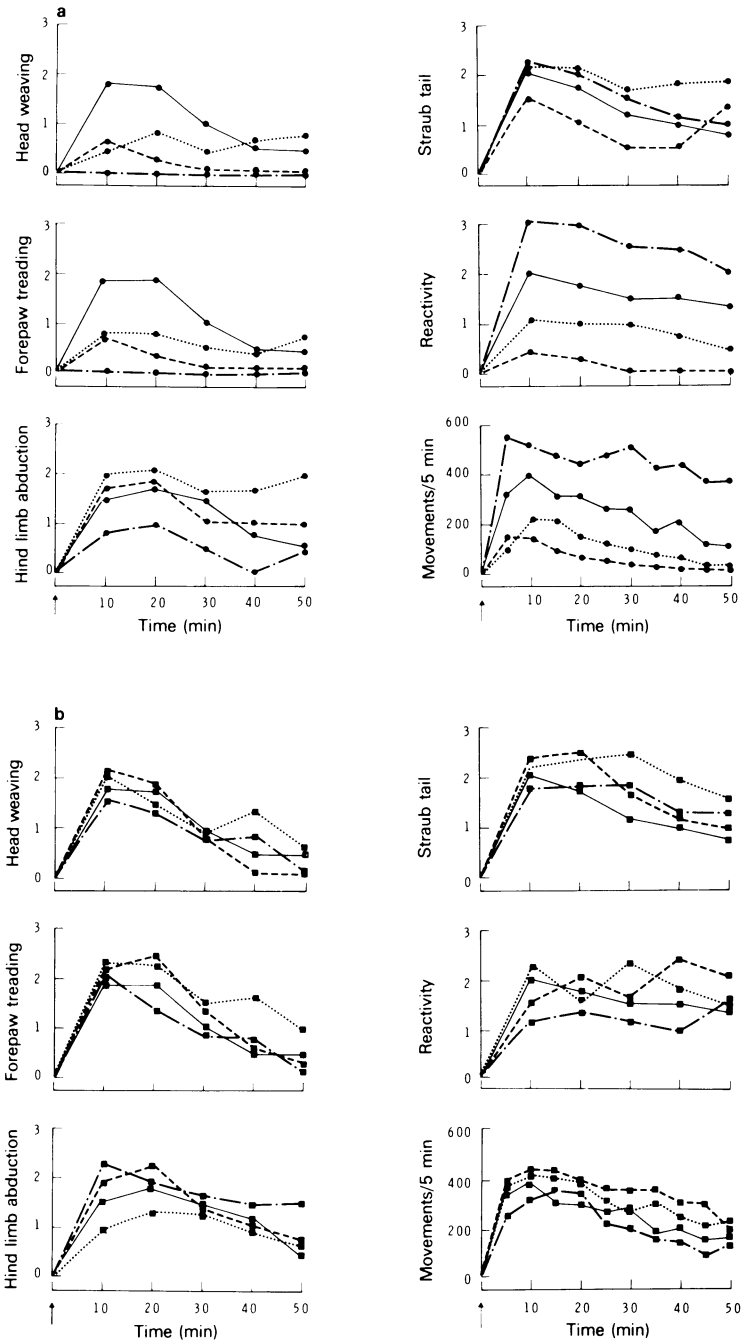


Figure 3(a) Effects of methysergide, methiothepin and (−)-propranolol on behaviour elicited by tranylcypromine and 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT) in rats. 5-MeODMT (2 mg/kg) injected 30 min after tranylcypromine (10 mg/kg) in each group. For experimental details see legend to Figure 2a. (b) Effects of cyproheptadine, cinanserin and mianserin on behaviour elicited by tranylcypromine and 5-MeODMT in rats. Key as in Figure 2b. For experimental details see legend to Figure 2a.

