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# A behavioural and biochemical study in mice and rats of putative selective agonists and antagonists for 5-$HT_1$ and 5-$HT_2$ receptors

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**1** Radioligand binding techniques have demonstrated the existence of 5-hydroxytryptamine (5-HT) binding subtypes: 5-$HT_2$, 5-$HT_{1A}$ and 5-$HT_{1B}$. These techniques have also indicated that certain drugs appear to show sub-type specificity: 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), a 5-$HT_{1A}$ agonist; 5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)1-H indole (RU 24969), a 5-$HT_{1B}$ agonist; and ritanserin, a 5-$HT_2$ antagonist. (−)-Propranolol is a 5-$HT_1$ antagonist of uncertain sub-type specificity.

**2** An examination has been made in mice and rats of the behavioural and biochemical effects of these drugs to determine whether the binding sites have physiological functions and further characterise the behavioural models.

**3** Administration of carbidopa (25 mg $kg^{-1}$) plus 5-hydroxytryptophan (100 mg $kg^{-1}$) produced head-twitch behaviour in mice which was antagonized by ritanserin ($ED_{50}$ = 65 μg $kg^{-1}$) but not (−)-propranolol (20 mg $kg^{-1}$). 8-OH-DPAT (1–10 mg $kg^{-1}$ s.c.) and RU 24949 (5 mg $kg^{-1}$ i.p.) did not produce head-twitch behaviour. 8-OH-DPAT decreased 5-HTP- but not 5-methoxy-*N*-*N*-dimethyltryptamine (5 mg $kg^{-1}$)-induced head-twitch by a (−)-propranolol-insensitive mechanism.

**4** Locomotor activity produced in mice by RU 24969 (3 mg $kg^{-1}$) was antagonized by (−)-propranolol (20 mg $kg^{-1}$) but not the (+)-isomer. (−)-Propranolol did not antagonize the behaviour induced in rats.

**5** In mice, both 8-OH-DPAT and RU 24969 markedly inhibited whole brain 5-HT synthesis and this effect was not antagonized by (−)-propranolol.

**6** In rats, 8-OH-DPAT (3 mg $kg^{-1}$ s.c.) produced all the behavioural changes seen after quipazine (25 mg $kg^{-1}$). (−)-Propranolol inhibited the behaviour changes produced by both agonists, while ritanserin antagonized the behaviour produced by quipazine but not 8-OH-DPAT. It is concluded, therefore, that the 5-$HT_{1A}$ receptor exists between the 5-$HT_2$ receptor and the behavioural effectors.

**7** 8-OH-DPAT (at 20°C ambient temperature) rapidly decreased rat body temperature, an effect antagonized by (−)-propranolol but not ritanserin. Quipazine (at 27°C ambient temperature, but not 20°C) increased body temperature but the effect was not blocked by either antagonist.

**8** Ritanserin does not antagonize apomorphine-induced locomotion in either species.

**9** We suggest that 5-HT-induced head-twitch behaviour in mice is a useful 5-$HT_2$ receptor model and the temperature change following 8-OH-DPAT injection in rats may be a 5-$HT_{1A}$ model. While (−)-propranolol antagonizes 8-OH-DPAT effects in rat, it does not inhibit 8-OH-DPAT effects in mice, and instead antagonizes RU 24969-induced locomotion. Its status as a 5-$HT_1$ antagonist remains ill-defined.

## Introduction

5-Hydroxytryptamine (5-HT)-mediated behavioural models have proved valuable in the examination of the effects of drugs on 5-HT function (see review by Green & Heal, 1985). The observations on the existence of subpopulations of 5-HT binding sites using ligand receptor binding (Peroutka & Snyder, 1979; Leysen, 1985) has led to the desire to link these sub-types with receptors mediating the various behavioural changes induced in rodents by 5-HT and its agonists; both to refine further the behavioural models and to clarify

whether the binding sites identified by radioligand binding have any functional significance.

The first experiments of this type were those of Peroutka *et al.* (1981) who concluded that the head-twitch behaviour in mice following administration of 5-hydroxytryptophan (5-HTP) was 5-$HT_2$-receptor-mediated. This hypothesis was supported by the work of Ortmann *et al.* (1982) who, like Peroutka *et al.* (1981), examined the relationship between the potency of drugs to inhibit the behaviour and to inhibit [$^3$H]-spiperone binding in frontal cortex. Middlemiss (1982), however, was unable to show a relationship between these two parameters. Using a rather different approach, Green *et al.* (1983) also concluded that the head twitch response was 5-$HT_2$-receptor-mediated on the basis of the potency of the selective 5-$HT_2$ antagonist pirenperone in inhibiting the behaviour.

Green *et al.* (1983) also observed that several behavioural changes induced by 5-HT agonists in rats were inhibited by pirenperone and suggested that these behavioural changes (forepaw treading, head-weaving and hind-limb abduction) were 5-$HT_2$-receptor-mediated while the locomotor behaviour and hyper-reactivity which were not antagonized by pirenperone were not 5-$HT_2$-receptor-mediated. These observations are not without controversy since Lucki *et al.* (1983) did not observe inhibition of head-weaving and forepaw treading after high doses of another 5-$HT_2$ antagonist, ketanserin, while Tricklebank (1984a,b) could inhibit them using much lower doses of the same drug. Nevertheless, the division was strengthened by the observation that hyperlocomotion and hyper-reactivity but not forepaw treading, head-weaving and hind-limb abduction could be induced by the putative 5-$HT_1$ agonist, 5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)1-H indole (RU 24969).

