Characterization of 8-OH-DPAT-induced hypothermia in mice as a 5-HT_{1A} autoreceptor response and its evaluation as a model to selectively identify antidepressants

¹Keith F. Martin, Ian Phillips, Mitchell Hearson, Michael R. Prow & David J. Heal

Boots Pharmaceuticals Research Department, Nottingham NG2 3AA

- 1 8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) dose-dependently induced hypothermia in mice.
- 2 The 5-HT_{1A} receptor partial agonists, buspirone, gepirone and ipsapirone, also dose-dependently induced hypothermia.
- 3 The 8-OH-DPAT temperature response was antagonized by the 5-HT₁ receptor antagonists quipazine (2 mg kg⁻¹, i.p.), (±)-propranolol (10 mg kg⁻¹, i.p.), (±)-pindolol (5 mg kg⁻¹, i.p.), spiroxatrine (0.5 mg kg⁻¹, i.p.) and metitepine (0.05 mg kg⁻¹, i.p.), but not by 5-HT₂ (ketanserin) or 5-HT₃ (MDL 72222, GR 38032F) receptor antagonists.
- 4 The response was also antagonized by the dopamine D₂ receptor antagonists, haloperidol and BRL 34778. No other catecholamine or muscarinic receptors were involved in mediating the response.
- 5 Destruction of 5-hydroxytryptamine (5-HT)-containing neurones with the neurotoxin, 5,7-dihydroxytryptamine (75 µg, i.c.v.), abolished the response to 8-OH-DPAT indicating that the 5-HT_{1A} receptors involved were located on 5-HT neurones.
- 6 Chronic antidepressant treatment down-regulated this 8-OH-DPAT response. In addition, chronic administration of anxiolytics and neuroleptics was also effective in this respect. Down-regulation was also observed following repeated administration of 8-OH-DPAT (0.5 mg kg⁻¹, s.c.), (±)-pindolol (10 mg kg⁻¹, i.p.) and ketanserin (0.5 mg kg⁻¹, i.p.).
- 7 In conclusion, these data confirm that 8-OH-DPAT-induced hypothermia is mediated by 5-HT_{1A} autoreceptors. They also indicate that the response involves D₂ receptors. The present study also shows that a wide range of antidepressant drugs down-regulate this response although this property is not restricted to antidepressant treatments. Therefore, care should be exercised when interpreting data from this paradigm.

Keywords: 8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT); hypothermia; mice; antidepressants; 5-HT_{1A} receptors

Introduction

In 1983, Middlemiss & Fozard showed that 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) was a selective agonist at 5-HT_{1A} receptors. Subsequently, it was found that peripheral administration of 8-OH-DPAT induced hypothermia in mice and rats (Hjorth, 1985; Goodwin & Green, 1985; Goodwin *et al.*, 1985a; Gudelsky *et al.*, 1986; Higgins *et al.*, 1988; Wozniack *et al.*, 1988). The studies in mice have shown that this response is dose-dependent and sigmoidal (e.g. Goodwin *et al.*, 1985a). Pharmacological evaluation of the hypothermic response to 8-OH-DPAT led these authors to suggest that it was specifically mediated by 5-HT_{1A} receptors located on 5-hydroxytryptaminergic neurones. Goodwin and his coworkers based this hypothesis on inhibition of the response by ipsapirone (Goodwin *et al.*, 1986), a partial agonist at 5-HT_{1A} receptors (Martin & Mason, 1986), low doses of quipazine (Goodwin *et al.*, 1985a) and abolition of the response by destruction of 5-HT neurones (Goodwin *et al.*, 1985a). However, these authors were unable to prevent the effects of 8-OH-DPAT with a range of other 5-HT₁-like receptor antagonists. Furthermore, haloperidol, a potent D₂ dopamine receptor antagonist, was also found to inhibit this response (Goodwin *et al.*, 1985a). The data suggest, therefore, that the pharmacology of 8-OH-DPAT-induced hypothermia in mice may not be as straight-forward as first thought.

Goodwin *et al.* (1985b) have reported that 8-OH-DPAT-induced hypothermia in mice was selectively attenuated after repeated administration of antidepressant drugs and electroconvulsive shock (ECS). However, as the types of antidepressant tested were limited and the authors failed to investigate the effects of other psychotropic agents, their conclusion that this adaptation was specific to antidepressants required further confirmation.

The aims of the work described here were two fold: first, to extend the pharmacological characterization of the 8-OH-DPAT-induced hypothermia in mice and second, to determine whether the adaptive down-regulation of this response was specific to antidepressant treatment.

Methods

Animals

Adult male C57/Bl/6Ola mice (Olac, Bicester) weighing 25–30 g were used throughout this study. They were housed in groups of 10 on a 12:12 h light:dark cycle (lights on 07 h 00 min) and had free access to food and water. The ambient temperature was maintained at 20 ± 1°C and humidity was approximately 50%.

Agonist studies

Core temperature was measured by inserting the probe of a digital thermometer approximately 2.5 cm into the rectum

¹ Author for correspondence.

while lightly restraining the animal 10 min prior to (t_{-10}), immediately before (t_0) and 20 min after (t_{20}) an s.c. injection of saline or 5-HT_{1A} receptor agonist and the difference in temperature between t_0 and t_{20} was calculated.

