Evidence that RU 24969-induced locomotor activity in C57/B1/6 mice is specifically mediated by the 5-HT_{1B} receptor

¹S.C. Cheetham & D.J. Heal

Boots Pharmaceuticals Research Department, Nottingham, NG2 3AA

- 1 The behavioural effects of the 5-HT_{1B} receptor agonists, RU 24969 and CGS 12066B, have been investigated in C57/B1/6 mice.
- 2 RU 24969 (1–30 mg kg⁻¹) produced intense and prolonged hyperlocomotion and other behavioural changes.
- 3 CGS 12066B caused similar effects, but they were much less pronounced, inconsistent and transient irrespective of whether this drug was given i.p. (1–15 mg kg⁻¹) or i.c.v. (0.2–40 µg). However, CGS 12066B (7.5 and 15 mg kg⁻¹) caused a dose-related inhibition of RU 24969 (7.5 mg kg⁻¹)-induced hyperlocomotion indicating that the former is a 5-HT_{1B} partial agonist.
- 4 RU 24969 (7.5 mg kg⁻¹ i.p.)-induced hyperlocomotion was inhibited by the (–)-, but not (+)-isomers of pindolol (4 mg kg⁻¹) and propranolol (20 mg kg⁻¹) but not by metoprolol (10 mg kg⁻¹) or ICI 118,551 (5 mg kg⁻¹), consistent with an involvement of 5-HT_{1A} or 5-HT_{1B} receptors.
- 5 The response was not altered by the selective 5-HT_{1A} receptor antagonist, WAY 100135 (5 mg kg⁻¹, s.c.), the 5-HT_{2A}/5-HT_{2C} receptor antagonist, ritanserin (0.1 mg kg⁻¹), the selective 5-HT₃ receptor antagonist, ondansetron (1 mg kg⁻¹) or the non-selective 5-HT receptor antagonists methysergide (3 mg kg⁻¹) and metergoline (3 mg kg⁻¹).
- 6 Although spiroxatrine (0.1 mg kg⁻¹) and ketanserin (1 mg kg⁻¹) inhibited RU 24969-induced hyperlocomotion, these effects were probably due to antagonism of dopamine D₂ receptors and α₁-adrenoceptors respectively.
- 7 Taken together, these results indicate that RU 24969-induced hyperlocomotion results specifically from activation of central 5-HT_{1B} receptors.
- 8 Lesioning of 5-HT neurones with 5,7-dihydroxytryptamine (75 µg, i.c.v.) or depletion with *p*-chlorophenylalanine (200 mg kg⁻¹, i.p. for 14 days) had no effect on RU 24969-induced hyperlocomotion demonstrating that the 5-HT_{1B} receptors involved are postsynaptic and that they do not show supersensitivity.
- 9 The involvement of other monoamine neurotransmitter systems in RU 24969-induced hyperlocomotion was also examined. The response was inhibited by the α₁-adrenoceptor antagonist, prazosin (1 mg kg⁻¹), the dopamine D₁ receptor antagonist, SCH 23390 (0.05 mg kg⁻¹) and the dopamine D₂ receptor antagonist, BRL 34778 (0.03 mg kg⁻¹), but not by the α₂-adrenoceptor antagonist, idazoxan (1 mg kg⁻¹). Lesioning noradrenergic neurones with *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (100 mg kg⁻¹) markedly attenuated this behaviour. These results show that the hyperlocomotion is expressed via noradrenergic and dopaminergic neurones acting on α₁-adrenoceptors, D₁ and D₂ receptors.
- 10 RU 24969 decreased brain concentrations of 5-hydroxyindoleacetic acid whilst simultaneously increasing 5-HT, consistent with the reduction of 5-HT neuronal activity by activation of 5-HT_{1A} and 5-HT_{1B} autoreceptors. RU 24969 increased brain 3-methoxy-4-hydroxyphenylglycol, but not noradrenaline, concentrations which supports the involvement of noradrenergic neurones in the expression of hyperlocomotion. RU 24969 did not alter dopamine, dihydroxyphenylacetic acid or homovanillic acid concentrations in the nucleus accumbens suggesting that the dopaminergic neurones terminating there are not directly involved.

Keywords: RU 24969; CGS 12066B; hyperlocomotion; locomotor activity in mice; 5-HT_{1B} receptors; behavioural model; dopamine; noradrenaline

Introduction

5-Hydroxytryptamine (5-HT) receptors were first classified as 5-HT₁ and 5-HT₂ by Peroutka *et al.* (1981) based on their respective affinities for [³H]-5-HT and [³H]-spiperone. It is now well established that there are at least five subtypes of 5-HT₁ receptor (1A, 1B, 1D, 1E, 1F) (reviewed by Humphrey *et al.*, 1993). RU 24969 (5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole succinate) was initially described as a selective 5-HT₁ receptor agonist (Hunt & Oberlander, 1981; Green *et al.*, 1984) and subsequent evaluation revealed that RU 24969 has high and approximately equal affinity for 5-HT_{1B} and 5-HT_{1A} receptors *in vitro* (Hoyer *et al.*, 1985). The most obvious behavioural consequence of RU 24969

administration to mice or rats is the induction of intense locomotor activity without production of either the 5-HT_{1A}-mediated 5-HT syndrome or 5-HT_{2A}-mediated head-twitch behaviour (Green *et al.*, 1984; Nisbet & Marsden, 1984). RU 24969-induced hyperlocomotion has been pharmacologically characterized extensively in rats where evidence for specific mediation by central 5-HT_{1B} receptors is poor and conflicting (Goodwin & Green, 1985; Tricklebank *et al.*, 1986). By contrast, although the early investigations in mice appeared to be more promising (Green *et al.*, 1984; Goodwin & Green, 1985), this behavioural response was not studied further. In view of this, work has now been carried out to characterize RU 24969-induced hyperlocomotion in mice using a wide range of 5-HT receptor subtype-selective antagonists. In addition the synaptic location of the receptors mediating this

¹ Author for correspondence.

response has been determined by the use of selective 5-HT neuronal lesioning and neurotransmitter depletion techniques. The involvement of other monoamines in the expression of the locomotor behaviour has also been elucidated with selective antagonists, lesioning and neurotransmitter depletion and turnover methods.

Recently, CGS 12066B (7-trifluoromethyl-4-(4-methyl-1-piperazinyl)-pyrrolo[1,2a]quinoxaline dimaleate) has been claimed to have selective 5-HT_{1B} receptor agonist activity (Neale *et al.*, 1987) and the behavioural consequences of administering this compound to mice have also been studied.

Methods

Animals

Adult male C57/B1/601a mice (Olac) weighing 22–30 g were used. They were housed in groups of 8–10 on a 12 h light/dark cycle (commencing 06.00 h) at a temperature of 21°C and 55% humidity. Mice were allowed food and water *ad libitum*. Experiments were performed between 08.00 and 13.00 h.