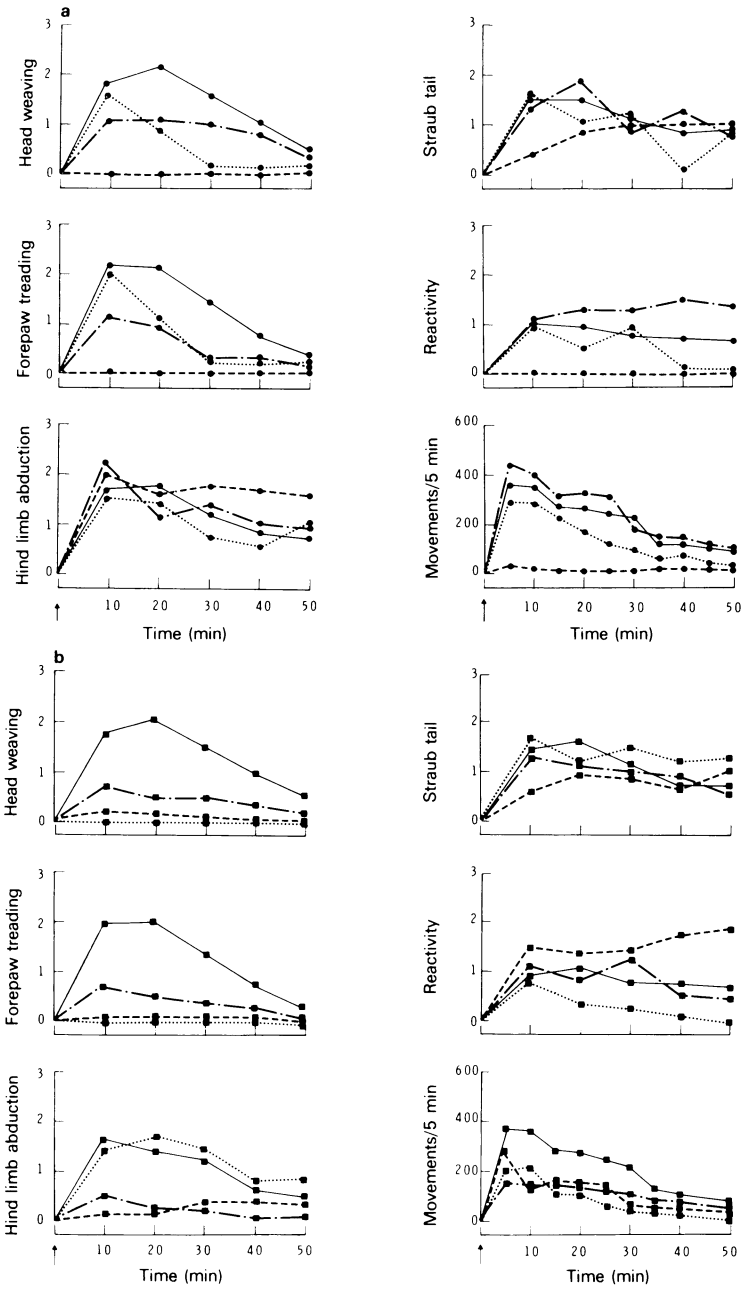


Figure 4(a) Effects of methysergide, methiothepin and (−)-propranolol on behaviour elicited by quipazine in rats. Key as in Figure 2a. Quipazine (50 mg/kg) was injected 60 min after all antagonists except (−)-propranolol which was given 45 min earlier. For experimental details see legend to Figure 2a. Differences between saline and drug pretreated animals were significant for head weaving and forepaw treading at $P < 0.05$ or better for all drugs. In addition, methiothepin inhibited and methysergide enhanced reactivity at 3 or more time points ($P < 0.05$ or better). (b) Effects of cyproheptadine, cinanserin and mianserin on behaviour elicited by quipazine in rats. Key as in Figure 2b. Quipazine (50 mg/kg) was injected 60 min after all antagonists except cyproheptadine which was given 120 min earlier. For experimental details see caption to Figure 2a. Differences between saline and pretreated animals were significant for head weaving and forepaw treading at $P < 0.05$ or better for all drugs. In addition, only cinanserin failed to inhibit hind limb abduction whilst cinanserin inhibited and cyproheptadine enhanced reactivity ($P < 0.05$ or better).

failed to inhibit the behaviour and no longer produced the increased reactivity or locomotor response (Figure 5).

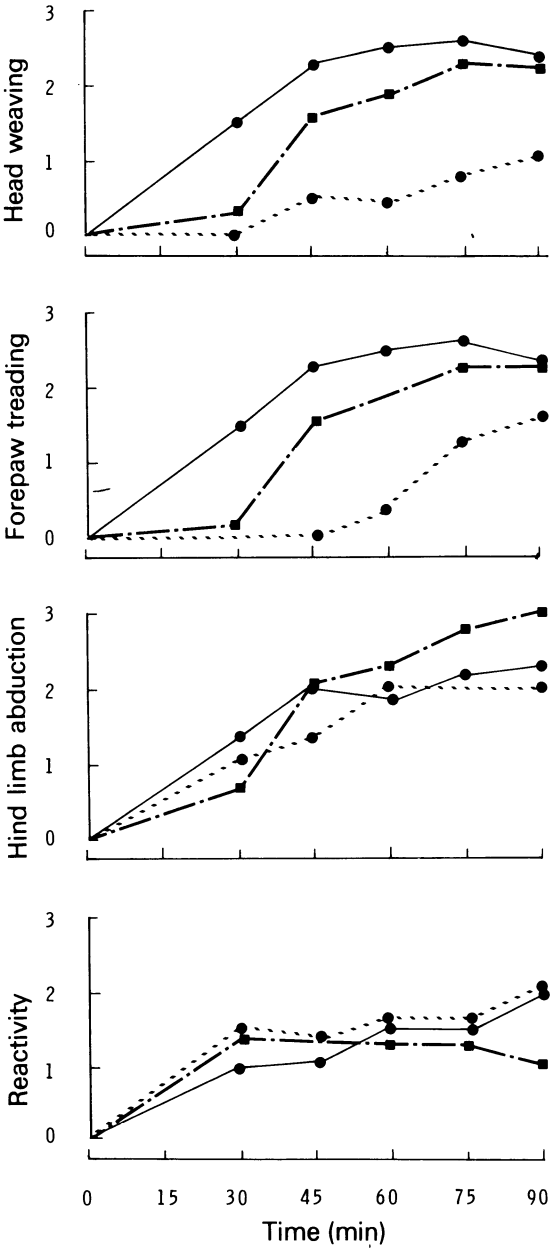


Figure 5 Effects of methysergide and cyproheptadine on the behaviour elicited by tranylcypromine and L-tryptophan in rats. (–O–) Saline pretreated; (–·–■–·–) methysergide (7.5 mg/kg) pretreated; (–––●–––) cyproheptadine (20 mg/kg) pretreated. Tranylcypromine and tryptophan administration and other experimental details as in legend to Figure 2a.