Some data are now appearing suggesting, on the basis of ligand-receptor binding, that the 5-$HT_1$-receptor can be further sub-classified into 5-$HT_{1A}$ and 5-$HT_{1B}$ (Pedigo *et al.*, 1981). Middlemisss & Fozard (1983) found in rat cortex that the 5-$HT_{1A}$ site defined by displacement of [$^3$H]-5-HT with spiperone selectively bound 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). It was of particular interest, therefore, that Tricklebank (1984a,b) found that 8-OH-DPAT at low doses produced in rats all the behaviours seen after injection of non-selective agonists (e.g. quipazine) and also that these behaviours were antagonized by (−)-propranolol, a drug reported several years ago to inhibit the behaviour induced by 5-HT and its agonists (Green & Grahame-Smith, 1976; Deakin & Green, 1978; Weinstock, 1980; Green *et al.*, 1981). This implies that most of the behavioural changes induced in rats by 5-HT may be 5-$HT_{1A}$-receptor-mediated.

The effects of (−)-propranolol and other β-adrenoceptor antagonists may therefore be associated with their high affinity for 5-$HT_1$ binding sites (Nahorski & Willcocks, 1983) although detailed information upon their relative affinity for A and B sub-types is not yet available.

We have now examined the behaviour induced in rats and mice by the non-selective 5-HT agonists, quipazine and 5-methoxy-*N*,*N*-dimethyltryptamine, the putative 5-$HT_{1A}$ agonist 8-OH-DPAT (Middlemiss & Fozard, 1983) and the suggested (Cortés *et al.* 1984) 5-$HT_{1B}$ agonist, RU 24969 and studied the effects on these behaviours of 'selective' antagonists, namely ritanserin (5-$HT_2$) and (−)-propranolol (5-$HT_1$). We have also examined the effect of the agonists and antagonists on body temperature and the rate of 5-HT synthesis.

## Methods

### *Animals*

Mice were male C57/black/6/Ola (Olac, Bicester) weighing 22–28 g at the time of study, housed in groups of 8–10 and given modified 41B pellets and tap water *ad libitum*. Rats were male Sprague-Dawley derived (Charles River, Margate) weighing 150–200 g, housed in groups of 6 and fed as above. All rodents were kept in conditions of controlled temperature (20°C ± 1°C) and lighting (dark period 19 h 00 min–07 h 00 min)

### *Behavioural studies in mice*

Head-twitch behaviour was studied after either precursor loading or agonist administration. In the precursor loading studies, mice were injected with carbidopa (25 mg kg$^{-1}$, i.p.) followed 15 min later by 5-hydroxytryptophan (5-HTP, 100 mg kg$^{-1}$ i.p.). The total number of head-twiches in a 2 min period was counted 15 min later. When an agonist was used, mice were injected with 5-methoxy-*N*,*N*,dimethyltryptamine (5-MeODMT; 5 mg kg$^{-1}$, i.p.) and the total number of head-twitches measured in the next 6 min. The observer was 'blind' to the pretreatment drug.

Locomotor activity was measured in 2 pairs of mice (control and experimental) using LKB Animex activity meters (sensitivity and tuning; 30 μA).

### *Behavioural studies in rats*

Behavioural changes in rats were scored with the method previously used by Deakin & Green (1978) and Green *et al.* (1981) whereby a particular behaviour is rated as being absent (0), just present (1), definite (2) or severe (3). Behaviours rated were: reciprocal forepaw treading, head-weaving, hind-limb abduction and flat body posture. The scorer was unaware of the pretreatment schedule.

Locomotor activity was measured by means of Animex meters as described above.

*Temperature measurement in rats*

Body temperature was measured with a Comark thermocouple with digital read-out and rectal probe inserted 3 cm into the colon, the animal being lightly restrained in the hand during measurement. The probe was lubricated with handcream before each use.

*Measurement of brain 5-hydroxyindoleacetic acid and estimation of rate of 5-HT synthesis*

Whole mouse brain 5-hydroxyindoleacetic acid (5-HIAA) was measured fluorimetrically by the method of Curzon & Green (1970). Synthesis was estimated by measurement of the accumulation of 5-HIAA 60 min after injection of probenecid (200 mg $kg^{-1}$, i.p.) as described by Costa & Neff (1970).

*Drugs*

Drugs were obtained from the following sources (in parentheses): 5-methoxy-*N*,*N*-dimethyltryptamine, 5-hydroxytryptophan and probenecid (Sigma, Poole) carbidopa (Merck, Sharp & Dohme, Hoddesdon), 8-OH-DPAT (Merrell-Dow, Strasbourg), (+)-and (−)-propranolol (ICI Pharmaceuticals, Alderley Edge), quipazine (Miles Research Laboratories, Stoke Poges), RU 24969 (Roussel-Uclaf, Romainville), ritanserin (Janssen Pharmaceutical, Wantage). All drugs were dissolved in 0.9% NaCl (saline) and injected i.p. except where stated otherwise.

*Statistics*

Locomotor responses and temperature changes are reported as mean ± s.e.mean and have been analysed using Student's *t* test (unpaired). All other behavioural scores were analysed with the Mann Whitney rank