Antagonist studies

Animals received either saline (10 mg kg⁻¹, s.c.) or 8-OH-DPAT (0.5 mg kg⁻¹, s.c.) 30 min after an i.p. injection of saline or antagonist. Rectal temperature was measured immediately before the first injection (t_{-30}) and prior to the second injection of saline or 8-OH-DPAT (t_0). A further measurement was taken 20 min after the second injection (t_{20}). The difference in temperature between t_0 and t_{20} was calculated.

Effect of chronic drug treatments

Groups of mice received either 14 daily injections (i.p.) of drugs or saline. The temperature decrease following an injection of 8-OH-DPAT (0.5 mg kg⁻¹, s.c.) was determined 24 h after the first and last treatments.

5-Hydroxytryptamine-containing neurone lesioning studies

The 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT; 75 µg) or saline-ascorbate (4 µl) was injected i.c.v. into halothane anaesthetized mice, pretreated with desipramine (5 mg kg⁻¹, i.p.) to protect noradrenergic neurones, by the method of Heal (1984). The temperature response to 8-OH-DPAT (0.5 mg kg⁻¹, i.p.) was measured seven days later as described above.

Noradrenaline-containing neurone lesioning studies

Mice were given an i.p. injection of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4, 100 mg kg⁻¹) 30 min after zimeldine (5 mg kg⁻¹, i.p.). Seven days later this procedure was repeated. The temperature response to 8-OH-DPAT (0.5 mg kg⁻¹, i.p.) was measured 12 days after the first DSP-4 injection as described above.

Statistics

Data were analysed by Student's unpaired *t* test and the null hypothesis was rejected when $P < 0.05$. However, when two treatment groups were compared with a single control group, the level of significance for rejection of the null hypothesis was set at $P < 0.01$.

Drugs

The following drugs were used (abbreviation if any and source shown in parentheses): N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), 8-hydroxy-2-(di-*n*-propylamino)tetralin HBr (8-OH-DPAT); ondansetron (GR 38032F), 1 α H,3 α ,5 α H-tropan-3-yl-3,5-dichlorobenzoate (MDL 72222), (+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine maleate (SCH 23390), spiroxatrine (Research Biochemicals Inc.), dothiepin HCl₂, gepirone (HCl)₂, ipsapirone (HCl)₂, sibutramine HCl (Boots Pharmaceuticals), zimeldine (HCl)₂ (Astra), *exo-N*-(9-[(4-fluorophenyl)methyl]-9-azabicyclo [3.3.1]non-3-yl)-4-amino-5-chloro-2-methoxybenzamide (BRL 34778) (Beecham), maprotiline (Ciba-Geigy), metitepine, metergoline (Farmitalia), *erythro*-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol (ICI 118,551) (I.C.I.), ketanserin tartrate (Janssen Pharmaceutica), citalopram (Lundbeck), quipazine (Miles Labs.), mianserin HCl (Organon), prazosin (Pfizer), idazoxan (Reckitt & Colman), chlordiazepoxide, diazepam (Roche), haloperidol (Searle), amitriptyline HCl, buspirone HCl, metoprolol, desipramine HCl, (±)-pindolol, (±)-propranolol (Sigma), tranlycypromine sulphate (Smith Kline & French).

All drugs were dissolved in 0.9% saline and injected intraperitoneally (i.p.) with the exception of 8-OH-DPAT, buspirone, gepirone and ipsapirone which were given subcutaneously (s.c.).

Results

Effects of 5-HT_{1A} receptor agonists on rectal temperature

When mice were injected with increasing doses of 8-OH-DPAT (0.05 to 5.0 mg kg⁻¹, s.c.), this 5-HT_{1A} receptor agonist produced a dose-dependent decrease in temperature (Figure 1). Time-course studies showed that the maximum hypothermic effect was observed 20 min after 8-OH-DPAT (data not shown). Similarly, the 5-HT_{1A} receptor partial agonists, buspirone, gepirone and ipsapirone, also dose-dependently induced hypothermia in mice (Figure 1).

Effects of 5-HT receptor antagonists

The putative 5-HT₁/5-HT_{1A} receptor antagonists quipazine (2 mg kg⁻¹), (±)-propranolol (10 and 20 mg kg⁻¹), (±)-pindolol (5 and 10 mg kg⁻¹), spiroxatrine (0.5 and 5 mg kg⁻¹) and metitepine (0.05 mg kg⁻¹) significantly attenuated the hypothermic response to 8-OH-DPAT (Table 1). Only quipazine had any significant effect (a small increase cf. control) when given alone (Table 1). Metitepine antagonized the response at a dose of 0.05 mg kg⁻¹, which itself had no effect on core temperature. However at a dose of 0.5 mg kg⁻¹, metitepine caused a marked reduction in temperature ($3.85 \pm 0.79^\circ\text{C}$; $P < 0.001$). There was no further fall in temperature induced by 8-OH-DPAT. The non-selective 5-HT receptor antagonist, metergoline had similar effects (Table 1).

Ketanserin (0.2 mg kg⁻¹), a 5-HT₂/5-HT_{1C} receptor antagonist, did not significantly alter body temperature itself, nor did it affect the hypothermia induced by 8-OH-DPAT (Table 1).