Effect of mianserin on the behaviour produced by lysergic acid diethylamide

Trulson *et al.* (1976a) have shown that high doses of lysergic acid diethylamide (LSD) produce all the behavioural changes produced by administration of tranylcypromine/L-tryptophan. The effect of mianserin on the behaviour produced by LSD (1 mg/kg) was examined.

Mianserin (10 mg/kg) effectively inhibited LSD-induced forepaw treading and head weaving but had no effect on hind-limb abduction or reactivity. The effect of mianserin on LSD-induced behaviour was thus very similar to its action on quipazine-induced behaviour.

Effect of p*-chlorophenylalanine pretreatment on the behaviour produced by quipazine or 5-methoxy-*N,N*-dimethyl tryptamine*

Trulson, Eubanks & Jacobs (1976b) and Deakin & Green (1978) have demonstrated that following denervation, 5-HT-induced behavioural responses (as demonstrated by 5-MeODMT responses) became enhanced or 'supersensitive'. However Trulson *et al.* (1976b) and Green (1977) were unable to show increased responses to 5-MeODMT following *p*-chlorophenylalanine (PCPA) pretreatment.

It seemed possible that enhanced responses after synthesis inhibition were not demonstrable following 5-MeODMT but might be following quipazine since these two agonists appeared to have different pharmacological profiles (this paper).

A different protocol for PCPA administration was used in this study from that employed in this laboratory previously or by Trulson *et al.* (1976b). Rats were injected with PCPA (200 mg/kg as the methyl ester HCl) or the saline vehicle on Day 1 and Day 2. On Day 6 the treated rats were given either quipazine (25 mg/kg) or tranylcypromine (10 mg/kg) followed 30 min later by 5-MeODMT (1 mg/kg) and the resultant behaviour scored.

Following PCPA pretreatment some of the behavioural changes (forepaw treading and head weaving) and the automated activity recordings following either quipazine or 5-MeODMT were enhanced significantly (Figure 6).

Structure activity requirements for 5-hydroxytryptamine agonists

The possibility existed that the differential effects of 5-HT antagonists on the behavioural responses of 5-HT receptor agonists were the result of quipazine binding to 5-HT receptors that were in a different conformational state from when they were stimulated by 5-HT or 5-MeODMT; quipazine not having

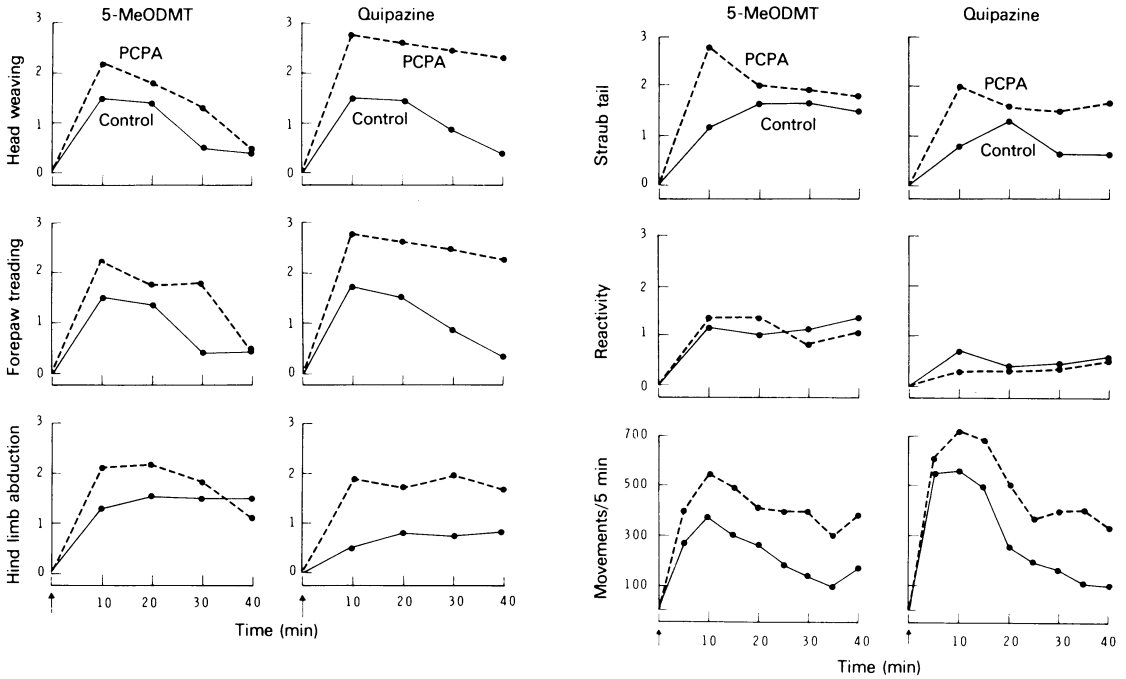


Figure 6 Effect of *p*-chlorophenylalanine (PCPA) pretreatment on behaviour elicited by tranylcypromine and 5-methoxy-*N,N*-dimethyltryptamine (5-MeODMT) or by quipazine: (–●–) saline pretreated; (---●---) PCPA pretreated (see Results). PCPA (200 mg/kg) was injected before tranylcypromine (10 mg/kg) and 5-MeODMT (1 mg/kg) or with quipazine (25 mg/kg). Head weaving, forepaw treading, hind limb abduction and automated activity counts were enhanced ($P < 0.05$) following 5-MeODMT. The same behaviours were also enhanced ($P < 0.01$) following quipazine.

an indole structure (Figure 7). We therefore examined a range of compounds closely related to quipazine.