Behavioural studies

Effect of RU 24969 on locomotor activity Locomotor activity was measured using perspex boxes (48 × 26.5 × 30 cm) with a wire grid floor. Three horizontal infrared photocell beams were located along the short axis of the cage and one along the long axis approximately 4.5 cm above the floor. Photocell beam interruptions were recorded by an on-line microcomputer (BBC Master Series). This system was designed, built and programmed in-house by the Bioelectronics Group, Boots Pharmaceuticals Research Department.

As in the previously described protocol by De Souza *et al.* (1986) for the measurement of hyperlocomotion after intracerebroventricular (i.c.v.) injection of RU 24969, pairs of mice were used. These were allowed 60 min habituation to the new environment and then administered RU 24969 (1–30 mg kg⁻¹), CGS 12066B (1–15 mg kg⁻¹ or 0.2–40 µg, i.c.v.) or vehicle. Antagonists or the appropriate vehicle were given 30 min prior to RU 24969 (7.5 mg kg⁻¹) or saline to determine the effects on hyperlocomotion and control activity. Antagonist doses are based on results of previous behavioural experiments. Locomotor activity was recorded for 60 min after the final injection and data are expressed as the accumulated number of photocell interruptions during this period.

The dose of RU 24969 required to produce 50% of the maximum response was determined by linear regression analysis following log transformation of the data. Comparisons between antagonist and vehicle-treated groups were made by Student's *t* test (two-tailed, unpaired).

Comparison of the behavioural effects of RU 24969 and CGS 12066B Pairs of mice were administered RU 24969 (7.5 or 75 mg kg⁻¹) and CGS 12066B (10 or 25 mg kg⁻¹). Fifteen minutes later mice were assessed for the following behavioural parameters: locomotor activity (including pattern of activity), posture, sniffing, grooming, piloerection, Straub tail, forepaw treading, hindlimb abduction, head-bobbing, head-twitching, tremor and shivering and jumping. Each behavioural parameter was rated as absent, mild, moderate or intense with the exception of posture which was described as normal, lowered or hunched. Rectal temperature was measured before and 60 min after drug administration and is expressed as decrease in °C.

Neuronal lesioning and monoamine depletion experiments Depletion of brain 5-HT concentrations was achieved either by destruction of 5-HT neurones with the neurotoxin, 5,7-

dihydroxytryptamine (5,7-DHT), or by inhibition of 5-HT synthesis with *p*-chlorophenylalanine (PCPA).

For lesioning experiments, mice were pretreated with desipramine (25 mg kg⁻¹) to protect central noradrenaline-containing neurones. Fifteen minutes later, the animals were anaesthetized with hexobarbitone (50 mg kg⁻¹). After a further 15 min, 5,7-DHT (75 µg) dissolved in ice-cold saline containing 0.4 mg ml⁻¹ ascorbic acid was administered i.c.v. in a volume of 4 µl by use of the stereotaxic injector described by Heal (1984). Sham-lesioned mice were injected with vehicle (4 µl, i.c.v.). Mice were then housed individually and allowed to recover for 14 days. The synthesis inhibitor PCPA (200 mg kg⁻¹) was given once daily for 14 days. Control mice received saline.

Verification of substantial 5-HT depletion was initially performed by measurement of 5-methoxy-*N,N*-dimethyltryptamine (5-MeODMT)-induced head-twitches because there is a good correlation between this response and the extent of 5-HT denervation (Heal *et al.*, 1985). In the PCPA experiments, the head-twitch responses were determined 6 h prior to the final dose of PCPA and in the 5,7-DHT experiments on day 15. Mice showing satisfactory responses (75% increase in the head-twitch response to 5-MeODMT above mean control value) were allowed 24 h to recover before locomotor testing.

Destruction of brain noradrenergic neurones was carried out with the selective neurotoxin, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4). Mice were pretreated with zimeldine (5 mg kg⁻¹) to protect central 5-HT-containing neurones and DSP-4 (100 mg kg⁻¹) was administered 30 min later. Sham-lesioned mice received saline. Mice were then allowed to recover for 7 days.

Depletion of brain noradrenaline, dopamine and 5-HT concentrations was achieved by inhibition of neurotransmitter uptake into the storage vesicles by reserpine (0.25–2 mg kg⁻¹ in 50 mg ml⁻¹ ascorbic acid). Control mice were injected with vehicle. Mice were then allowed to recover for 18 h.

Verification of the selectivity and extent of monoamine depletion was determined by measurement of brain 5-HT, noradrenaline and dopamine concentrations by high performance liquid chromatography (h.p.l.c.) with electrochemical detection by methods described below.

Comparisons between appropriate vehicle and 5,7-DHT, PCPA or DSP-4 treated groups were made with Student's *t* test (two-tailed, unpaired). Statistical comparisons between vehicle- and reserpine-treated groups were made by analysis of variance (Armitage & Berry, 1987) followed by Williams' test (Williams, 1972).

Biochemical studies

Brain monoamine and metabolite concentrations were determined 60 min after RU 24969 (3.75–10 mg kg⁻¹) by h.p.l.c. with electrochemical detection.

5-HT, 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline, dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured in whole brain (5-HT, 5-HIAA and noradrenaline) or nucleus accumbens (dopamine, DOPAC, and HVA). Tissue was homogenized in 0.1 M perchloric acid containing 0.4 mM sodium metabisulphite (antioxidant) and 0.8 µM isoprenaline (internal standard). Following centrifugation at 1100 g for 15 min at 4°C and 15000 g for 5 min at 4°C, 30 µl of the resulting supernatant was injected onto a 5 µm 25 cm Hypersil ODS 1 column maintained at 45°C. The mobile phase was 0.1 M sodium dihydrogen orthophosphate-orthophosphoric acid buffer, pH 3.2 containing 16% v/v methanol, 2.8 mM 1-octanesulphonic acid sodium salt and 0.7 mM EDTA. Monoamines and metabolites were detected with a BAS LC-4A detector (+0.75 V versus an Ag/AgCl reference electrode). Full details are given in Heal *et al.* (1991).

3-Methoxy-4-hydroxyphenylglycol (MHPG) was measured

in whole brain minus cerebellum. Tissue was homogenized in 5 volumes (w/v) of 0.1 M perchloric acid containing 0.1 μ M iso-MHPG (internal standard) and 0.4 mM sodium metabisulphite (antioxidant). After centrifugation, 1100 g for 15 min at 4°C and 11600 g for 5 min at room temperature, MHPG and iso-MHPG were extracted in ethyl acetate, 0.08 M potassium bicarbonate was added and the organic phase evaporated to dryness at 40°C. The residues were dissolved in mobile phase (0.1 M sodium acetate/citric acid buffer, pH 4.4 containing 8% v/v methanol and 4.6 mM 1-octanesulphonic acid sodium salt) and injected onto a 5 μ m 25 cm Spherisorb ODS 1 column maintained at 45°C. MHPG and iso-MHPG were detected with a BAS LC-4B detector (+ 0.75 V versus an Ag/AgCl reference electrode). Full details are given in Heal *et al.* (1989).