At moderate doses, the 5-HT₃ receptor antagonists, MDL 72222 and GR 38032F, did not alter the temperature response to 8-OH-DPAT (Table 1).

Effects of catecholamine and muscarinic receptor antagonists

The α_1 -adrenoceptor antagonist, prazosin (0.1 mg kg⁻¹) did not attenuate the response to 8-OH-DPAT (0.5 mg kg⁻¹) (Table 2). However, at high dose (1 mg kg⁻¹), it induced a marked fall in core temperature itself; this was not altered by 8-OH-DPAT (Table 2). The α_2 -adrenoceptor antagonist,

Figure 1 The effect of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), buspirone, gepirone and ipsapirone on the rectal temperature of C57/B1/6Ola mice. Measurements were made immediately before and 20 min after s.c. drug injection. The doses used, in mg kg⁻¹, are shown under each column. Columns represent mean temperature decrease with s.e. mean shown by vertical bars for $n = 10$ in each group.

Table 1 The effects of 5-hydroxytryptamine (5-HT) receptor antagonists on the hypothermic response to 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, 0.5 mg kg⁻¹, s.c.)

Antagonist (mg kg ⁻¹ , i.p.)	Change in temperature 20 min after	
	Saline	8-OH-DPAT
Saline	-0.37 ± 0.01 (78)	-1.63 ± 0.02 (78)
Quipazine (2)	+0.07 ± 0.11 (6)*	+0.53 ± 0.25 (6)***
(±)-Propranolol (10)	-0.38 ± 0.08 (6)	-1.16 ± 0.07 (5)*
(±)-Propranolol (20)	-0.42 ± 0.07 (6)	-0.28 ± 0.29 (6)**
(±)-Pindolol (5)	-0.27 ± 0.04 (6)	-1.07 ± 0.24 (6)**
(±)-Pindolol (10)	-0.65 ± 0.18 (6)	-0.78 ± 0.14 (6)**
Spiroxatrine (0.5)	-0.5 ± 0.11 (6)	-0.35 ± 0.21 (6)**
Spiroxatrine (5)	-0.12 ± 0.09 (5)*	+0.66 ± 0.10 (5)**
Metitepine (0.05)	-0.38 ± 0.13 (6)	-0.62 ± 0.21 (6)**
Metitepine (0.5)	-3.85 ± 0.79 (6)***	-3.8 ± 1.01 (6)*
Metergoline (0.5)	-1.33 ± 0.20 (6)**	-2.73 ± 0.44 (6)*
Ketanserin (0.2)	-0.43 ± 0.07 (6)	-2.15 ± 0.41 (6)
MDL 72222 (1)	-0.66 ± 0.13 (5)	-1.98 ± 0.13 (5)
GR 38032F (0.1)	-0.48 ± 0.07 (6)	-2.08 ± 0.17 (6)

Mice received i.p. injections of the antagonists and were injected 30 min later with 8-OH-DPAT (0.5 mg kg⁻¹, s.c.) or saline (10 ml kg⁻¹, s.c.). The temperature change 20 min after the 8-OH-DPAT/saline injection was determined. Data are presented as mean ± s.e.mean (*n*). Each treated group was compared with its own control but these have been pooled for the purpose of clarity. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 versus saline pretreatment group (Student's *t* test).

Table 2 Effects of catecholamine and muscarinic receptor antagonists on the hypothermic response to 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, 0.5 mg kg⁻¹, s.c.)

Antagonist (mg kg ⁻¹ , i.p.)	Change in temperature 20 min after	
	Saline	8-OH-DPAT
Saline	-0.35 ± 0.02 (60)	-1.60 ± 0.03 (60)
Prazosin (0.1)	-0.46 ± 0.08 (5)	-2.90 ± 0.29 (5)**
Prazosin (1)	-2.35 ± 0.14 (6)***	-2.57 ± 0.28 (6)**
Idazoxan (0.1)	-0.62 ± 0.03 (6)*	-2.18 ± 0.36 (6)
Idazoxan (1)	-0.58 ± 0.07 (6)	-2.22 ± 0.20 (6)
(±)-Propranolol (2)	-0.16 ± 0.03 (5)	-1.48 ± 0.09 (5)
ICI 118,551 (3)	-0.26 ± 0.10 (5)	-1.53 ± 0.09 (5)
Metoprolol (3)	-0.22 ± 0.11 (5)	-1.43 ± 0.06 (5)
ICI 118,551 (3) + Metoprolol (3)	-0.25 ± 0.14 (5)	-1.63 ± 0.06 (5)
Haloperidol (0.1)	+0.22 ± 0.17 (6)**	+0.08 ± 0.22 (5)***
Haloperidol (1)	-0.65 ± 0.14 (6)	-0.72 ± 0.12 (6)**
BRL 34778 (0.05)	-0.67 ± 0.11 (6)*	-0.84 ± 0.10 (5)*
SCH 23390 (0.1)	-0.68 ± 0.12 (6)	-1.55 ± 0.13 (6)
Atropine (0.1)	-0.24 ± 0.06 (5)	-2.26 ± 0.10 (5)
Atropine (1)	-0.28 ± 0.02 (5)	-2.38 ± 0.03 (5)