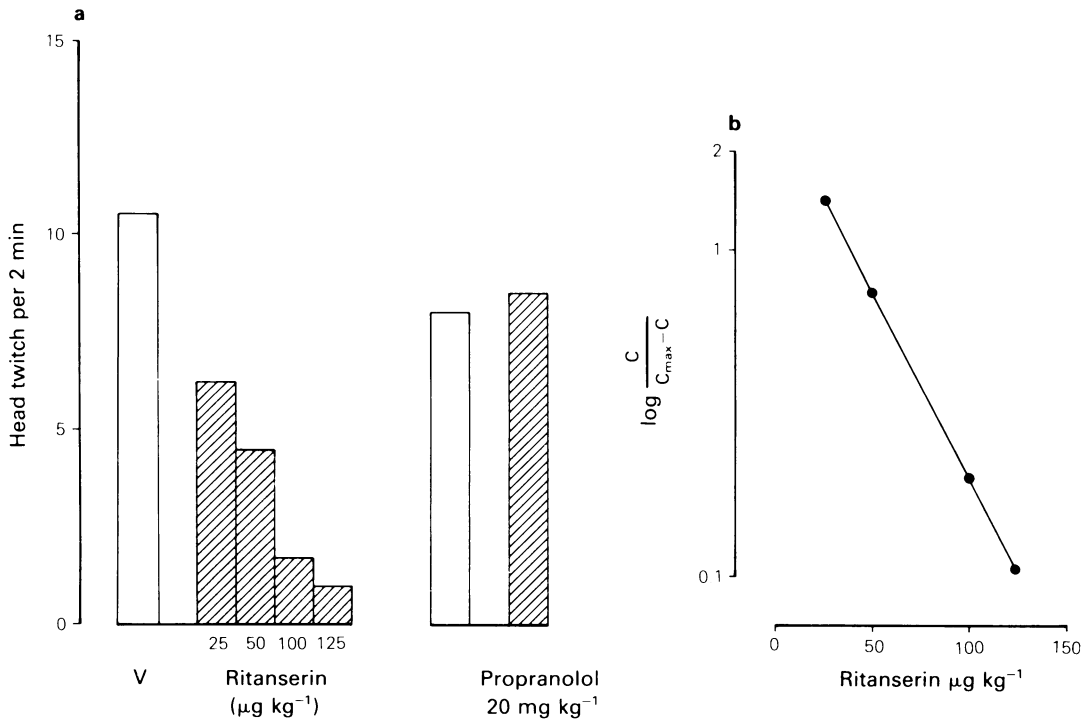


**Figure 1** Effect of ritanserin and propranolol on 5-hydroxytryptophan (5-HTP)-induced head-twitch behaviour in mice. (a) Ritanserin was given at the doses shown 60 min before carbidopa (25 mg $kg^{-1}$). 5-HTP (100 mg $kg^{-1}$) was given after a further 15 min and the head-twitches measured over a 2 min period after a further 15 min. Results show mean of 6 animals in each group. (−)-Propranolol (20 mg $kg^{-1}$) was given 15 min before the carbidopa and the experiment repeated as above. (b) The $ED_{50}$ for ritanserin calculated from Figure 1 b was 65 μg $kg^{-1}$; C = head-twitches in drug-treated mice; $C_{max}$ = head-twitches in the vehicle-treated mice. Ritanserin-treated mice significantly different from vehicle-injected (V): $P < 0.01$ or better at all doses examined. Propranolol-treated mice not significantly different from saline-injected controls.

order test for non-parametric data with the exception of data in Figure 2, where the ranked sign test for differences (Wilcoxon) was employed.

## Results

*Studies on 5-hydroxytryptamine function in mice*

*Effect of ritanserin and (−)-propranolol on head-twitch behaviour* Mice were injected with saline or one of 4 doses of ritanserin (25, 50, 100 and 125 $\mu g\,kg^{-1}$) and the head-twitch response to 5-HTP determined 60 min later. Ritanserin produced a dose-dependent inhibition of the behaviour (Figure 1a) with an $ED_{50}$ of 65 $\mu g\,kg^{-1}$ (Figure 1b). In contrast, a dose of (−)-propranolol of 20 $mg\,kg^{-1}$ (a dose previously found to inhibit markedly quipazine-induced behaviour; Green *et al.*, 1981) given 15 min before the carbidopa (see Methods) produced no alteration in the behavioural response (Figure 1a).

*Effect of ritanserin and propranolol on RU 24969-induced locomotor response* In confirmation of an earlier report (Green *et al.*, 1984) RU 24969 (3 $mg\,kg^{-1}$) produced a marked increase in locomotor activity (Table 1). Pretreatment with ritanserin (100 $\mu g\,kg^{-1}$) 50 min before RU 24969 did not alter this response. (−)-Propranolol (20 $mg\,kg^{-1}$) given 45 min before the RU 24969 inhibited the locomotor response, confirming an earlier study (Green *et al.*, 1984) while in contrast (+)-propranolol (20 $mg\,kg^{-1}$) had no effect on RU 24969-induced locomotion (Table 1).

**Table 1** Effect of propranolol and ritanserin on the locomotor activity induced in rodents by RU 24969

| *Injected* | *Recorded activity counts per 40 min* *Mice* | *Rats* |
|---|---|---|
| Saline | 2673 ± 795 (4) | 1594 ± 296 (3) |
| Ritanserin | 2539 ± 759 (4) | 2551 ± 445 (3)** |
| (−)-Propranolol | 562 ± 119 (4)* | 1710 ± 418 (3) |
| (+)-Propranolol | 3647 ± 731 (3) | ND |

Rodents were injected with saline, ritanserin (100 $\mu g\,kg^{-1}$ for mice; 200 $\mu g\,kg^{-1}$ for rats, 50 min earlier) or propranolol (20 $mg\,kg^{-1}$ of respective isomer, 45 min earlier) followed by RU 24969 (3 $mg\,kg^{-1}$) and the resultant locomotor activity in pairs of rodents measured on meters for the next 40 min. Results show mean ± s.d. with number of experiments in parentheses. Different from saline-injected: $*P<0.05$; $*P<0.001$; ND: not determined. Responses of rodents to saline only injection, given in Table 3.

*Effect of 8-OH-DPAT administration to mice* No head-twitch behaviour was observed in mice given subcutaneous doses of 8-OH-DPAT from 0.5–10 $mg\,kg^{-1}$. There was a small increase in locomotion but this did not clearly increase with dose and was not observed after 20 min. No other marked behavioural changes, such as piloerection, flat body posture, straub tail or proptosis, were seen.