Statistical comparisons between control and RU 24969-treated groups were made by analysis of variance (Armitage & Berry, 1987) followed by Williams' test (Williams, 1972).

Drugs and reagents

Drugs were obtained from the following sources (in parentheses): 1(-)-ascorbic acid (FSA, Loughborough); PCPA, desipramine HCl, 5,7-DHT, hexobarbitone, 5-MeODMT, metoprolol tartrate, ($\pm$)-pindolol, ($\pm$)-propranolol HCl, (+)- and (-)-propranolol HCl, reserpine (Sigma, Poole); 7-trifluoromethyl-4-(4-methyl-1-piperazinyl)-pyrrolo[1,2a]-quinoxaline dimaleate (CGS 12066B), DSP-4, idazoxan HCl, prazosin HCl, ritanserin, (R)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH 23390), spiroxatrine (Research Biochemicals Incorporated, St. Albans); ketanserin tartrate (Janssen Pharmaceuticals, Beersse); metergoline (Farmitalia, Milan); methysergide maleate, (+)- and (-)-pindolol (Sandoz, Horsforth); ondansetron (Medicinal Chemistry, Boots Pharmaceuticals Research Department); 5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole succinate (RU 24969) (Roussel-Uclaf, Romainville); exo-4-amino-5-chloro-2-methoxy-N-[9-(4-fluorophenyl methyl)-9-azabicyclo(3.3.1)non-3-yl]-benzamide (BRL 34778) (SmithKline Beecham Pharmaceuticals, Harlow); erythro-($\pm$)-1-(7-methylindan-4-yloxy)-3-isopropyl-aminobutan-2-ol (ICI 118,551) (ICI, Macclesfield) and (+)-N-tert-butyl-3-4-(2-methoxyphenyl)piperazin-1-yl-2-phenylpropanamide 2 HCl ((+)-enantiomer of WAY 100135).

Drugs were dissolved in 0.9% w/v sodium chloride solution (saline) whenever possible. ($\pm$)-Pindolol and its enantiomers, BRL 34778, CGS 12066B, ICI 118,551, metergoline, ritanserin and spiroxatrine were dissolved in a few drops of either glacial acetic acid or hydrochloric acid and made up to volume with saline. Prazosin was dissolved in distilled water. All drugs were injected intraperitoneally (i.p.), unless other-

wise stated, in weight-related doses (0.1 ml 10 g⁻¹ body weight). Control mice were given the appropriate vehicle.

Drugs and reagents for h.p.l.c. analysis were of the highest purity available and were obtained from the following sources: dopamine HCl, 5-hydroxytryptamine creatinine sulphate (5-HT), isoprenaline HCl, noradrenaline HCl (Sigma, Poole); perchloric acid, orthophosphoric acid, potassium bicarbonate, sodium metabisulphite (BDH, Poole); 1-octanesulphonic acid sodium salt, sodium dihydrogen orthophosphate, citric acid, sodium hydroxide (FSA, Loughborough); ethylenediaminetetraacetic acid disodium salt (EDTA; Aldrich, Gillingham); ethyl acetate, methanol (Rathburn Chemicals, Walkerburn) and 3-hydroxy-4-methoxyphenylglycol hemipiperazine (iso-MHPG; Medicinal Chemistry, Boots Pharmaceuticals Research Department). All water was distilled and deionised before use.

Results

When pairs of mice were injected with RU 24969 (1–75 mg kg⁻¹, i.p.) marked hyperlocomotion occurred in bursts accompanied by sniffing, grooming, infrequent head-bobbing and Straub tail (Figure 1, Table 1). The maximum locomotor effects of RU 24969 were observed at 10 mg kg⁻¹ and the ED₅₀ for this response was 2.6 mg kg⁻¹ (Figure 1). Doses

Figure 1 Dose-response relationship for RU 24969-induced hyperlocomotion in C57/B1/6 mice. Pairs of mice were injected with RU 24969 (1–30 mg kg⁻¹, i.p.). Results are plotted as mean $\pm$ s.e.mean; n = 9–12 groups of mice. ED₅₀ = 2.6 mg kg⁻¹ (95% confidence limits = 1.9–4.3 mg kg⁻¹). Correlation coefficient = 0.97.

Table 1 Comparison of the behavioural effects of RU 24969 and CGS 12066B

Behavioural parameter	RU 24969		CGS 12066B	
	7.5 mg kg ⁻¹	75 mg kg ⁻¹	10 mg kg ⁻¹	25 mg kg ⁻¹
Locomotor activity	Moderate	Intense	Mild	Mild
Pattern of activity	Intermittent and co-ordinated. Random pattern in cage	Constant, side to side locomotion. Random pattern in cage	Intermittent and co-ordinated. Random pattern in cage	Intermittent and co-ordinated. Random pattern in cage
Posture	Hunched	Normal	Hunched	Hunched
Sniffing	Moderate	Intense	Mild	Moderate
Grooming	Moderate	Mild	Moderate	Absent
Piloerection	Absent	Absent	Moderate	Moderate
Straub tail	Mild	Mild	Absent	Mild
Forepaw treading	Absent	Absent	Absent	Absent
Hindlimb abduction	Absent	Absent	Absent	Absent
Head-bobbing	Mild	Moderate	Absent	Absent
Head-twitching	Absent	Absent	Absent	Absent
Tremor and shivering	Absent	Moderate	Moderate	Moderate
Jumping	Absent	Absent	Absent	Absent
Temperature decrease	1.9°C	5.6°C	1.6°C	3.2°C

greater than 10 mg kg^{-1} induced smaller increases in locomotor activity which was increasingly displaced by the stereotyped behavioural components of sniffing, head-bobbing and tremor (Table 1). RU 24969 did not induce head-twitches or any component of the 5-HT syndrome (forepaw treading, hindlimb abduction or flat body posture) at any dose. This 5-HT₁ agonist did reduce the core body temperature of the mice and this effect was marked at high dosage (Table 1).

CGS 12066B produced a similar pattern of behavioural changes to those observed with RU 24969 (Table 1). However, the behavioural components were less intense than

those induced by RU 24969 and the locomotor effect was small and inconsistent when observed after either i.p. ($1\text{--}15 \text{ mg kg}^{-1}$) or i.c.v. ($0.2\text{--}40 \mu\text{g}$) injection (Figure 2).

Administration of CGS 12066B (7.5 and 15 mg kg^{-1}) 10 min prior to RU 24969 (7.5 mg kg^{-1}) inhibited the hyperlocomotor response induced by the latter drug by 30% and 54%, respectively (Figure 3). The saline response of control mice was not altered by pretreatment with CGS 12066B (15 mg kg^{-1} , data not shown).