Mice received i.p. injections of the antagonists and were injected 30 min later with 8-OH-DPAT (0.5 mg kg⁻¹, s.c.) or saline (10 ml kg⁻¹, s.c.). The temperature change 20 min after the 8-OH-DPAT/saline injection was determined. Data are presented as mean ± s.e.mean (*n*). Each treated group was compared with its own control but these have been pooled for the purpose of clarity. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 versus saline pretreatment group (Student's *t* test).

idazoxan (0.1 and 1 mg kg⁻¹) had no effect on basal temperature and did not alter the hypothermia induced by 8-OH-DPAT (Table 2). When (±)-propranolol was given at the low dose of 2 mg kg⁻¹, the response to 8-OH-DPAT was not altered (Table 2). In addition, the selective β₁- and β₂-adrenoceptor antagonists, ICI 118,551 (3 mg kg⁻¹) and metoprolol (3 mg kg⁻¹), did not modify the response to 8-OH-DPAT (Table 2). These two β-adrenoceptor sub-type antagonists were also without effect on the response to 8-OH-DPAT when used in combination (Table 2). Haloperidol (0.1 and 1 mg kg⁻¹) and BRL 34778 (0.05 mg kg⁻¹), both potent D₂-receptor antagonists, significantly inhibited the effects of 8-OH-DPAT (Table 2). By contrast, SCH 23390 (0.1 mg kg⁻¹) was without effect (Table 2).

Atropine (1 mg kg⁻¹), a muscarinic receptor antagonist, did not alter the effects of 8-OH-DPAT on body temperature (Table 2).

Effects of 5,7-DHT and DSP-4 lesioning

The temperature response to 8-OH-DPAT (0.5 mg kg⁻¹, i.p.) was markedly attenuated following an i.c.v. injection of 5,7-DHT (Figure 2). Furthermore, basal temperatures were not significantly altered (Figure 2). Table 3 shows that the depletions of whole brain 5-HT, noradrenaline and dopamine were respectively 71% (*P* < 0.001), 37% (*P* < 0.001) and +1% (not significant).

Lesioning of the noradrenergic system with DSP-4 had no effect on the temperature response to 8-OH-DPAT (0.5 mg kg⁻¹, i.p., Figure 2) although basal temperatures were significantly lower in the lesioned animals (Figure 2). DSP-4 lesioning decreased whole brain noradrenaline concentrations by 75% (*P* < 0.001, Table 3). This treatment also resulted in a minor reduction in dopamine (16%, *P* < 0.001) content but had no effect on 5-HT content.

Figure 2 The effects of 5,7-dihydroxytryptamine (5,7-DHT, a) and N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4, b) treatment on the hypothermic response to 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, 0.5 mg kg⁻¹, i.p.). (a) Mice were given desipramine (5 mg kg⁻¹, i.p.) 30 min before 5,7-DHT (75 µg, i.c.v.) or saline-ascorbate (4 µl, i.c.v.) under halothane anaesthesia. Seven days later the temperature change in the 20 min following 8-OH-DPAT (0.5 mg kg⁻¹, i.p.) was measured. (b) Mice were given zimeldine (5 mg kg⁻¹, i.p.) 30 min before DSP-4 (100 mg kg⁻¹, i.p.) or saline (0.25 ml, i.p.). This was repeated seven days later and 12 days after the first dose of DSP-4 the temperature response to 8-OH-DPAT (0.5 mg kg⁻¹, i.p.) was measured. Each column represents the mean rectal temperature with s.e.mean shown by vertical bars. The number of animals is shown at the base of each column. Data were analysed by Student's paired (within subjects) or unpaired (between subjects) *t* test.

NS = not significant; ***P* < 0.01; ****P* < 0.001.

Effects of acute and chronic treatments

Antidepressants A single dose of maprotiline (20 mg kg⁻¹) or citalopram (20 mg kg⁻¹) attenuated the response to 8-OH-DPAT given 24 h later (temperature decrease: -1.88 ± 0.08°C, *n* = 10, saline; -0.92 ± 0.04°C, *n* = 10, maprotiline; -0.69 ± 0.03°C, *n* = 10, citalopram). None of the other drugs tested had any effect after a single dose (data not shown). By contrast, after 14 days of treatment, all of the drugs reduced the magnitude of the hypothermia induced by 8-OH-DPAT (0.5 mg kg⁻¹) (Table 4). In some cases (sibutramine HCl, desipramine, maprotiline, citalopram and zimeldine) this effect was only apparent with relatively large doses.

In common with the established antidepressant drugs, those with more novel mechanisms of action, such as idazoxan, buspirone and gepirone, were also effective in this paradigm (Table 4).

Anxiolytics Repeated administration of diazepam (1 mg kg⁻¹) and chlordiazepoxide (3 mg kg⁻¹) reduced the size of the response to 8-OH-DPAT (0.5 mg kg⁻¹) (Table 5). By contrast, a single dose of these drugs was without effect (data not shown).