*Effect of RU 24969 and 8-OH-DPAT on the head-twitch response induced by 5-HTP and 5-MeODMT administration* There is evidence that the presynaptic 5-HT receptor is of the $5\text{-}HT_1$ sub-type, although there are enough pharmacological discrepancies not to be able to state this categorically (see Moret, 1985). We therefore examined whether it could be shown that the putative $5\text{-}HT_1$ agonists, 8-OH-DPAT and RU 24969, inhibited release. To do this the following protocol was adopted. Mice were injected with carbidopa (25 $mg\,kg^{-1}$) followed 15 min later by 5-HTP (100 $mg\,kg^{-1}$). The precursor-induced head-twitch response was measured for 2 min after a further 15 min (Figure 2, test 1). The mice were then injected with either saline (control), RU 24969 (3 $mg\,kg^{-1}$) or 8-OH-DPAT (3 $mg\,kg^{-1}$ s.c.) and the head-twitch res-

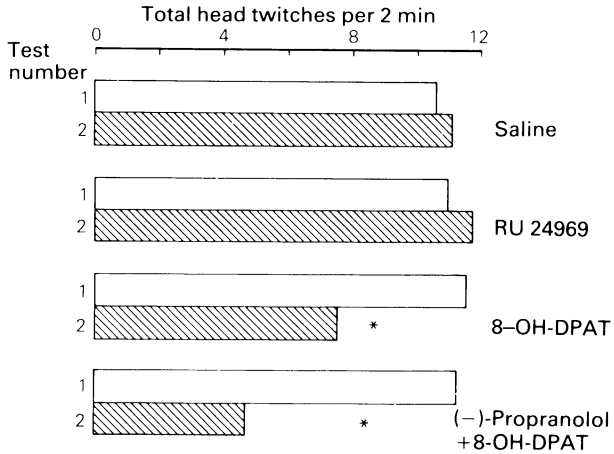


**Figure 2** Mean head-twitch of mice 15 min and 30 min after 5-hydroxytryptophan (5-HTP). Mice were given carbidopa (25 $mg\,kg^{-}$) with 5-HTP (100 $mg\,kg^{-1}$) 15 min later. After a further 15 min the total number of head-twitches was measured (Test 1). Mice were then injected with saline, 5-methoxy-3(1,2,3, 6-tetrahydropyridin-4-yl)1-H-indole (RU 24969, 3 $mg\,kg^{-1}$) or 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT, 3 $mg\,kg^{-1}$) and the mean head-twitch response measured again after a further 15 min (Test 2). The head-twitch response was inhibited ($*P<0.05$) by 8-OH-DPAT and this effect was not altered by (−)-propranolol (20 $mg\,kg^{-1}$) pretreatment 15 min before the carbidopa; data analysed by the ranked sign test for differences (Wilcoxon).

ponse again measured for 2 min after a further 15 min (Figure 2, test 2).

There was no significant change in the head-twitch response in the control (saline-injected) animals during the two observation periods (Figure 2). Administration of RU 24969 between the two periods also left the response unchanged (Figure 2) even though, as expected, the locomotor response increased. However, injection of 8-OH-DPAT (3 mg kg$^{-1}$) produced an inhibition of the second head-twitch response (Figure 2).

Because it has been suggested that (−)-propranolol is an antagonist of the biochemical and behavioural effects of 8-OH-DPAT, a further group of mice were pretreated with (−)-propranolol (20 mg kg$^{-1}$) 15 min before the carbidopa and the experiment repeated to see whether it would block the 8-OH-DPAT-induced decrease in head-twitch response. In confirmation of the previous experiment (Figure 1a) the propranolol pretreatment did not alter the head-twitch response; however, it also failed to block the decrease in the head-twitch response produced by the 8-OH-DPAT injection (Figure 2).

To check that the 8-OH-DPAT was inhibiting the head-twitch response because of an alteration in pre- rather than postsynaptic receptor function, the experiment with 8-OH-DPAT was repeated using the postsynaptic agonist 5-MeODMT to induce the head-twitch.

Mice were injected with saline or 8-OH-DPAT (10 mg kg$^{-1}$) and injected with 5-MeODMT (5 mg kg$^{-1}$) 8 min later. Head-twitch behaviour was measured for 6 min starting immediately after the 5-MeODMT injection. Pretreatment with 8-OH-DPAT even at this high dose did not alter the response when induced by the agonist 5-MeODMT (mean control response following saline pretreatment: 11.6 head twitches in 6 min, $n = 8$; response following 8-OH-DPAT pretreatment: 12.3 head twitches in 6 min $n = 8$).

*Effects of RU 24969 and 8-OH-DPAT on the rate of synthesis of brain 5-HT* The rate of brain 5-HT synthesis was determined by the measurement of 5-HIAA accumulation following probenecid injection as described in the Methods.

Mice were injected with saline or RU 24969 (3 mg kg$^{-1}$). Sixty minutes later half the number of each group were killed, the brain removed and 5-HIAA measured. The other half of each of the two groups were injected with probenecid (200 mg kg$^{-1}$) and killed after a further 60 min when the brain was dissected out and 5-HIAA measured.

RU 24969 produced a small decrease in basal 5-HIAA content and a marked decrease in 5-HIAA accumulation (Table 2).

In a further experiment mice were given either saline or 8-OH-DPAT (3 mg kg$^{-1}$ s.c.) and either killed 15 min later for determination of brain 5-HIAA or injected with probenecid (200 mg kg$^{-1}$). Sixty minutes after the probenecid both groups were killed and brain 5-HIAA determined. 8-OH-DPAT also markedly decreased the rate of 5-HIAA accumulation compared with the control (saline-injected) group (Table 2). Administration of (−)-propranolol 20 mg kg$^{-1}$, 5 min before either agonist did not prevent the decrease in the rate of 5-HT synthesis produced by either 8-OH-DPAT or RU 24969 injection (Table 2).