Whilst MK212 has been reported to produce the same behavioural changes as quipazine and 5-MeODMT (Clineschmidt, 1979) none of the other compounds shown (W3428A, W3368A, 2-PP and NPP) produced any behavioural response at doses as high as 50 mg/kg. This did not appear to be due to poor penetration into the brain as in a fundic strip preparation, 5-HT produced a full-scale contraction at a concentration of 10^{-9}M, quipazine a 50% deflection at 5×10^{-9}M whilst W3428A, W3368A, 2-PP and NPP produced no contraction at a dose of 10^{-7}M.

Effect of 5-hydroxytryptamine antagonists on the concentration of 5-HT and 5-hydroxyindoleacetic acid in the brain and spinal cord and the rate of 5-HT synthesis

Putative 5-HT antagonists have been reported to have various effects on brain 5-HT concentrations and synthesis (see Discussion). The effects of the antagonists used on these parameters was therefore examined to see whether they produced effects that correlated with their ability to inhibit 5-HT-mediated behaviour. In particular, the effects of the drugs on spinal cord 5-HT were examined since both the head weaving and forepaw treading behaviour appear to be spinally mediated (Jacobs & Klemfuss, 1975; Deakin & Green, 1978).

All the drugs under investigation failed to produce a statistically significant effect on basal concentrations of 5-HT in the whole brain (Table 1) or spinal cord (Table 2) when given as pretreatment. When the rate of 5-HT synthesis was examined by the method of Neff & Tozer (1968; see Methods) no change in rate was detected in the brain following any drug except methergoline and methiothepin, both of which decreased the rate slightly (Table 1). The apparent, although non-significant, rise in basal 5-HT concentration produced by all drugs except methysergide in the cord meant that when the synthesis rate was calculated all drugs except methysergide had decreased the rate of accumulation of 5-HT following monoamine oxidase inhibition and thus, by inference, the rate of 5-HT synthesis (Table 2).

With the exception of methergoline, which low-

ered 5-HIAA concentration in the spinal cord, no significant effects of the antagonists were seen on the 5-HIAA concentration in brain or cord (Table 3).

There was no correlation between those drugs that inhibited 5-HT-mediated behaviour and those that altered 5-HT synthesis rates in brain or spinal cord.

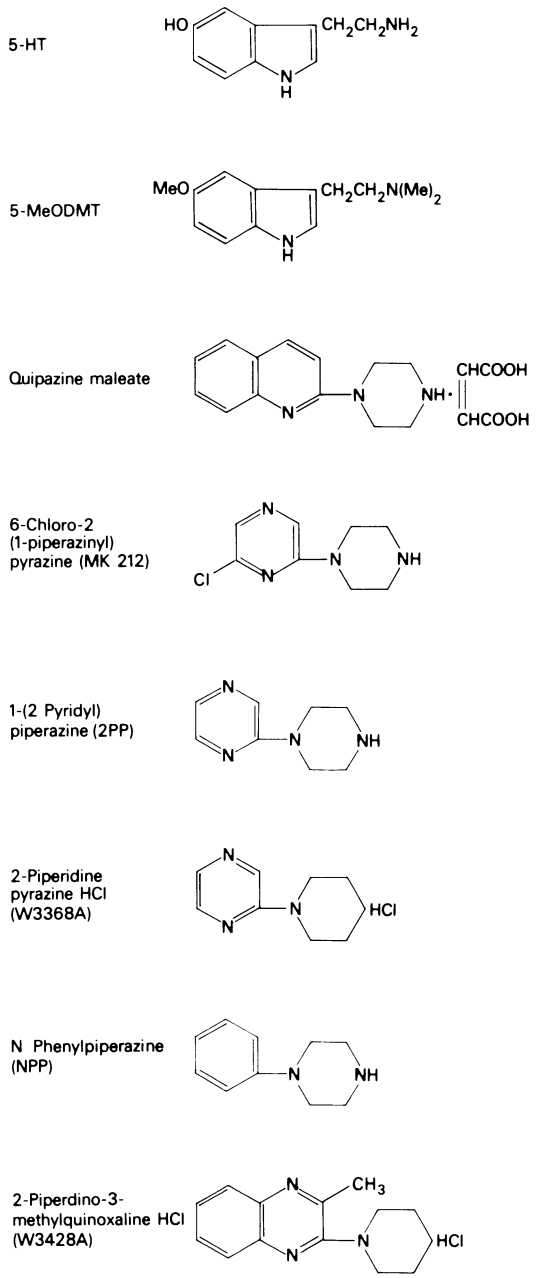


Figure 7 Structure of putative 5-hydroxytryptamine (5-HT) and 5-HT receptor agonists investigated.

The effect of 5-hydroxytryptamine antagonists on the behaviour produced by apomorphine

Since 5-HT-mediated behaviours require intact dopaminergic function for their expression (Green & Grahame-Smith, 1974; Deakin & Green, 1978), the possible effects of the 5-HT antagonists on dopamine-mediated responses were investigated by examining their action on the locomotor response produced by apomorphine (0.25 mg/kg s.c. into the neck).