Neuroleptic and 5-HT receptor antagonists

Four other psychotropic agents were tested: haloperidol (1 mg kg⁻¹), 8-OH-DPAT (0.5 mg kg⁻¹), (±)-pindolol (10 mg kg⁻¹) and ketanserin (0.5 mg kg⁻¹). Repeated haloperidol and ketanserin weakly attenuated the response, whereas 8-OH-DPAT and (±)-pindolol markedly reduced it (Table 5). None of the drugs had any effect after a single dose (data not shown).

Discussion

In this study, 8-OH-DPAT produced dose-dependent hypothermia in mice in agreement with previously reported findings with this species (Goodwin *et al.*, 1985a) or rats (Goodwin *et al.*, 1986; Hutson *et al.*, 1987). Similarly, the ED₅₀ value of approximately 0.5 mg kg⁻¹ agrees well with that found by Goodwin *et al.* (1985a). We have also extended these findings by showing that the 5-HT_{1A} receptor partial agonists, buspirone, gepirone and ipsapirone, also dose-dependently induce hypothermia.

Mediation of the 8-OH-DPAT-induced hypothermia by 5-HT_{1A} receptors was confirmed with various 5-hydroxytryptamine and other neurotransmitter receptor antagonists. The 5-HT₁ receptor antagonists, (±)-propranolol (Middle-

Table 3 The effects of 5,7-dihydroxytryptamine (5,7-DHT) and N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) treatment on whole mouse brain noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5-HT) concentrations

Treatment	Brain monoamine concentration (ng g ⁻¹ tissue wet wt.)			
	n	NA	DA	5-HT
5,7-DHT				
Control	9	506 ± 7	1014 ± 14	677 ± 17
5,7-DHT	8	316 ± 20***	1020 ± 18	196 ± 39***
% change		- 37	+ 1	- 71
DSP-4				
Control	8	463 ± 10	1146 ± 14	882 ± 13
DSP-4	7	117 ± 6***	969 ± 13***	859 ± 18
% change		- 75	- 16	- 3

5,7-DHT lesioning Mice were given an i.p. injection of desipramine (5 mg kg⁻¹) 30 min before an i.c.v. injection of 5,7-DHT (75 µg) or saline-ascorbate (4 µl) under halothane anaesthesia. Seven days later, the mice were given a s.c. injection of 8-OH-DPAT (0.5 mg kg⁻¹) and the temperature change in the following 20 min determined.

DSP-4 lesioning Mice were given an i.p. injection of zimeldine (5 mg kg⁻¹) 30 min before DSP-4 (100 mg kg⁻¹, i.p.) or saline (0.25 ml). This procedure was repeated seven days later. Twelve days after the first dose of DSP-4 the temperature changes of the mice in the 20 min following 8-OH-DPAT (0.5 mg kg⁻¹, s.c.) were determined. Twenty-four hours later, the mice were killed and brain NA, DA and 5-HT concentrations (ng g⁻¹ tissue wet wt.) were determined by h.p.l.c.-e.c.d. Results are expressed as mean ± s.e.mean and were analysed by Student's unpaired *t* test.

****P* < 0.001 compared with control.

Table 4 The effects of 14 day treatment with antidepressant drugs on the hypothermic response to 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, 0.5 mg kg⁻¹, s.c.)

Treatment (mg kg ⁻¹ , i.p.)	Principle pharmacological action	Decrease in temp. 20 min after 8-OH-DPAT
Control (0.25 ml, i.p.)	—	1.23 ± 0.02 (127)
Amitriptyline (10)	NA + 5-HT uptake	0.87 ± 0.07 (10)***
Sibutramine HCl (10)	NA + 5-HT uptake	1.13 ± 0.07 (10)*
Dothiepin (30)	NA + 5-HT uptake	0.77 ± 0.09 (10)***
Desipramine (10)	NA uptake	1.24 ± 0.09 (10)
Desipramine (20)	NA uptake	0.86 ± 0.10 (7)***
Maprotiline (10)	NA uptake	1.26 ± 0.11 (8)
Maprotiline (20)	NA uptake	0.50 ± 0.05 (10)***
Citalopram (10)	5-HT uptake	1.33 ± 0.10 (9)
Citalopram (20)	5-HT uptake	0.70 ± 0.10 (10)***
Zimeldine (10)	5-HT uptake	1.26 ± 0.10 (9)
Zimeldine (20)	5-HT uptake	0.82 ± 0.06 (9)***
Tranlycypromine (5)	MAO inhibition	0.64 ± 0.04 (10)***
Mianserin (5)	α ₂ /5-HT ₂ antagonist	0.83 ± 0.10 (10)***
Idazoxan (0.1)	α ₂ -antagonist	1.01 ± 0.04 (10)*
Idazoxan (1)	α ₂ -antagonist	0.99 ± 0.04 (10)***
Buspirone (10)	5-HT _{1A} agonist	1.0 ± 0.05 (10)**
Buspirone (30)	5-HT _{1A} agonist	0.82 ± 0.07 (10)***
Gepirone (10)	5-HT _{1A} agonist	0.69 ± 0.06 (8)***
Gepirone (30)	5-HT _{1A} agonist	0.63 ± 0.07 (6)**

Mice received i.p. injections of the drugs indicated above. Twenty-four hours after the final dose, 8-OH-DPAT (0.5 mg kg⁻¹, s.c.) was administered and the temperature change in the following 20 min determined. Data are mean ± s.e.mean (n). Each treated group was compared with its own control but these have been pooled for the purposes of clarity.