(−)-Propranolol alone has previously been shown not to alter the rate of 5-HT synthesis (Green & Grahame-Smith, 1976).

**Table 2** Effect of 5-methoxy-3(1,2,3, 6-tetrahydropyridin-4-yl)1-H-indole (RU 24969) and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) on the synthesis rate of mouse brain 5-hydroxytryptamine (5-HT)

| *Injected* | *Saline* | *Probenecid* | *5-HIAA accumulation* (μg g$^{-1}$ h$^{-1}$) |
|---|---|---|---|
| Saline | 0.63 ± 0.06 (6) | 0.96 ± 0.14 (6) | 0.33 |
| RU 24969 | 0.57 ± 0.08 (6) | 0.71 ± 0.11 (4)* | 0.14 |
| RU 24969 + (−)-propranolol | 0.56 ± 0.07 (6) | 0.67 ± 0.09 (6)* | 0.11 |
| 8-OH-DPAT | 0.61 ± 0.03 (8) | 0.75 ± 0.04 (6)* | 0.14 |
| 8-OH-DPAT + (−)-propranolol | 0.51 ± 0.07 (6) | 0.65 ± 0.06 (5)* | 0.14 |

The rate of 5-hydroxyindoleacetic acid (5-HIAA) synthesis was estimated by examining the rate of 5-HIAA accumulation following the administration of probenecid (200 mg kg$^{-1}$) as described in Methods. Mice were injected with saline or RU 24969 (3 mg kg$^{-1}$) and either killed 60 min later or probenecid-injected. After a further 60 min the probenecid-treated mice were killed. When 8-OH-DPAT (3 mg kg$^{-1}$ s.c.) was injected, mice were either killed 15 min later or probenecid-injected with death after a further 60 min. (−)-Propranolol (20 mg kg$^{-1}$) was given 5 min before the 8-OH-DPAT or RU 24969.

*Different from concentration in saline-treated rats given probenecid: $P < 0.01$.

*Studies on 5-hydroxytryptamine function in rats*

*Effect of ritanserin and (−)-propranolol on RU 24969-induced locomotor activity* Initial experiments on the effects of ritanserin on the behavioural changes induced by quipazine (see below) indicated that consistent effects on the behaviour were observed at a dose of 200 μg kg$^{-1}$. This dose was therefore used in all studies on rats.

Rats were injected with ritanserin (200 μg kg$^{-1}$) followed by RU 24969 (3 mg kg$^{-1}$) 60 min later. The locomotor response to RU 24969 was enhanced by the

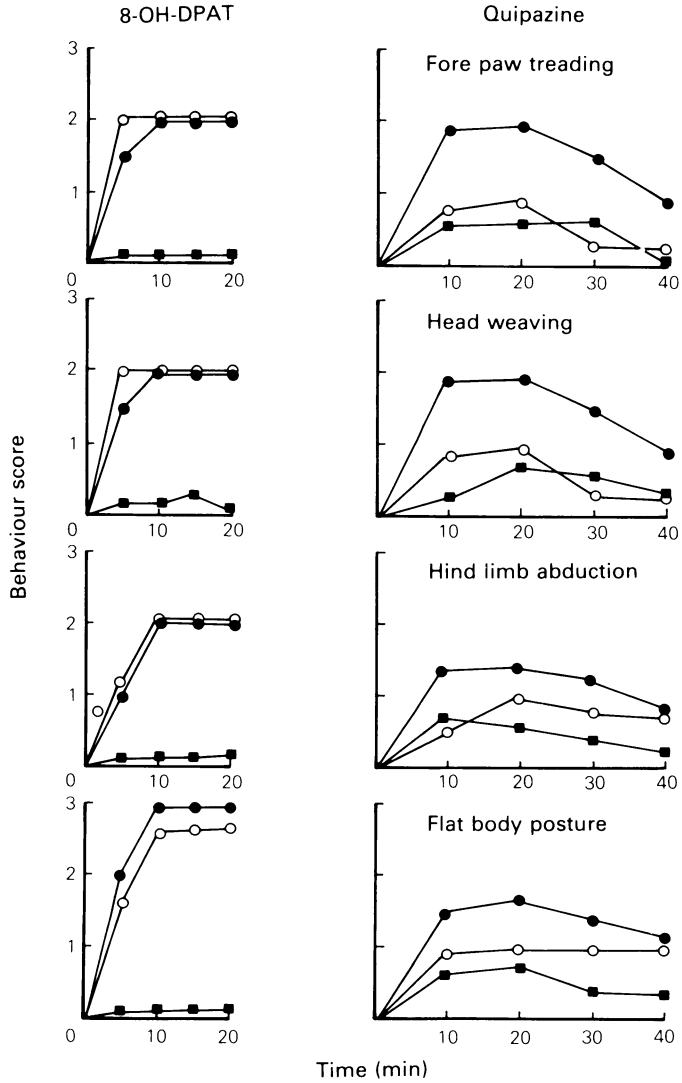


**Figure 3** Effects of ritanserin or propranolol on the behavioural responses of rats following administration of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) or quipazine. Rats were given saline (●), ritanserin (200 μg kg$^{-1}$ (○)) or (−)-propranolol (20 mg kg$^{-1}$ (■)) and subsequently (45 min after propranolol, 60 min after ritanserin) injected with 8-OH-DPAT (3 mg kg$^{-1}$, left panel) or quipazine (25 mg kg$^{-1}$, right panel). Subsequent behavioural changes were rated as described in Methods. Ritanserin was without effect on the 8-OH-DPAT-induced behaviour while (−)-propranolol totally abolished the behavioural changes at all time points. (Behaviour significantly different from saline-injected animals $P<0.001$ at all time points). Ritanserin and propranolol significantly inhibited quipazine-induced forepaw treading and head-weaving at all time points ($P<0.01$). Propranolol also inhibited hind-limb abduction and flat body posture at all time points ($P<0.05$ or better) while ritanserin inhibited hind-limb abduction at 10 min ($P<0.05$) and flat body posture at 10 and 20 min ($P<0.05$).

ritanserin pretreatment (Table 1). Pretreatment with (−)-propranolol (20 mg kg$^{-1}$) 45 min before RU 24969 had no effect on the locomotor response (Table 1).