Pretreatment 1 h beforehand with any of the antagonists, except methiothepin, produced a statistically significant enhancement of the locomotor response that follows apomorphine administration. Methiothepin pretreatment resulted in an almost total inhibition of the locomotor activity (Figure 8).

Discussion

Alteration of the behaviours produced by 5-hydroxytryptamine, 5-methoxy-N,N-dimethyl tryptamine or quipazine by putative 5-HT antagonists

Table 4 summarizes and simplifies the major behavioural changes and the effects of the antagonists

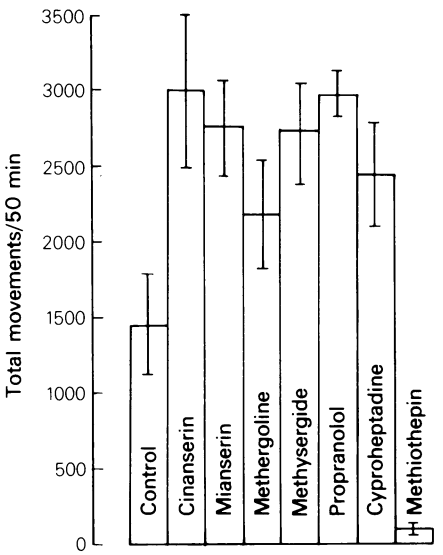


Figure 8 The effect of putative 5-hydroxytryptamine (5-HT) antagonists on the locomotor behaviour produced by apomorphine. Rats were pretreated with the antagonists; all at a dose of 10 mg/kg except (−)-propranolol (20 mg/kg) 60 min before apomorphine (0.25 mg/kg s.c.). Results are shown as total recorded movements on Automex meters during the next 50 min. All drugs significantly enhanced ($P < 0.025$ or better) responses except methiothepin, which significantly inhibited the activity ($P < 0.001$).

Table 1 Effect of putative 5-hydroxytryptamine (5-HT) antagonists on brain 5-HT concentration and the rate of accumulation of 5-HT during 1 h following tranylcypromine (20 mg/kg)

	Brain 5-hydroxytryptamine (μg 5-HT/g brain)		*Synthesis rate of 5-HT* ($\mu g\ g^{-1}\ h^{-1}$)
Injected	*Saline*	*Tranylcypromine*	
Saline	0.39 ± 0.04 (6)	0.71 ± 0.05 (6)	0.32
Cinanserin	0.42 ± 0.01 (6)	0.69 ± 0.08 (8)	0.27
Cyproheptadine	0.37 ± 0.03 (6)	0.69 ± 0.08 (8)	0.32
Methergoline	0.41 ± 0.02 (5)	0.61 ± 0.06 (8)*	0.20
Methiothepin	0.39 ± 0.04 (5)	0.63 ± 0.06 (7)*	0.24
Methysergide	0.38 ± 0.05 (6)	0.67 ± 0.06 (6)	0.29
Mianserin	0.40 ± 0.05 (6)	0.67 ± 0.10 (10)	0.27
Propranolol	0.43 ± 0.03 (6)	0.69 ± 0.07 (5)	0.26

Results expressed as mean ± s.d. with number of observations in parentheses.
*Different from saline-injected control given tranylcypromine $P < 0.001$.
All drugs given at a dose of 10 mg/kg except (−)-propranolol (20 mg/kg) and methiothepin (5 mg/kg), and injected 60 min before either saline or tranylcypromine with measurement after a further 60 min.

on them. With regard to the behaviour produced by 5-HT (i.e. after tranylcypromine and L-tryptophan) it can be seen that methysergide, methergoline, methiothepin and propranolol decrease head weaving, forepaw treading and hind-limb abduction. Both propranolol and methiothepin decrease hyperreactivity but this behaviour is greatly enhanced by methysergide and methergoline.

In marked contrast, none of these behaviours is in any way modified by cinanserin, mianserin or cyproheptadine.

All these drugs have essentially the same effects or lack of effects on the behaviours produced by the agonist 5-MeODMT.

Methiothepin has been reported to have quite considerable neuroleptic activity (Lloyd & Barthollini, 1974) and we propose that this might go some way towards explaining its action in decreasing all responses since haloperidol (5 mg/kg) pretreatment has the same effect (Table 4) and methiothepin was the only drug used that inhibited apomorphine-induced locomotor activity (Figure 8). It has been shown that a dopaminergic system is involved, postsynaptically in this 5-HT-mediated behavioural response. Sloviter, Drust & Connor (1978) have recently argued against this on the basis of the failure of both pimozide and α-methyl-*p*-tyrosine pretreatment to attenuate the response. However, it had already been shown that pimozide did not inhibit the 5-HT-mediated behavioural response, in marked contrast to a whole range of other neuroleptics (Heal, Green, Boullin & Grahame-Smith, 1976) and indeed does not appear to inhibit dopamine-sensitive adenylate cyclase either *in vitro* (Clement-Cormier, Kebabian, Petzold & Greengard, 1974) or *in vivo* (Heal, Green, Bloomfield & Grahame-Smith, 1978).