The effects of 5-HT receptor antagonists on RU 24969-induced hyperlocomotion

Various 5-HT receptor antagonists or the appropriate vehicle were administered 30 min before injection of RU 24969 (7.5 mg kg^{-1}). The resulting locomotor response was attenuated by ($\pm$)-pindolol (4 mg kg^{-1}), ($\pm$)-propranolol (20 mg kg^{-1}), spiroxatrine (0.1 mg kg^{-1}) and ketanserin (1 mg kg^{-1}) (Table 2). The (+)-enantiomer of WAY 100135 (5 mg kg^{-1} , s.c.), ritanserin (0.1 mg kg^{-1}), ondansetron (1 mg kg^{-1}), metergoline (3 mg kg^{-1}) and methysergide (3 mg kg^{-1}) were without effect (Table 2). At these doses, metergoline was the only compound that inhibited spontaneous locomotor

Figure 2 Dose-response relationship for CGS 12066B-induced locomotor activity in C57/B1/6 mice. Pairs of mice were injected with CGS 12066B, $1\text{--}15 \text{ mg kg}^{-1}$ i.p. (a) or $0.2\text{--}40 \mu\text{g}$ i.c.v. (b). Control mice were injected with vehicle ($0.25 \text{ ml i.p. (a), or } 2 \mu\text{l i.c.v. (b)}$). Results are plotted as mean $\pm$ s.e.mean; $n = 4$ groups of mice.

Figure 3 The effects of CGS 12066B on RU 24969-induced hyperlocomotion. Groups of two mice were injected with CGS 12066B (7.5 and 15 mg kg^{-1} , i.p.) 10 min prior to RU 24969 (7.5 mg kg^{-1} , i.p.) shown by the hatched columns. Control mice received vehicle (0.25 ml i.p.) as shown by the open column. Results are the accumulated number of photocell interruptions in $60 \text{ min} \pm \text{s.e.mean}$ with the number of groups tested shown in each column. Statistical evaluations were performed with Student's t test. Although control data have been pooled for clarity, statistical comparisons were made against the appropriate subgroup of control values. Significantly different from appropriate control. * $P < 0.05$; *** $P < 0.001$.

Table 2 Effect of various 5-HT receptor antagonists on RU 24969-induced hyperlocomotion in mice

Antagonist	5-HT receptor selectivity	Dose (mg kg^{-1})	RU 24969 activity		Saline activity	
			Control	Antagonist-treated	Control	Antagonist-treated
($\pm$)-Pindolol	5-HT ₁	4	5070 ± 220	$3217 \pm 272^{***}$	231 ± 46	130 ± 47
($\pm$)-Propranolol	5-HT ₁	20	5567 ± 212	$2346 \pm 306^{***}$	211 ± 76	304 ± 64
Spiroxastrine	5-HT _{1A}	0.1	4918 ± 358	$2265 \pm 134^{***}$	421 ± 151	307 ± 81
(+)-WAY 100135	5-HT _{1A}	5	4246 ± 378	4652 ± 251	16 ± 9	68 ± 25
Ketanserin	5-HT _{2A}	1	4947 ± 266	$2991 \pm 123^{***}$	134 ± 48	70 ± 23
Ondansetron	5-HT ₃	1	4961 ± 263	5257 ± 276	175 ± 39	107 ± 24
Ritanserin	5-HT _{2A/2C}	0.1	5191 ± 278	5123 ± 301	83 ± 39	76 ± 35
Methysergide	5-HT _{2A/2C}	3	5026 ± 239	5370 ± 243	145 ± 46	183 ± 42
Metergoline	5-HT _{1A/1D/2A/2C}	3	4713 ± 310	4325 ± 372	198 ± 56	$67 \pm 18^*$

Pairs of mice were given an intraperitoneal or in the case of the (+)-enantiomer of WAY 100135 subcutaneous injection of antagonist or vehicle. Thirty minutes later these mice received either RU 24969 (7.5 mg kg^{-1} , i.p.) or saline (0.25 ml i.p.) to determine the effects on hyperlocomotion and control activity, respectively. Results are mean $\pm$ s.e.mean of the photocell interruptions in 60 min; $n = 7\text{--}12$ groups. Results were analysed by Student's t test. Significantly different from appropriate control: * $P < 0.05$; *** $P < 0.001$.

activity in control mice (Table 2). The stereoselectivity of the inhibition produced by pindolol and propranolol was also determined. The locomotor response to RU 24969 (7.5 mg kg⁻¹) was markedly inhibited by the (-)-isomers of pindolol (4 mg kg⁻¹) and propranolol (20 mg kg⁻¹), but at identical

doses, the (+)-isomers were without effect (Figure 4). The saline responses of control mice were not altered by administration of the isomers of pindolol or propranolol given at 4 mg kg⁻¹ and 20 mg kg⁻¹ respectively (data not shown).

The effects of catecholamine receptor antagonists on RU 24969-induced hyperlocomotion

The locomotor activity of pairs of mice induced by RU 24969 (7.5 mg kg⁻¹) was measured 30 min after i.p. injection of various catecholamine receptor antagonists or appropriate vehicle. Prazosin (1 mg kg⁻¹), SCH 23390 (0.05 mg kg⁻¹) and BRL 34778 (0.03 mg kg⁻¹) inhibited the hyperlocomotion without impairing the saline control response (Table 3). Idazoxan (1 mg kg⁻¹), metoprolol (10 mg kg⁻¹) and ICI 118,551 (5 mg kg⁻¹) had no effect on RU 24969-induced hyperlocomotion (Table 3).

Effects of selective neuronal lesions and monoamine depletion on RU 24969-induced hyperlocomotion and 5-MeODMT-induced head-twitches

5-HT-containing neurones were selectively lesioned by administration of 5,7-DHT (75 µg, i.c.v.). This procedure reduced the 5-HT content of whole brain by 73% with only minor effects on noradrenaline and dopamine levels (Table 4). Head-twitch and locomotor responses to 5-MeODMT and RU 24969 were measured on two consecutive days commencing 15 days after 5,7-DHT lesioning. Although this procedure increased the head-twitch response to 5-MeODMT (2 mg kg⁻¹) by 139%, it did not modify the hyperlocomotion produced by RU 24969 at doses of 3.75 or 7.5 mg kg⁻¹ (Figure 5). In a second study, the 5-HT content of the brain was reduced by 14 days of administration of the synthesis inhibitor, PCPA (200 mg kg⁻¹). This treatment, which produced a pronounced and selective 75% reduction of 5-HT concentrations (Table 4), also markedly increased 5-MeODMT (2 mg kg⁻¹)-induced head-twitches by 155%, but had no effect on RU 24969 (3.75 or 7.5 mg kg⁻¹)-induced locomotor activity (Figure 5).

Noradrenergic neurones were lesioned by administration of DSP-4 (100 mg kg⁻¹) which reduced noradrenaline concentrations in whole brain by 72% with no effect on dopamine or 5-HT levels (Table 4). This noradrenergic lesion attenuated RU 24969-induced hyperlocomotion by 39% at 3.75 mg kg⁻¹ and 45% at 7.5 mg kg⁻¹ when these responses were tested 8 days after DSP-4 administration (Figure 6).