*Effect of ritanserin and (−)-propranolol on the behavioural responses to quipazine* Rats were pretreated with either saline (control group), ritanserin (200 μg kg$^{-1}$, 60 min) or (−)-propranolol (20 mg kg$^{-1}$, 45 min) followed by quipazine (25 mg kg$^{-1}$). The behavioural changes induced by quipazine were inhibited by pretreatment with either drug (Figure 3).

*Effect of ritanserin and (−)-propranolol on the behavioural responses to 8-OH-DPAT* The experiment above was repeated as described except that 8-OH-DPAT (3 mg kg$^{-1}$ s.c.) was given instead of quipazine. 8-OH-DPAT produced a qualitatively identical behavioural syndrome to that seen after quipazine. The only difference observed was that the animals seemed more 'aware' of their surroundings, moving away from any object, such as a hand advanced towards them (quipazine-treated animals seemed unresponsive to such stimuli). In addition, the rats displayed mounting behaviour even though all rats tested together were males.

Pretreatment with (−)-propranolol completely abolished all the behavioural changes (Figure 3), the animals looking essentially normal, albeit with some increase in locomotor activity. In contrast, pretreatment with (−)-ritanserin did not alter the behavioural changes induced in the rats by 8-OH-DPAT (Figure 3).

*Effect of quipazine, RU 24969 and 8-OH-DPAT on body temperature and the effect of (−)-propranolol and ritanserin on the resultant changes* It has been known for many years that increasing brain 5-HT content in rats can produce hyperthermia (e.g. Grahame-Smith, 1971a). The piperazine agonists such as quipazine and MK 212 will also elevate body temperature, although the effect is clearer in a raised ambient temperature (see Vetulani *et al.*, 1981).

RU 24969 (15 mg kg$^{-1}$) has previously been found

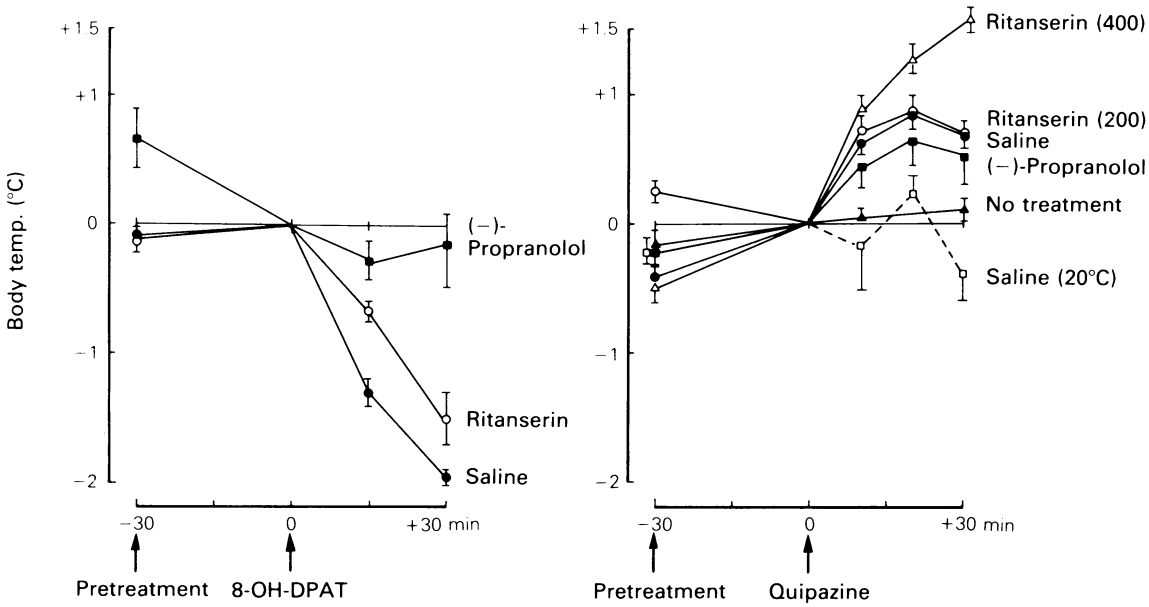


**Figure 4** Effect of ritanserin and propranolol on the change in body temperature induced in rats by administration of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and quipazine. Rats were injected with ritanserin (200 or 400 μg kg$^{-1}$) or (−)-propranolol (10 mg kg$^{-1}$) 30 min before 8-OH-DPAT (3 mg kg$^{-1}$). Temperature was measured rectally at the times shown and values have been expressed in terms of the mean ± s.e.mean change from the time the agonist was given. The 8-OH-DPAT experiment was measured at normal (20°C) ambient temperature and the quipazine at high ambient temperature (27°C) as described in Methods. The response in animals pretreated with saline and injected with quipazine (25 mg kg$^{-1}$) at 20°C ambient temperature is shown by the dashed line and labelled saline (20°C).

(−)-Propranolol abolished the temperature decrease induced by 8-OH-DPAT (different from saline-injected animals, $P < 0.01$ at both time points) while ritanserin had no effect. The temperature rise induced by quipazine was unaltered by propranolol or ritanserin (200 μg kg$^{-1}$) while the 20 min value for ritanserin (400 μg kg$^{-1}$) was significantly greater than the saline-injected group ($P < 0.05$).