Table 2 Effect of putative 5-hydroxytryptamine (5-HT) antagonists on the spinal cord 5-HT concentration and the rate of accumulation of 5-HT during 1 h following tranylcypromine (20 mg/kg)

	Cord 5-hydroxytryptamine (μg 5-HT/g brain)		*Synthesis rate of 5-HT* ($\mu g\ g^{-1}\ h^{-1}$)
Injected	*Saline*	*Tranylcypromine*	
Saline	0.89 ± 0.06 (6)	1.13 ± 0.18 (6)	0.24
Cinanserin	0.93 ± 0.11 (8)	1.10 ± 0.19 (6)	0.17
Cyproheptadine	0.96 ± 0.15 (7)	1.13 ± 0.13 (6)	0.17
Methergoline	0.98 ± 0.08 (5)	1.17 ± 0.22 (5)	0.19
Methiothepin	1.01 ± 0.13 (8)	1.17 ± 0.11 (6)	0.16
Methysergide	0.86 ± 0.15 (7)	1.15 ± 0.17 (6)	0.29
Mianserin	0.94 ± 0.13 (8)	1.14 ± 0.10 (5)	0.20
Propranolol	0.99 ± 0.18 (7)	1.21 ± 0.15 (5)	0.22

Results expressed as mean ± s.d. with number of observations in parentheses. All drugs given at a dose of 10 mg/kg except (−)-propranolol (20 mg/kg) and methiothepin (5 mg/kg), and injected 60 min before either saline or tranylcypromine with measurement after a further 60 min.

Table 3 Concentration of brain and spinal cord 5-hydroxyindoleacetic acid (5-HIAA) 1 h following administration of putative 5-hydroxytryptamine (5-HT) antagonists

Injected	*Tissue 5-hydroxyindoleacetic acid* (μg 5-HIAA/g brain) *Brain*	*Spinal cord*
Saline	0.35 ± 0.03 (5)	0.24 ± 0.08 (12)
Cinanserin	0.31 ± 0.03 (6)	0.25 ± 0.10 (10)
Cyproheptadine	0.33 ± 0.05 (6)	0.24 ± 0.04 (10)
Methergoline	0.33 ± 0.03 (5)	0.16 ± 0.02 (5)*
Methiothepin	0.33 ± 0.05 (8)	0.25 ± 0.05 (9)
Methysergide	0.27 ± 0.04 (5)	0.25 ± 0.11 (10)
Mianserin	0.35 ± 0.03 (6)	0.21 ± 0.07 (10)
Propranolol	0.33 ± 0.03 (6)	0.21 ± 0.06 (9)

Results expressed as mean ± s.d. with number of observations in parentheses.
*Different from saline injected control, $P < 0.05$.
All drugs given at a dose of 10 mg/kg except (−)-propranolol (20 mg/kg) and methiothepin (5 mg/kg), and injected 60 min before measurement.

Furthermore, inhibition of the 5-HT response shown by Green & Grahame-Smith (1974) and Deakin & Green (1978) is in good accord with the observation that intraventricular 6-hydroxydopamine abolished the behaviour (Foldes & Costa, 1975).

The remarkable aspect of this study is that cinanserin, cyproheptadine and mianserin, having been totally without effect on forepaw treading and head weaving produced by 5-HT or 5-MeODMT, were extremely effective in inhibiting this behaviour when it was produced with a similar degree of severity by injection of quipazine. Furthermore only mianserin also failed to inhibit hind-limb abduction.

Methysergide, methergoline, methiothepin and propranolol all inhibited quipazine-induced forepaw treading and head weaving although methysergide

Table 4 Summary of the effects of putative 5-hydroxytryptamine (5-HT) receptor agonists on the putative 5-HT antagonists studied

5-HT or 5-MeODMT	*HW*	*FT*	*HLA*	*R*
Cyproheptadine	—	—	—	—
Cinanserin	—	—	—	—
Mianserin	—	—	—	—
Methysergide	↓	↓	↓	↑
Methergoline	↓	↓	↓	↑
Propranolol	↓	↓	↓*	↓
Methiothepin	↓	↓	↓*	↓
Haloperidol	↓	↓	↓	↓

*No decrease using 5-MeODMT

Quipazine	*HW*	*FT*	*HLA*	*R*
Cyproheptadine	↓	↓	↓	↑
Cinanserin	↓	↓	↓	—
Mianserin	↓	↓	—	↓
Methysergide	↓	↓	—	↑
Methergoline	↓	↓	↓	↑
Propranolol	↓	↓	—	↓
Methiothepin	↓	↓	—	↓
Haloperidol	↓	↓	—	↓

HW, headweaving; FT, forepaw treading; HLA, hind-limb abduction; R, reactivity.
Direction of arrow indicates increase or decrease of behaviour, fine lines indicate small response. —Indicates no effect.

appeared somewhat less potent in this respect than after 5-HT or 5-MeODMT. Hind-limb abduction produced by quipazine was less affected by these drugs and reactivity was again somewhat increased by methysergide and methergoline.

The experiment with mianserin and LSD would suggest that LSD and quipazine act in a similar way. This interpretation is supported by the experiments of Kuhn, White & Appel (1978) who showed that animals trained to discriminate saline from LSD would not discriminate between quipazine and LSD, though they could discriminate between LSD and other procedures thought to increase brain 5-HT function (e.g. chlorimipramine and L-tryptophan injection).

The failure of various antagonists to inhibit 5-HT-mediated behaviour in our systems, mirrors other reports on their failure to act on suggested 5-HT-mediated behaviours. Thus 5-HTP- and 5-HT-facilitated flexor reflex is not antagonized by cyproheptadine (Martin & Eades, 1970); and tryptamine-induced behavioural excitation and forepaw treading in rats and rabbits is not antagonized by cinanserin or cyproheptadine (although hyperthermia induced by 5-HTP was affected by these drugs) (Quock & Weick, 1978).