P* < 0.05; *P* < 0.01; ****P* < 0.001 versus saline pretreatment group (Student's *t* test).

Table 5 The effects of 14 days treatment with psychotropic drugs on the hypothermia induced by 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, 0.5 mg kg⁻¹, s.c.)

Treatment (mg kg ⁻¹ , i.p.)	Principle pharmacological action	Decrease in temp. 20 min after 8-OH-DPAT
Control	—	1.36 ± 0.03 (54)
Diazepam (1)	BDZ agonist	0.84 ± 0.04 (9)**
Diazepam (2.5)	BDZ agonist	0.83 ± 0.07 (9)**
Chlordiazepoxide (1)	BDZ agonist	1.15 ± 0.08 (10)
Chlordiazepoxide (3)	BDZ agonist	0.97 ± 0.05 (10)**
Haloperidol (1)	D ₂ /α ₁ antagonist	1.14 ± 0.07 (10)*
8-OH-DPAT (0.5 s.c.)	5-HT _{1A} agonist	0.74 ± 0.06 (8)**
(±)-Pindolol (10)	β/5-HT ₁ antagonist	0.78 ± 0.06 (8)**
Ketanserin (0.5)	5-HT ₂ /5-HT _{1C} antagonist	1.06 ± 0.09 (8)*

Mice received i.p. injections of the drugs indicated above. Twenty-four hours after the final dose, 8-OH-DPAT (0.5 mg kg⁻¹, s.c.) was administered and the temperature change in the following 20 min determined. Data are mean ± s.e.mean (n). Each treated group was compared with its own control but these have been pooled for the purposes of clarity. BDZ = benzodiazepine receptor.

P* < 0.05; *P* < 0.01; ****P* < 0.001 versus saline-treatment group (Student's *t* test).

miss, 1984), (±)-pindolol (Hamon *et al.*, 1986), spiroxatrine (Nelson & Taylor, 1986) and quipazine (Moret, 1985), inhibited 8-OH-DPAT-induced hypothermia. In addition, metitepine, which is reported to show some 5-HT₁ selectivity (Hibert & Middlemiss, 1986), also inhibited the response at low doses. Goodwin *et al.* (1985a) previously reported that 8-OH-DPAT hypothermia was antagonized only by quipazine and that propranolol, pindolol and metitepine were without effect. Our data confirm that quipazine is an antagonist, but disagree with respect to the latter antagonists. In the case of metitepine, the failure to observe inhibition of 8-OH-DPAT hypothermia was probably because at the dose used by Goodwin *et al.* (1985a), metitepine itself produced a marked reduction in core temperature; a finding confirmed by the present study. However, this argument does not explain the differences in respect of propranolol and pindolol and methodological variations are unlikely to be responsible because the identical strain and supplier of mice were used and a similar experimental protocol was employed. Further evidence for the 5-HT_{1A} receptor-mediation of 8-OH-DPAT hypothermia is provided by the observations that ketanserin (5-HT₂/5-HT_{1C} antagonist) and MDL 72222 and GR 38032F (5-HT₃ receptor antagonists) had no effect.

In addition to demonstrating that 8-OH-DPAT hypothermia was a 5-HT_{1A} receptor-mediated response, the involvement of other neurotransmitters in its expression was also determined. Our findings clearly implicate dopamine receptors because the hypothermia was inhibited by the D₂ receptor antagonists, haloperidol and BRL 34778. However, D₁ receptor involvement can be discounted because the highly selective antagonist, SCH 23390, had no effect on this response.

The hypothermia induced by 8-OH-DPAT was not modified by administration of α₁-, α₂- or selective β-adrenoceptor antagonists and these data argue that the response is unlikely to be expressed via any of the above receptor subtypes. These findings are in agreement with those of Goodwin *et al.* (1985a), but are at variance with the reported potentiation of 8-OH-DPAT hypothermia by the β-adrenoceptor agonist, clenbuterol (Green *et al.*, 1986). Since it has been suggested that 8-OH-DPAT produces hypothermia by activating somatodendritic autoreceptors in the median raphe (Higgins *et al.*, 1988), these observations are also incompatible with the involvement of α₂-adrenoceptors in the 8-OH-DPAT-induced inhibition of 5-HT release (Marsden & Martin, 1986).

Destruction of 5-HT neurones with the neurotoxin, 5,7-DHT, abolished the hypothermia induced by 8-OH-DPAT. This finding confirms the observations of Goodwin *et al.* (1985a), who reported that inhibition of 5-HT synthesis by chronic administration of *p*-chlorophenylalanine also prevented the decrease in temperature induced by 8-OH-DPAT. By contrast, lesioning of the noradrenergic system with DSP-4 had no effect on the temperature response to 8-OH-DPAT. This finding confirms that noradrenaline is not involved in the mediation of this response.

Viewed overall, therefore, the data indicate that 8-OH-DPAT induces hypothermia via an action on 5-HT_{1A} receptors located on 5-HT neurones. These receptors are probably located on 5-HT cell bodies in the midbrain raphe complex (Verge *et al.*, 1985; Higgins *et al.*, 1988).