**Table 3** Effect of ritanserin on apomorphine-induced locomotor activity in mice and rats

| 1st injection | 2nd injection | Total recorded activity (counts per 40 min) Mice | Rats |
|---|---|---|---|
| Saline | Saline | 566 ± 233 (4) | 590 ± 570 (4) |
| Saline | Apomorphine | 1268 ± 581 (7) | 1700 ± 357 (4) |
| Ritanserin | Apomorphine | 915 ± 398 (7) | 1557 ± 70 (4) |

Mice were injected with saline or ritanserin (100 $\mu g\,kg^{-1}$) and rats with ritanserin (200 $\mu g\,kg^{-1}$) 60 min before saline or apomorphine (mice: 2 $mg\,kg^{-1}$ i.p.; rats: 0.2 $mg\,kg^{-1}$ s.c.).
Results expressed as mean ± s.d. with number of observations in parentheses. Ritanserin did not significantly inhibit the apomorphine-induced locomotor response.

not to alter body temperature at ambient temperature of 20°C (Green *et al.*, 1984). It was therefore of interest to examine the effect of 8-OH-DPAT and examine the pharmacology of the quipazine and 8-OH-DPAT-induced body temperature changes.

Body temperature was measured rectally as described in Methods. Antagonist drugs were always given 30 min before the agonists and results have been 'normalised' to indicate a change from the temperature at the time of agonist administration (time = 0; Figure 4).

Experiments on the quipazine-induced change in body temperature were conducted in an ambient temperature of 27°C ± 0.5°C. Administration of quipazine (25 $mg\,kg^{-1}$) following saline pretreatment produced a rapid increase in body temperature, the maximum increase being 0.8°C 20 min after injection (Figure 4). Pretreatment with (−)-propranolol (10 $mg\,kg^{-1}$) had no effect on this temperature rise. Ritanserin (200 $\mu g\,kg^{-1}$) pretreatment was also without effect on the temperature rise and a higher dose (400 $\mu g\,kg^{-1}$), far from blocking the increase, produced an enhancement of it (Figure 4).

The experiments with 8-OH-DPAT were conducted at the normal animal house temperature of 20°C ± 1°C. At this temperature quipazine (25 $mg\,kg^{-1}$) was without consistent hyperthermic effect (Figure 4).

8-OH-DPAT (3 $mg\,kg^{-1}$, s.c.) given 30 min after saline produced a rapid and dramatic fall in body temperature (Figure 4). Ritanserin (200 $\mu g\,kg^{-1}$) pretreatment did not prevent this fall in body temperature, while (−)-propranolol (10 $mg\,kg^{-1}$) totally inhibited it (Figure 4).

*Effect of ritanserin on apomorphine-induced locomotor activity in mice and rats*

In the previous study on pirenperone (Green *et al.*, 1983) it was found that the drug was a potent dopamine antagonist. Since dopamine antagonists can inhibit the quipazine-induced behaviour in rats (see review of Green & Heal, 1984) and high doses of neuroleptics will inhibit the head-twitch behaviour in mice (Maj *et al.*, 1978) the final experiments in this study were to examine whether ritanserin would alter the locomotor response which follows apomorphine administration.

Pairs of rats or mice were injected with ritanserin (100 $\mu g\,kg^{-1}$ for mice, 200 $\mu g\,kg^{-1}$ for rats) or vehicle. After 60 min the control and ritanserin-treated groups were injected with apomorphine (for dose, see legend to Table 3) and the locomotor response measured over the next 40 min. Ritanserin pretreatment to rats did not alter the response (Table 3). In mice, however, the mean value was somewhat decreased although this failed to reach statistical significance (Table 3).

## Discussion

One of the major changes in 5-HT neuropharmacology that has occurred in the last few years has been the identification of binding sites which may reflect the existence of receptor sub-types. However, as often happens, the binding sites have rapidly become referred to as 'receptors'. Before the advent of ligand-receptor binding, receptor sub-types were identified by the functional responses of physiological systems to specific drugs. In this study, therefore, we have attempted to discover whether drugs shown to be selective at 5-HT binding sites on the basis of radioligand binding do have physiological effects.

*Studies in mice*

It has previously been suggested that the head-twitch behaviour in mice is 5-$HT_2$-receptor-mediated and our current results support this strongly for the following reasons: (1) the behaviour is blocked by the 5-$HT_2$ antagonist ritanserin at low dose, but not by the 5-$HT_1$ antagonist (−)-propranolol at high dose; (2) 8-OH-

DPAT and RU 24969 neither produce head-twitch behaviour nor facilitate it.

With regard to 5-$HT_1$-mediated behaviour in mice, RU 24969 did produce a striking motility syndrome, which was unaffected by ritanserin but was inhibited by (−)-propranolol but not by (+)-propranolol. The motility syndrome has been previously reported (Gardner & Guy, 1983; Green *et al.*, 1984) and it is significant that 5-$HT_1$ antagonist properties reside only in the (−)-propranolol isomer (Nahorski & Willcocks, 1983; Middlemiss, 1984a).

8-OH-DPAT, in contrast, did not produce any clear and easily measureable behavioural change.

The assumption has been made in both this and an earlier publication (Green *et al.*, 1984) that the 5-$HT_1$ agonist drugs are acting postsynaptically to produce the behavioural changes and in the rat there is evidence for this (Blackburn *et al.*, 1984). However, also in the rat, there is evidence that the prejunctional autoreceptor has many of the characteristics of 5-$HT_1$ type (see Moret, 1985). Engel *et al.* (1983) have further suggested that the potency of drugs for the autoreceptor correlate well with the low affinity component of 5-$HT_1$ binding that is, presumably, the 5-$HT_{1B}$ type. In support of this contention, Middlemiss (1984b) has found that RU 24969 will inhibit $K^+$-evoked 5-HT release from rat frontal cortex slices, while 8-OH-DPAT is without effect (Middlemiss, 1984c). It should be noted, however, that Gozlan *et al.* (1983) did obtain inhibition with 8-OH-DPAT and the reasons for this discrepancy are not clear (see Middlemiss, 1984c).