Reserpine (0.25–2 mg kg⁻¹) dose-dependently reduced brain 5-HT, noradrenaline and dopamine concentrations (Table 4). Although 5-HT and noradrenaline concentrations were significantly reduced at all doses of reserpine and dopamine concentrations were significantly reduced at 0.5, 1 and 2 mg kg⁻¹, marked reductions (>60%) were only obtained at doses of 1 and 2 mg kg⁻¹. Reserpine did not significantly modify the hyperlocomotion induced by RU

Figure 4 The effects of the isomers of pindolol and propranolol on RU 24969-induced hyperlocomotion. Groups of two mice were injected with the (-)- and (+)-isomers of (a) pindolol (4 mg kg⁻¹, i.p.) or (b) propranolol (20 mg kg⁻¹, i.p.) shown by the solid and hatched columns, respectively. Control mice received vehicle (0.25 ml, i.p.) as shown by the open columns. Results are the accumulated number of photocell interruptions in 60 min $\pm$ s.e.mean with the number of groups tested shown in each column. Statistical evaluations were performed with Student's *t* test. Although control data have been pooled for clarity, statistical comparisons were made against the appropriate subgroup of control values. Significantly different from appropriate control. ****P* < 0.001.

Table 3 Effects of various catecholamine receptor antagonists on RU 24969-induced hyperlocomotion

Antagonist	Catecholamine receptor selectivity	Dose (mg kg ⁻¹ , i.p.)	RU 24969 activity		Saline activity	
			Control	Antagonist-treated	Control	Antagonist-treated
Prazosin	α_1	1	5044 $\pm$ 211	2496 $\pm$ 290***	67 $\pm$ 27	42 $\pm$ 16
Idazoxan	α_2	1	5331 $\pm$ 437	4426 $\pm$ 213	210 $\pm$ 37	264 $\pm$ 75
Metoprolol	β_1	10	4379 $\pm$ 222	4003 $\pm$ 208	192 $\pm$ 65	160 $\pm$ 54
ICI 118,551	β_2	5	4353 $\pm$ 444	3852 $\pm$ 343	65 $\pm$ 23	170 $\pm$ 77
SCH 23390	D ₁	0.05	4734 $\pm$ 158	1100 $\pm$ 232***	141 $\pm$ 49	141 $\pm$ 70
BRL 34778	D ₂	0.03	5162 $\pm$ 266	1133 $\pm$ 170***	145 $\pm$ 58	125 $\pm$ 31

Pairs of mice were given an intraperitoneal injection of antagonist or vehicle. Thirty minutes later these mice received either RU 24969 (7.5 mg kg⁻¹, i.p.) or saline (0.25 ml, i.p.) to determine the effects on hyperlocomotion and control activity, respectively. Results are mean $\pm$ s.e.mean of the locomotor counts accumulated in 60 min; *n* = 7–11 groups. Results were analysed by Student's *t* test. Significantly different from appropriate control: ****P* < 0.001.

Table 4 Effects of lesioning and monoamine depleting agents on 5-HT, noradrenaline and dopamine concentrations in mouse brain

Treatment	n	Dose	5-HT	Noradrenaline	Dopamine
Vehicle	36	4 μ l i.c.v.	890 $\pm$ 11	678 $\pm$ 7	1563 $\pm$ 16
5,7-DHT	28	75 μ g i.c.v.	242 $\pm$ 18*** (73%)	581 $\pm$ 12*** (14%)	1463 $\pm$ 20*** (6%)
Saline	36	0.25 ml i.p. $\times$ 14	888 $\pm$ 14	465 $\pm$ 7	1054 $\pm$ 17
PCPA	36	200 mg kg ⁻¹ i.p. $\times$ 14	220 $\pm$ 7*** (75%)	459 $\pm$ 8 (1%)	1062 $\pm$ 13 (+ 1%)
Saline	42	0.25 ml i.p.	950 $\pm$ 13	476 $\pm$ 13	950 $\pm$ 13
DSP-4	38	100 mg kg ⁻¹ i.p.	924 $\pm$ 11 (3%)	134 $\pm$ 5*** (72%)	924 $\pm$ 11 (3%)
Vehicle	20	0.25 ml i.p.	891 $\pm$ 10	551 $\pm$ 9	1170 $\pm$ 14
Reserpine	8	0.25 mg kg ⁻¹ i.p.	676 $\pm$ 31** (24%)	437 $\pm$ 21** (21%)	1099 $\pm$ 26 (7%)
	8	0.5 mg kg ⁻¹ i.p.	618 $\pm$ 46** (31%)	363 $\pm$ 51** (34%)	984 $\pm$ 51** (16%)
	8	1 mg kg ⁻¹ i.p.	329 $\pm$ 28** (63%)	101 $\pm$ 14** (82%)	450 $\pm$ 50** (62%)
	10	2 mg kg ⁻¹ i.p.	210 $\pm$ 24** (76%)	61 $\pm$ 18** (89%)	203 $\pm$ 50** (83%)

Groups of mice were injected with the above depleting agents or appropriate vehicle as described in Methods. Monoamine concentrations in mouse whole brain are reported as mean $\pm$ s.e.mean in ng g⁻¹ wet weight; percentage depletion in parentheses. Statistical comparisons between appropriate vehicle and 5,7-DHT, PCPA or DSP-4 treated groups were made with Student's *t* test, ****P* < 0.001. Comparisons between vehicle- and reserpine-treated groups were made by analysis of variance followed by Williams' test, ***P* < 0.01. For abbreviations, see text.

Figure 5 The effects of (a) 5,7-dihydroxytryptamine (5,7-DHT) lesioning and (b) repeated *p*-chlorophenylalanine (PCPA) administration on RU 24969-induced hyperlocomotion. Groups of mice were injected with: (a) 5,7-DHT (75 μ g, i.c.v.; hatched columns) or saline-ascorbate vehicle (4 μ l, i.c.v.; open columns); (b) PCPA (200 mg kg⁻¹, i.p.; solid columns) or saline (0.25 ml, i.p.; open columns) once daily for 14 days. The head-twitch response of all mice were determined using 5-methoxy-*N,N*-dimethyltryptamine (5-MeODMT) (2 mg kg⁻¹, i.p.) 15 days after 5,7-DHT lesioning or on the day of the final PCPA injection. Twenty-four h later pairs of control and 5-HT-depleted mice were tested for their locomotor response to RU 24969 (3.75 or 7.5 mg kg⁻¹, i.p.). Results are shown as mean $\pm$ s.e.mean with the number of observations shown in each column. Statistical comparisons were made with Student's *t* test. Significantly different from appropriate control. ****P* < 0.001.

24969 (7.5 mg kg⁻¹) at the lower doses of 0.25 and 0.5 mg kg⁻¹, but, virtually abolished the response at 1 and 2 mg kg⁻¹ by 95% and 98%, respectively (Figure 6). In both the DSP-4-treated and reserpine-treated mice hyperlocomotion was not replaced by any other behavioural effect.

Effects of RU 24969 administration on brain concentrations of monoamines and metabolites

Mice were injected with 3.75–10 mg kg⁻¹ of RU 24969 60 min before determination of the concentrations of various monoamine neurotransmitters and their metabolites. RU 24969 increased the concentration of 5-HT in whole brain and correspondingly decreased the levels of the major metabolite, 5-HIAA (Table 5).