In the second part of this study, we have re-evaluated the hypothesis put forward by Goodwin *et al.* (1985b) that down-regulation of 5-HT_{1A} receptors initiating 8-OH-DPAT hypothermia is an important adaptive response to antidepressant treatment.

We have now confirmed that repeated administration of desipramine, zimeldine, tranylcypromine and mianserin down-regulated this response. In addition, we have extended the findings by demonstrating identical effects with maprotiline, citalopram, amitriptyline, dothiepin, sibutramine, idazoxan, buspirone and gepirone. Overall, therefore, it is clear that antidepressant treatments with diverse modes of pharmacological effect all attenuate this 5-HT_{1A} receptor-mediated response. However, the present findings also demonstrate that this adaptation is produced by repeated treatment with psychotropic drugs which are not antidepressants. Thus the anxiolytic agents diazepam and chlordiazepoxide, attenuated 8-OH-DPAT hypothermia. While they are not claimed to be antidepressants, they have been shown to reduce significantly HAM-D scores in some clinical trials (Goldberg & Finnerty, 1979). This does not argue that these compounds are antidepressants, rather it indicates that drugs which are used to treat disorders with an affective component may be efficacious in this paradigm. In addition to the anxiolytics, haloperidol, an antipsychotic drug, significantly attenuated the response to 8-OH-DPAT, although this effect was weak in comparison to that observed after antidepressant treatment. We have clearly shown that D₂-receptors are involved in the expression of this hypothermic response and

therefore, the apparent attenuation by haloperidol may be due to antagonism of D₂-receptors by the haloperidol still present 24 h after the final dose. Alternatively, a direct effect of haloperidol on 5-HT_{1A} receptors cannot be ruled out, because this drug and other butyrophenones have weak affinity for 5-HT_{1A} sites (Pedigo *et al.*, 1981; Martin, unpublished data).

The temperature response to 8-OH-DPAT was markedly attenuated following its repeated administration. This observation indicates that chronic stimulation of the 5-HT_{1A} receptors involved in the response results in tachyphylaxis and is entirely in keeping with pharmacological principles. However, the effects of (±)-pindolol were surprising as this drug is generally considered to be an antagonist at 5-HT_{1A} receptors and chronic administration would be expected to produce up-regulation. Similar effects of ritanserin on 5-HT₂ receptor binding have been reported (Leysen *et al.*, 1986) and this phenomenon may represent an idiosyncrasy of 5-HT receptor regulation. Alternatively, Hjorth & Carlsson (1986) have reported that pindolol is a partial agonist at 5-HT_{1A} receptors and if this is correct, it would satisfactorily explain the down-regulation of 5-HT_{1A} receptors following repeated administration of pindolol.

The findings of the second part of the present study are consistent with previous studies using this mouse model (Goodwin *et al.*, 1985b). Furthermore, they are also in agreement with the results of electrophysiological studies in rats. Thus, Blier and co-workers (1984, 1987) have consistently shown that antidepressant treatments attenuate the responses of 5-HT neurones to stimulation of somatodendritic 5-HT_{1A} receptors in the midbrain raphe complex. This effect has been shown to have the overall effect of increasing 5-hydroxytryptaminergic neurotransmission (Blier & de Montigny, 1987).

In conclusion, we have confirmed that the hypothermic response of mice to 8-OH-DPAT is mediated by 5-HT_{1A} receptors, the anatomical location of these sites being on the 5-HT-containing neurones. In addition, we have shown that a wide range of antidepressants attenuate this response. However, the apparent down-regulation of these receptors by other classes of drugs indicates that care should be exercised when interpreting data from this model.

We would like to thank Astra, Farmitalia, Lundbeck, I.C.I., Miles Labs., Organon, Pfizer, Reckitt and Colman, Roche, Searle and SmithKline Beecham for generous supplies of drugs.