We have tried two rather different *in vivo* approaches in mice to try and determine possible presynaptic actions of the selective agonists. From behavioural studies it was found that 8-OH-DPAT, but not RU 24969, inhibited the head-twitch response evoked by precursor loading. This inhibition could have occurred through one of two mechanisms; either because release was being inhibited by the 8-OH-DPAT or because a 5-$HT_{1A}$-receptor exists which is inhibitory on the postsynaptic pathway involved in the response. The latter is rendered unlikely by the observation that 8-OH-DPAT did not affect the behavioural response when it was produced by the agonist 5-MeODMT. Interestingly, the 5-$HT_1$ antagonist (−)-propranolol did not prevent the action of 8-OH-DPAT in inhibiting the head-twitch.

The second approach was to examine the effects of both agonists on the synthesis rate of 5-HT. Consistent with previous observations in rats with both RU 24969 (Euvrard & Boissier, 1980; Green *et al.*, 1984) and 8-OH-DPAT (Hjöth *et al.*, 1982), both drugs greatly decreased the rate of 5-HT synthesis. However, this effect was not antagonized by (−)-propranolol, which suggests that however these drugs are inhibiting synthesis, it is not through a (−)-propranolol-sensitive receptor and this is consistent with a study in rats, at least with RU 24969 (Green *et al.*, 1984).

In mice, therefore, we have clear evidence for differential effects of 8-OH-DPAT and RU 24969 with the possibility that only presynaptic changes occur with 8-OH-DPAT. Identification of these responses with 5-$HT_{1A}$ and 5-$HT_{1B}$ binding sites requires further work in the same species. The need for care in translating from one species to another is underlined by the absence of a motor syndrome in mice following 8-OH-DPAT and the different selectivity of (−)-propranolol towards the effects of either agonist seen in rats and mice (see below).

*Studies in rats*

In confirmation of the reports of Hjörth *et al.* (1982) and Tricklebank *et al.* (1984), 8-OH-DPAT produced all the behavioural changes previously observed in rats given a monoamine oxidase inhibitor and L-tryptophan or various 5-HT agonists or releasing drugs (see review by Green & Heal, 1985). In addition, as also reported by Hjöth *et al.* (1982), it produced vigorous mounting behaviour in the male rats being investigated. All these behavioural changes were antagonized by administration of (−)-propranolol, but not ritanserin. If, however, the same behaviours with the exception of mounting, which was not seen, were produced by quipazine administration both ritanserin and (−)-propranolol were effective in inhibiting the behaviour. This is in agreement with previous studies which have found that (−)-propranolol and pirenperone (another 5-$HT_2$ antagonist) inhibit quipazine-induced behavioural changes (Green *et al.*, 1981; 1984). However, the data with ritanserin are perhaps stronger than with pirenperone since this drug, unlike pirenperone, seems to have little antagonist action at either the dopamine receptor (this paper and Janssen Pharmaceutical, unpublished data) or the $\alpha_1$-adrenoceptor (Janssen Pharmaceutical, unpublished data).

The simplest explanation for these findings is that between the 5-$HT_2$-receptors and the behavioural effectors there exists a 5-$HT_{1A}$ link. 5-$HT_2$ initiated behaviour can thus be blocked by both 5-$HT_2$ and 5-$HT_{1A}$ antagonists while 5-$HT_{1A}$-induced behaviour can only be blocked by 5-$HT_{1A}$ antagonists.

The study with regard to 5-HT and temperature change in the rat appears to be somewhat complicated, although there are some conclusions that can be made. RU 24969 does not alter body temperature in the rat (Green *et al.*, 1984). In contrast, we have found that 8-OH-DPAT produces a rapid and marked decrease in body temperature; the potent antagonism of this change by (−)-propranolol and lack of effect of ritanserin does suggest that this response is 5-$HT_{1A}$-receptor-mediated and that it may provide a rapid and simple screen for the effects of drugs on 5-$HT_{1A}$-mediated function.

5-HT and 5-HT agonists can produce hyperthermia (see, for example, Grahame-Smith, 1971a; 1971b). The effect with the piperazine agonists seems most marked at a raised ambient temperature and it has been suggested that it is 5-$HT_2$-receptor-mediated (Vetulani *et al.*, 1981). While we did obtain an increase in body temperature with quipazine, this change was not blocked by either (−)-propranolol or ritanserin, which raises doubts as to the neuropharmacological basis of it being a 5-HT-receptor. Of course, further study is necessary on this problem since peripheral effects of the agonists may have a profound influence on temperature.

Finally, enhancement by ritanserin of the locomotion induced in rats by RU 24969 is consistent with the enhancement previously observed with methergoline and methysergide and is perhaps indicative of an inhibitory 5-$HT_2$ modulation of RU 24969-induced behaviour (Green *et al.*, 1984).

*General conclusions*

Much remains to be done to elucidate the action of 5-HT-receptor sub-type selective agonists and antagonists. Nevertheless, this study has demonstrated that the drugs examined have defined behavioural and biochemical effects in rats and mice, suggesting that the binding sites may indeed be functional receptors. We would suggest that the head-twitch response in mice and the hypothermic response in rats may respectively be 5-$HT_2$- and 5-$HT_{1A}$-receptor-mediated. Furthermore, the 5-$HT_{1A}$ site (as defined by the use of 8-OH-DPAT) is clearly not identical to either the 5-$HT_{1B}$ site (as defined by the use of RU 24969) or the 5-$HT_2$ site (antagonized by ritanserin).

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