Both cyproheptadine and mianserin decreased 5-HTP-induced head twitches and other behavioural changes. However, the effect was not dose-dependent and large doses were needed for complete suppression (van Riezen, 1972).

The LSD behavioural cue used for discrimination of LSD from saline in the experiments of Kuhn *et al.* (1978) was blocked by cinanserin, cyproheptadine, methiothepin and methysergide in a dose-related manner and various centrally mediated behavioural responses following quipazine have been shown to be blocked by cinanserin (Rodriguez, Rojas-Ramirez & Drucker-Colin, 1973), cyproheptadine (Hong, Sancilio, Vargas & Pardo, 1969) and mianserin (Maj, Sowinska, Baran, Gancarczyk & Rawtow, 1978). Mianserin also inhibits the 5-HTP-induced behavioural changes and the 5-HTP-induced head twitch (Maj *et al.*, 1978).

These data, therefore suggest that the antagonists under study have differential effects on various 5-HT-induced behavioural changes, a point made by Clineschmidt & Lotti (1974). The major point to be made in the current study is that they have a different effect on the *same* behaviour produced by different agonists.

Clearly the ability of methysergide to inhibit 5-HT- and 5-MeODMT-induced behaviour and the failure of cyproheptadine to act on the behaviour is not absolute but dose-related. If the dose of cyproheptadine is doubled a partial inhibition is seen (Figure 5).

It seemed possible that the ability of cyproheptadine, cinanserin and mianserin to antagonize the head weaving and forepaw treading induced by quipazine was the result of quipazine stimulating the 5-HT receptor when it was in a conformationally different state from that when being stimulated by indole compounds. Our data with the various compounds shown in Figure 6 suggested certain requirements for agonist action.

If the receptor site for 5-HT is shown diagrammatically, as in Figure 9, then we can draw certain conclusions. Both 5-HT and quipazine have R_1, R_2 and side $chain_2$ interactions. This can be seen if molecular models are made and rotated appropriately (Figure 9). MK212 has only one aromatic ring and whilst it could bind at R_1 or R_2 the chlorine forces the ring into R_2, producing an interaction at R_2^N and side chain-N and thus an agonist action. 2-PP lacks the chlorine and can thus bind in the R_1 inactive position.

The indole antagonists (Figure 1) can clearly bind strongly to the site but it seems that the methyl group at R_2^N confers antagonist action. This is not absolute since some biochemical indication of weak agonist actions were obtained (Table 2).

It is reasonable, therefore, to suggest that quipazine binds to the 5-HT receptor in a similar way to the indoles. However, it binds weakly, due both to its lack of the side $chain_1$ site and to a reduced hydrophobic interaction at R_1 and R_2 when the orientations of all these groups essential to agonist activity are optimized (Figure 9). Receptor ligand binding studies would tend to strengthen this argument. Tryptamine lacking the side $chain_1$ site binds 100 fold less strongly than 5-HT (Whitaker & Seeman, 1978).

Ligand-receptor binding studies suggest that propranolol, methysergide and methergoline bind strongly to the 5-HT receptor (that is a low IC_{50} for antagonizing [^{3}H]-5-HT binding) whilst mianserin, cinanserin and cyproheptadine bind weakly (Bennett & Snyder, 1976; Middlemiss, Blakebrough & Leather, 1977; Whitaker & Seeman, 1978; Peroutka & Snyder, 1979). Since quipazine also binds weakly to 5-HT receptors (Whitaker & Seeman, 1978) it is reasonable to suppose that the weak antagonists can displace the weakly binding agonist but not the strongly binding indole compounds.

While there is a neat division of the actions of mianserin, cyproheptadine and cinanserin on the head weaving and forepaw treading induced by quipazine versus that produced by 5-HT or 5-MeODMT, such a clear division does not exist when one examines the effects of these drugs on hind-limb abduction or reactivity. Thus the pharmacological profile of the 5-HT receptors initiating forepaw treading and head weaving is not the same as those initiating hind-limb abduction or reactivity. The exis-