RU 24969 had no effect on the concentrations of noradrenaline in whole brain, but this 5-HT₁ agonist increased levels of MHPG (Table 5). Since the primary pharmacological action of RU 24969 is to produce locomotor activity, its effects on dopamine and metabolites were determined in nucleus accumbens rather than whole brain. However, RU 24969 did not alter the concentrations of dopamine, DOPAC or HVA at any of the doses tested (Table 5).

Discussion

In this study, we observed that RU 24969 produces pronounced and sustained locomotor activity in mice and this finding is totally consistent with earlier reports (Green *et al.*, 1984; Goodwin & Green, 1985; De Souza *et al.*, 1986). However, CGS 12066B which is a selective, 5-HT_{1B} ligand (Neale *et al.*, 1987) produced only a very transient increase in locomotor activity whether given by the intraperitoneal or intracerebroventricular route. Moreover, CGS 12066B inhibited RU 24969-induced hyperlocomotion indicating that this compound is almost certainly a partial, rather than full, agonist at 5-HT_{1B} receptors. These results agree with the findings of Neale *et al.* (1987) which showed that CGS 12066B had relatively weak agonist efficacy.

Goodwin & Green (1985) have previously reported that the locomotor response to RU 24969 is inhibited by (–)-propranolol, but not (+)-propranolol or ritanserin suggesting that the hyperlocomotion is specifically mediated by 5-HT₁ receptors. We have now confirmed and extended these findings. Thus, RU 24969-induced locomotor activity was unaltered by the (+)-enantiomer of WAY 100135, a potent and selective 5-HT_{1A} antagonist (Bill *et al.*, 1993), ritanserin, a potent 5-HT_{2A/2C} antagonist (Hoyer, 1988), ondansetron, a potent 5-HT₃ antagonist (Kilpatrick *et al.*, 1987) and the (+)-isomers of pindolol and propranolol which have poor 5-HT₁ affinity. However, this response was markedly inhibited by (–)-pindolol and (–)-propranolol which are potent 5-HT₁ ligands (Middlemiss *et al.*, 1977; Hoyer *et al.*,

Figure 6 The effects of (a) N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) lesioning and (b) reserpine administration on RU 24969-induced hyperlocomotion. Groups of mice were injected with: (a) DSP-4 (100 mg kg⁻¹, i.p.; hatched columns) or saline (0.25 ml, i.p.; open columns); (b) Reserpine (0.25–2 mg kg⁻¹, i.p.; solid columns) or vehicle (0.25 ml, i.p. open columns). The locomotor responses of pairs of mice to RU 24969 (3.75 or 7.5 mg kg⁻¹, i.p., DSP-4 or saline; 7.5 mg kg⁻¹, i.p., reserpine or vehicle) were determined 7 days and 18 h after DSP-4 and reserpine, respectively. Results are shown as mean ± s.e.mean with the number of groups shown on each column. Statistical comparisons between saline and DSP-4 treated groups were made with Student's *t* test; ****P* < 0.001. Comparisons between vehicle- and reserpine-treated groups were made by analysis of variance followed by Williams' test; ***P* < 0.01.

1985) and by CGS 12066B which is 5-HT_{1B} receptor-selective (Neale *et al.*, 1987). This hypothesis is also supported by the observation that RU 24969 did not produce head-twitching in the mice which is a 5-HT_{2A}-mediated behavioural effect. Taken together, our results show that the locomotor effects of RU 24969 are mediated via 5-HT_{1B} receptors. The only results which would detract from this conclusion are the inhibition of RU 24969-induced hyperlocomotion by spiroxatrine and ketanserin, which have high affinity for 5-HT_{1A} and 5-HT_{2A} receptors, respectively (Janssen, 1983; Nelson & Taylor, 1986; Alexander & Wood, 1987). Since ritanserin had no effect on this response, the inhibition by ketanserin probably resulted from its known α₁-adrenoceptor antagonist activity (Janssen, 1983). It is likely that the inhibition by spiroxatrine was due to potent antagonism of dopamine D₂ receptors (IC₅₀ = 10.8 nM; Alexander & Wood, 1987).

Although the results argue that this locomotor response derives specifically from activation of central 5-HT_{1B} receptors, they do not preclude an action of RU 24969 on 5-HT_{1A} receptors. In fact, the observation that RU 24969 produced marked hypothermia in mice suggests that this agonist may simultaneously activate presynaptic 5-HT_{1A} receptors and it is, therefore, fortunate that this latter effect does not interfere with the locomotor response. The finding that RU 24969-induced hyperlocomotion in mice results solely from 5-HT_{1B} receptor activation contrasts with the contradictory findings which have been derived from studies using rats. For example, the response in rats was not inhibited by either racemic propranolol or its active (–)-isomer and it was potentiated by ritanserin (Green *et al.*, 1984; Goodwin & Green, 1985). In contrast, Tricklebank *et al.* (1986) found inhibition with propranolol and pindolol, but this inhibition was not stereoselective. It is obvious from these reports that there is no evidence to support the argument that RU 24969-induced hyperlocomotion in rats is a response specifically initiated by 5-HT_{1B} receptor activation.

Although 5-HT_{1B} receptors function as nerve terminal autoreceptors in rodent brain (Middlemiss, 1984a,b), the receptors responsible for the locomotor effects of RU 24969 are almost certainly postsynaptic as lesioning of 5-HT-containing neurones or depletion of 5-HT did not inhibit this behavioural response. This 5-HT_{1B}-mediated effect did not, however, show supersensitivity after these procedures, which contrasts with the marked potentiation of 5-HT_{2A}-mediated head-twitches observed in mice. Studies previously performed in rats have also found that the 5-HT receptors mediating the locomotor effects of RU 24969 are postsynaptic (Green *et al.*, 1984; Nisbet & Marsden, 1984; Tricklebank *et al.*, 1986; Oberlander *et al.*, 1986; 1987). However, there is disagreement over the induction of 5-HT_{1B} receptor supersensitivity in rats; Tricklebank *et al.* (1986) and Oberlander *et al.* (1987) reported its occurrence after 5,7-DHT lesioning, whilst Nisbet & Marsden (1984) failed to observe this effect.