References

- BLIER, P. & DE MONTIGNY, C. (1987). Modification of 5-HT neuron properties by sustained administration of the 5-HT_{1A} partial agonist gepirone: electrophysiological studies in the rat brain. *Synapse*, **1**, 470–480.
- BLIER, P., DE MONTIGNY, C. & CHAPUT, Y. (1987). Modifications of the serotonin system by antidepressant treatments: implications for the treatment response in major depression. *J. Clin. Psychopharmacol.*, **7**, (6 Suppl) 24S–35S.
- BLIER, P., DE MONTIGNY, C. & TARDIFF, D. (1984). Effects of the two antidepressant drugs mianserin and inadapine on the serotonergic system: single cell studies in the rat. *Psychopharmacology*, **84**, 242–249.
- GOLDBERG, H.L. & FINNERTY, R.J. (1979). The comparative efficacy of buspirone and diazepam in the treatment of anxiety. *Am. J. Psychiatry*, **136**, 1184–1187.
- GOODWIN, G.M., DE SOUZA, R.J. & GREEN, A.R. (1985a). The pharmacology of the hypothermic response in mice to 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT): a model of presynaptic 5-HT₁ function. *Neuropharmacology*, **24**, 1187–1194.
- GOODWIN, G.M., DE SOUZA, R.J. & GREEN, A.R. (1985b). Presynaptic serotonin receptor-mediated response in mice attenuated by antidepressant drugs and electroconvulsive shock. *Nature*, **317**, 531–533.
- GOODWIN, G.M., DE SOUZA, R.J. & GREEN, A.R. (1986). The effects of the 5-HT₁ receptor ligand ipsapirone (TVXQ 7821) on 5-HT synthesis and the behavioural effects of 5-HT agonists in mice and rats. *Psychopharmacology*, **89**, 382–387.
- 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., GOODWIN, G.M., DE SOUZA, R.J. & HEAL, D.J. (1986). The β_2 -adrenoceptor agonists clenbuterol and salbutamol enhance the hypothermic action of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) in mice by a central mechanism. *Neuropharmacology*, **25**, 21–24.
- GUDELSKY, G.A., KOENIG, J.J. & MELTZER, H.Y. (1986). Thermoregulatory responses to serotonin (5-HT) receptor stimulation in the rat. Evidence for opposing roles of 5-HT₂ and 5-HT_{1A} receptors. *Neuropharmacology*, **25**, 1307–1313.
- HAMON, M., COSSERY, J.-M., SPAMPINATO, U. & GOZLAN, H. (1986). Are there selective ligands for 5-HT_{1A} and 5-HT_{1B} receptor binding sites in brain? *Trends Pharmacol. Sci.*, **7**, 336–338.
- 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*, **2**, 1241–1251.
- HIBERT, M. & MIDDLEMISS, D.N. (1986). Stereoselective blockade at the 5-HT autoreceptor and inhibition of radioligand binding to central 5-HT recognition sites by the optical isomers of methiothepin. *Neuropharmacology*, **25**, 1–4.

- HIGGINS, G.A., BRADBURY, A.J., JONES, B.J. & OAKLEY, MR. (1988). Behavioural and biochemical consequences following activation of 5-HT₁-like and GABA receptors in the dorsal raphe nucleus of the rat. *Neuropharmacology*, **27**, 993–1001.
- HJORTH, S. (1985). Hypothermia in the rat induced by the potent serotonergic agent 8-OH-DPAT. *J. Neural Transm.*, **61**, 131–135.
- HJORTH, S. & CARLSSON, A. (1986). Is pindolol a mixed agonist-antagonist at central serotonin (5-HT) receptors? *Eur. J. Pharmacol.*, **129**, 131–138.
- HUTSON, P.H., DONOHOE, T.P. & CURZON, G. (1987). Hypothermia induced by the putative 5-HT_{1A} agonists LY-165163 and 8-OH-DPAT is not prevented by 5-HT depletion. *Eur. J. Pharmacol.*, **143**, 221–228.
- LEYSSEN, J.E., VAN GOMPEL, P., GOMMEREN, W., WOESTENBORGH, R. & JANSSEN, P.A.J. (1986). Down regulation of serotonin- S_2 receptor sites in rat brain by chronic treatment with the serotonin- S_2 antagonists: ritanserin and setoperone. *Psychopharmacology*, **88**, 434–444.
- MARSDEN, C.A. & MARTIN, K.F. (1986). Involvement of 5-HT_{1A}- and α_2 -receptors in the decreased 5-hydroxytryptamine release and metabolism in rat suprachiasmatic nucleus after intravenous 8-hydroxy-2-(n-dipropylamino)tetralin. *Br. J. Pharmacol.*, **89**, 227–286.
- MARTIN, K.F. & MASON, R. (1986). Ipsapirone is a partial agonist at 5-hydroxytryptamine 1A (5-HT_{1A}) receptors in the rat hippocampus: electrophysiological evidence. *Eur. J. Pharmacol.*, **141**, 479–483.
- MIDDLEMISS, D.N. (1984). 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. & FOZARD, J.R. (1983). 8-Hydroxy-2-(di-n-propylamino) tetralin discriminates between sub-types of the 5-HT₁ recognition site. *Eur. J. Pharmacol.*, **90**, 151–153.
- MORET, C. (1985). Pharmacology of the serotonin autoreceptor. In *Neuropharmacology of Serotonin* ed. Green, A.R. pp. 21–49. Oxford University Press: Oxford.
- NELSON, D.L. & TAYLOR, E.W. (1986). Spiroxatrine: a selective serotonin_{1A} receptor antagonist. *Eur. J. Pharmacol.*, **124**, 207–208.
- PEDIGO, N.W., YAMAMURA, H.I. & NELSON, D.L. (1981). Discrimination of multiple [³H]-5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. *J. Neurochem.*, **36**, 220–226.
- VERGE, D., DAVAL, G., PATEY, A., GOZLAN, H., EL MESTIKAWAY, S. & HAMON, M. (1985). Presynaptic 5-HT autoreceptors on serotonergic cell bodies and/or dendrites but not terminals are of the 5-HT_{1A} subtype. *Eur. J. Pharmacol.*, **113**, 463–464.
- WOZNIACK, K.M., AULAKH, C.S., HILL, J.L. & MURPHY, D.L. (1988). The effect of 8-OH-DPAT on temperature in the rat and its modification by chronic antidepressant treatments. *Pharmacol. Biochem. Behav.*, **30**, 451–456.

(Received October 11, 1991

Revised April 21, 1992

Accepted April 22, 1992)