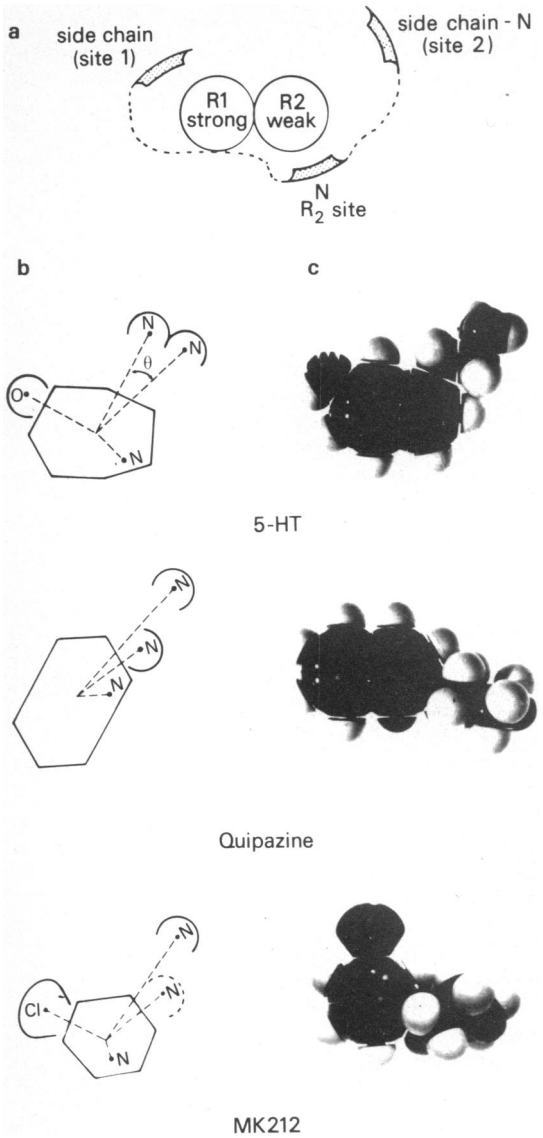


Figure 9 A comparison of the structures of 5-hydroxytryptamine (5-HT), quipazine and MK212 in relation to a putative receptor binding site for 5-HT. (a) Diagrammatic receptor binding site for 5-HT showing the important components. Sites R_2^N and side chain-N, site 2 are important for agonist activity while R_1, R_2 and side chain site 1 are important for binding. (b) Line diagrams of the models shown in (c) indicating the appropriate orientations for optimizing the agonist interactions. One angle θ indicates the limits of permissible side chain rotations for 5-HT. (c) Space-filling models of 5-HT, quipazine and MK212.

tence of more than one population of 5-HT receptors in the periphery has been recognized for many years (Gaddum & Hameed, 1954; Gaddum & Picarelli, 1957) and the data have recently been extended (Apperley, Feniuk, Humphrey & Levy, 1980). Evidence for different central 5-HT receptor populations has now also been provided (Peroutka & Snyder, 1979; Nelson, Herbert, Enjalbert, Bockaert & Hamon, 1980).

It was felt that the PCPA-treatment experiments might also differentiate between receptor types since PCPA pretreatment enhances automated locomotor recordings of quipazine-induced hyperactivity (Grabowska, Antkiewicz & Michaluk, 1974; Green *et al.*, 1976) but has been reported not to produce enhancement of 5-MeODMT-induced activity (Trulson *et al.*, 1976a, b; Green, 1977). However, when PCPA administration was terminated 4 days before testing, enhanced responses were now seen following administration of either quipazine or 5-MeODMT.

Effect of the 5-hydroxytryptamine antagonists on 5-HT concentrations and synthesis in brain and spinal cord

The published data on the effects of various 5-HT antagonists on 5-HT metabolism show no clear pattern of effect and it is probable that changes observed are at least in part dose-related. Methiothepin (20 mg/kg) accelerates brain 5-HT turnover (Monachon, Burkhard, Jalfre & Haefely, 1972; Bourgoin, Artaud, Enjalbert, Héry, Glowinski & Hamon, 1977; Bourgoin, Artaud, Bockaert, Héry, Glowinski & Hamon, 1978) because it increases the brain tryptophan concentration (Jacoby, Shabshelowitz, Fernstrom & Wurtman, 1975). However, at a dose that totally inhibits 5-HT behavioural changes (5 mg/kg), methiothepin actually decreased 5-HT synthesis, particularly in the spinal cord. Methergoline has a similar effect at a dose of 10 mg/kg (Bourgoin *et al.*, 1978), but not at a dose of 5 mg/kg. 5-HT synthesis decreases after administration of various ergot derivatives (Kehr, 1977) and other compounds which stimulate 5-HT receptors (see, for example, Hamon, Bourgoin, Enjalbert, Bockaert, Héry, Ternaux & Glowinski, 1976). This suggests that as well as antagonist actions, methiothepin and methergoline may have some agonist actions, particularly on the presynaptic receptor in the spinal cord, thereby leading to a decrease in 5-HT synthesis in this region. The doses employed and the affinity for the pre- and postsynaptic receptor would be expected to influence the observed action of these drugs, a point also discussed by Bourgoin *et al.* (1978). Sofia & Vassar (1976) found that methysergide stimulates 5-HT turnover but again they used

twice or four times the dose of the current study, which found methysergide to have little effect, in agreement with Deakin & Green (1978).

Mianserin had no significant effect on 5-HT turnover, confirming earlier studies (for example, see Kafoe, De Ridder & Leonard, 1976).

Effects of the 5-hydroxytryptamine antagonists on dopamine-mediated behaviour

There is no evidence that, with the exception of methiothepin, any of the antagonists inhibited 5-HT-mediated behaviours by inhibiting brain dopaminergic systems. Methiothepin has previously been reported to be a dopamine antagonist (Lloyd & Bartholini, 1974).

The enhanced apomorphine-induced locomotor response is in agreement with previous findings that inhibition of 5-HT function enhances this behaviour (Segal, 1976; Carter & Pycock, 1978). It is probable that the enhanced locomotor and reactivity seen after methysergide and methergoline pretreatment of tranylcypromine/L-tryptophan-treated rats results from the attenuation of the 5-HT inhibition normally acting on the dopaminergic component of the behaviour.

General conclusions

These data suggest that attempts can be made to correlate *in vitro* ligand binding data with functional effects of 5-HT antagonists. The data also suggest that different 5-HT receptor populations can be demonstrated behaviourally and that it is dangerous to try to characterize receptor populations as being 5-hydroxytryptaminergic on the basis of antagonist studies unless several antagonists have been examined.

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