The role of other monoaminergic neurones in the expression of RU 24969-induced hyperlocomotion has also been determined. Destruction of noradrenergic neurones with the selective neurotoxin, DSP-4 (Jaim-Etcheverry & Zieher, 1980; Jonsson *et al.*, 1981) and depletion of 5-HT, noradrenaline

Table 5 Effects of administration of RU 24969 on the concentrations of monoamines and their major metabolites in mouse brain

Treatment	5-HT	Whole brain 5-HIAA	Whole brain Noradrenaline	Whole brain minus cerebellum MHPG	Dopamine	Nucleus accumbens DOPAC	HVA
Saline	1209 ± 28	388 ± 28	514 ± 7	89 ± 2	5899 ± 184	767 ± 88	785 ± 40
RU 24969 (3.75)	1436 ± 30**	243 ± 11**	508 ± 4	102 ± 3*	5855 ± 208	783 ± 78	809 ± 19
RU 24969 (7.5)	1602 ± 53**	195 ± 17**	515 ± 12	104 ± 4**	5819 ± 143	770 ± 59	861 ± 33
RU 24969 (10)	1499 ± 34**	190 ± 9**	492 ± 10	114 ± 5**	5904 ± 322	877 ± 50	866 ± 33

Groups of mice were injected with RU 24969 (3.75–10 mg kg⁻¹, i.p.) or saline (0.25 ml, i.p.). Sixty minutes later brains were removed and the concentrations of monoamines and their metabolites were measured by h.p.l.c. with electrochemical detection as described in Methods. Results are reported as mean ± s.e.mean in ng g⁻¹ wet weight; *n* = 6–8. Statistical comparisons between saline and RU 24969 treated groups were made using analysis of variance followed by Williams' test. Significantly different from control. **P* < 0.05, ***P* < 0.01. For abbreviations, see text.

and dopamine concentrations by reserpine markedly inhibited this locomotor response demonstrating the involvement of catecholaminergic neurones. Receptor antagonist studies revealed mediation of RU 24969-induced hyperlocomotion via α_1 -adrenoceptors, and dopamine D₁ and D₂ receptors. These findings are consistent with and, in addition, complement earlier investigations performed on C57/B1/6 mice which have shown that activation of α_1 -adrenoceptors with phenylephrine or methoxamine produces intense locomotor activity and this response is in turn expressed via dopaminergic neurones (Heal, 1984). Other research workers have similarly reported that catecholaminergic neurones were involved in the expression of RU 24969-induced locomotor activity in the rat because the response was also inhibited by α_1 -adrenoceptor and dopamine receptor antagonists in this species (Tricklebank *et al.*, 1986; Oberlander *et al.*, 1986). In the rat, the influence of other monoamine neurotransmitter systems on RU 24969-induced hyperlocomotion may be more complex than in the mouse. For example, Tricklebank *et al.* (1986) found that monoamine depletion with reserpine resulted in the progressive release of behavioural components of the 5-HT syndrome, an effect not seen in the mouse, whilst Oberlander *et al.* (1986) reported that lesioning the globus pallidus to disrupt motor outputs of the dopamine system enhanced the RU 24969 locomotor response and suppressed the inhibitory effect of dopamine receptor antagonists.

The effects of RU 24969 administration on the concentrations of monoamine neurotransmitters and their metabolites in the brain were almost entirely consistent with the observed behavioural effects of this 5-HT_{1A/1B} agonist. In keeping with its potent actions as a 5-HT_{1A/1B} receptor agonist, RU 24969 markedly decreased the concentration of the 5-HT metabolite, 5-HIAA, while simultaneously increasing the concentration of the parent monoamine. These effects are almost certainly due to a reduction of 5-HT neuronal activity as a result of the concurrent activation of inhibitory 5-HT_{1B} terminal autoreceptors and inhibitory 5-HT_{1A} somatodendritic autoreceptors, and this has been reported by numerous earlier workers (Euvrard & Boissier, 1980; Green *et al.*, 1984;

Goodwin & Green, 1985; Carli *et al.*, 1988). Although noradrenaline concentrations were unaltered by RU 24969, levels of MHPG were increased. In mice, this metabolite is a good index of noradrenaline turnover and functional utilization (Heal *et al.*, 1989). By contrast, RU 24969 had no effect on the concentrations of dopamine or its metabolites in the nucleus accumbens which is involved in the generation of locomotor activity (Pijnenburg & Van Rossum, 1973; Costall & Naylor, 1976). Taken together, these results suggest that RU 24969-induced locomotor activity is mediated via noradrenergic neurones acting on α_1 -adrenoceptors and, subsequently, via dopaminergic neurones acting on dopamine D₁ and D₂ receptors. This sequence of events has previously been demonstrated for phenylephrine-induced activity in mice (Heal, 1984).

In conclusion, the results presented demonstrate that RU 24969 produces a syndrome of pronounced locomotor activity in mice which is specifically initiated via 5-HT_{1B} receptor activation and, consequently, this response may serve as a valuable model for monitoring 5-HT_{1B} receptor function *in vivo*. The 5-HT_{1B} receptors involved are post-synaptic and do not show supersensitivity following prolonged and extensive 5-HT depletion or neuronal lesioning. Catecholaminergic neurones are involved in the expression of this locomotor response.

Note added in proof

5-HT receptors have recently been reclassified (Humphrey *et al.*, 1993). Of particular relevance to this paper is that the increasingly recognised similarity between the 5-HT_{1C} and 5-HT₂ receptor has led to the former being reclassified as the 5-HT_{2C} receptor. The receptor previously referred to as the 5-HT₂ receptor is now termed the 5-HT_{2A} receptor. These names have been used in the text.

The authors wish to thank Mike Prow and Sarah Butler for performing the h.p.l.c. analyses on the brain samples, and Jo Hilliard and Jean Smith for preparing the manuscript. We also gratefully acknowledge the donation of drugs by the following pharmaceutical companies: Farmitalia, ICI, Janssen, Roussel-Uclaf, Sandoz and SmithKline Beecham.

References

- ALEXANDER, B.S. & WOOD, M.D. (1987). Monoamine receptor binding profile of the 5-HT_{1A} antagonist spiroxatrine. *Br. J. Pharmacol.*, **91**, 449P.
- ARMITAGE, P. & BERRY, G. (1987). Comparison of several groups. In *Statistical Methods in Medical Research*, 2nd edition. pp. 186–196. Oxford: Blackwell.
- BILL, D.J., BROWN, G. & FLETCHER, A. (1993). Stereospecific blockade of 8-OH-DPAT-induced hypothermia in rodents by the selective 5-HT_{1A} receptor antagonist, WAY 100135. *Br. J. Pharmacol.*, **108**, 94P.
- CARLI, M., INVERNIZZI, R., CERVO, L. & SAMANIN, R. (1988). Neurochemical and behavioural studies with RU-24969 in the rat. *Psychopharmacology*, **94**, 359–364.
- COSTALL, B. & NAYLOR, R.J. (1976). Antagonism of the hyperactivity induced by dopamine applied intracerebrally to the nucleus accumbens septi by typical neuroleptics and by clozapine, sulphide and thioridazine. *Eur. J. Pharmacol.*, **35**, 161–168.
- DE SOUZA, R.J., GOODWIN, G.M., GREEN, A.R. & HEAL, D.J. (1986). Effect of chronic treatment with 5-HT₁ agonist (8-OH-DPAT and RU 24969) and antagonist (isapirone) drugs on the behavioural responses of mice to 5-HT₁ and 5-HT₂ agonists. *Br. J. Pharmacol.*, **89**, 377–384.
- EUVRARD, C. & BOISSIER, J.R. (1980). Biochemical assessment of the central 5-HT agonist activity of RU 24969 (a piperidinyl indole). *Eur. J. Pharmacol.*, **63**, 65–72.
- GOODWIN, G.M. & GREEN, A.R. (1985). A behavioural and biochemical study in mice and rats of putative selective agonists and antagonists for 5-HT₁ and 5-HT₂ receptors. *Br. J. Pharmacol.*, **84**, 743–753.
- GREEN, A.R., GUY, A.P. & GARDNER, C.R. (1984). The behavioural effects of RU 24969, a suggested 5-HT₁ receptor agonist in rodents and the effect on the behaviour of treatment with antidepressants. *Neuropharmacology*, **23**, 655–661.
- HEAL, D.J. (1984). Phenylephrine-induced activity in mice as a model of central α_1 -adrenoceptor function. Effects of acute and repeated administration of antidepressant drugs and electroconvulsive shock. *Neuropharmacology*, **23**, 1241–1251.
- HEAL, D.J., PHILPOT, J., MOLYNEUX, S.G. & METZ, A. (1985). Intracerebroventricular administration of 5,7-dihydroxytryptamine to mice increases both head-twitch response and the number of cortical 5-HT₂ receptors. *Neuropharmacology*, **24**, 1201–1205.
- HEAL, D.J., PROW, M.R. & BUCKETT, W.R. (1989). Measurement of 3-methoxy-4-hydroxyphenylglycol (MHPG) in mouse brain by h.p.l.c. with electrochemical detection, as an index of noradrenaline utilisation and presynaptic α_2 -adrenoceptor function. *Br. J. Pharmacol.*, **96**, 547–556.
- HEAL, D.J., PROW, M.R. & BUCKETT, W.R. (1991). Determination of the role of noradrenergic and 5-hydroxytryptaminergic neurones in postsynaptic α_2 -adrenoceptor desensitization by desipramine and ECS. *Br. J. Pharmacol.*, **103**, 1865–1870.
- HOYER, D. (1988). Molecular pharmacology and biology of 5-HT_{1C} receptors. *Trends Pharmacol. Sci.*, **9**, 89–94.
- HOYER, D., ENGEL, G. & KALKMAN, H.O. (1985). Molecular pharmacology of 5-HT₁ and 5-HT₂ recognition sites in rat and pig brain membranes: radioligand binding studies with [³H]5-HT, [³H]8-OH-DPAT, (–)[¹²⁵I]iodocyanopindolol, [³H]mesulergine and [³H]ketanserin. *Eur. J. Pharmacol.*, **118**, 13–23.

- HUMPHREY, P.P.A., HARTIG, P. & HOYER, D. (1993). A proposed new nomenclature for 5-HT receptors. *Trends Pharmacol. Sci.*, **14**, 233–236.
- HUNT, P. & OBERLANDER, C. (1981). The interaction of indole derivatives with the serotonin receptor and non-dopaminergic circling behaviour. *Adv. Exp. Med. Biol.*, **133**, 547–561.
- JAIME-ETCHEVERRY, G. & ZIEHER, L.M. (1980). DSP-4: a novel compound with neurotoxic effects on noradrenergic neurons of adult and developing rats. *Brain Res.*, **188**, 513–523.
- JANSSEN, P.A.J. (1983). 5-HT₂ receptor blockade to study serotonin-induced pathology. *Trends Pharmacol. Sci.*, **4**, 198–206.
- JONSSON, G., HALLMAN, H., PONZIO, F. & ROSS, S. (1981). DSP4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine)- a useful denervation tool for central and peripheral noradrenaline neurons. *Eur. J. Pharmacol.*, **72**, 173–188.
- KILPATRICK, G.J., JONES, B.J. & TYERS, M.B. (1987). Identification and distribution of 5-HT₃ receptors in rat brain using radioligand binding. *Nature*, **330**, 746–748.
- MIDDLEMISS, D.N. (1984a). Stereoselective blockade at [³H]5-HT binding sites and at the 5-HT autoreceptor by propranolol. *Eur. J. Pharmacol.*, **101**, 289–293.
- MIDDLEMISS, D.N. (1984b). 8-Hydroxy-2-(di-n-propylamino) tetralin is devoid of activity at the 5-HT autoreceptor in the rat frontal cortex. Implications for the proposed link between the autoreceptor and the [³H]5-HT recognition site. *Naunyn-Schmied. Arch. Pharmacol.*, **327**, 18–22.
- MIDDLEMISS, D.N., BLAKEBOROUGH, L. & LEATHER, S.R. (1977). Direct evidence for an interaction of β -adrenergic blockers with the 5-HT receptor. *Nature*, **267**, 289–290.
- NEALE, R.F., FALLON, S.L., BOYAR, W.C., WASLEY, J.W.F., MARTIN, L.L., STONE, G.A., GLAESER, B.S., SINTON, C.M. & WILLIAMS, M. (1987). Biochemical and pharmacological characterisation of CGS 12066B, a selective serotonin-1B agonist. *Eur. J. Pharmacol.*, **136**, 1–9.
- NELSON, D.L. & TAYLOR, E.W. (1986). Spiroxitrine: a selective serotonin_{1A} receptor antagonist. *Eur. J. Pharmacol.*, **124**, 207–208.
- NISBET, A.P. & MARSDEN, C.A. (1984). Increased behavioural response to 5-methoxy-N, N-dimethyltryptamine but not to RU-24969 after intraventricular 5,7-dihydroxytryptamine administration. *Eur. J. Pharmacol.*, **104**, 177–180.
- OBERLANDER, C., BLAQUIÈRE, B. & PUJOL, J.-F. (1986). Distinct functions for dopamine and serotonin in locomotor behaviour: evidence using the 5-HT₁ agonist RU 24969 in globus pallidus-lesioned rats. *Neurosci. Letts.*, **67**, 113–118.
- OBERLANDER, C., DEMASSEY, Y., VERDU, A., VAN DE VELDE, D. & BARDELEY, C. (1987). Tolerance to the serotonin 5-HT₁ agonist RU 24969 and effects on dopaminergic behaviour. *Eur. J. Pharmacol.*, **139**, 205–214.
- PEROUTKA, S.J., LEBOVITZ, R.M. & SYNDER, S.H. (1981). Two distinct central serotonin receptors with differential physiological functions. *Science*, **212**, 827–829.
- PIJNENBURG, A.J.J. & VAN ROSSUM, J.M. (1973). Stimulation of locomotor activity following injection of dopamine into the nucleus accumbens. *J. Pharm. Pharmacol.*, **25**, 1003–1005.
- TRICKLEBANK, M.D., MIDDLEMISS, D.N. & NEILL, J. (1986). Pharmacological analysis of the behavioural and thermoregulatory effects of the putative 5-HT₁ receptor agonist, RU 24969, in the rat. *Neuropharmacology*, **25**, 877–886.
- WILLIAMS, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics*, **28**, 519–531.

(Received July 16, 1993

Revised August 18, 1993

Accepted September 1, 1993)