Direct evidence for an important species difference in the mechanism of 8-OH-DPAT-induced hypothermia

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1 Parallel series of experiments were carried out in the rat and mouse in order to investigate the mechanism(s) underlying the hypothermia induced in rodents by the selective 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT).

2 In the mouse, lesioning of central 5-hydroxytryptaminergic neurones (by use of the neurotoxin, 5,7-dihydroxytryptamine; 5,7-DHT) abolished the hypothermic response to 8-OH-DPAT, and depletion of brain 5-hydroxytryptamine (5-HT) levels (with the 5-HT synthesis inhibitor, *p*-chlorophenylalanine) markedly attenuated the response in this species. These pretreatments did not significantly attenuate 8-OH-DPAT-induced hypothermia in the rat, except for a significant attenuation of the response in 5,7-DHT-lesioned rats at the top dose of 8-OH-DPAT (1.0 mg kg⁻¹, s.c.).

3 Pharmacological pretreatments which facilitate 5-HT release (selective 5-HT uptake inhibitors, precursor (5-hydroxytryptophan) loading, or fenfluramine), markedly attenuated or abolished 8-OH-DPAT-induced hypothermia in the mouse. These pretreatments generally had no significant effect on 8-OH-DPAT-induced hypothermia in the rat.

4 The selective noradrenaline uptake inhibitor, desipramine, had no effect on the hypothermic response to 8-OH-DPAT in either species. The selective dopamine uptake inhibitor, nomifensin, significantly increased the hypothermic response to 8-OH-DPAT in the mouse, but did not affect the response in the rat except at high, motor stimulant doses, when the response was attenuated.

5 These data are consistent with the hypothesis that 8-OH-DPAT-induced hypothermia is mediated by presynaptic autoreceptors in the mouse and by postsynaptic 5-HT_{1A} receptors in the rat. Preliminary data also indicate an involvement of dopamine release in the mouse but not in the rat.

Keywords: 8-OH-DPAT-induced hypothermia; species difference (mouse, rat); presynaptic 5-HT_{1A} receptors; postsynaptic 5-HT_{1A} receptors

Introduction

The selective 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT; Hjorth *et al.*, 1982; Midlemis & Fozard, 1983) induces a centrally-mediated hypothermia in mice and rats (Goodwin *et al.*, 1985; Hjorth, 1985; Gudelsky *et al.*, 1986; Hutson *et al.*, 1987). However, the mechanism by which 8-OH-DPAT induces this effect on thermoregulation has not been elucidated. Central 5-HT_{1A} receptors are located both presynaptically (functioning as inhibitory somatodendritic autoreceptors on 5-hydroxytryptaminergic neurones in the raphe nuclei) and postsynaptically (Dourish *et al.*, 1986). As a first step in understanding the mechanism of 8-OH-DPAT-induced hypothermia, it is important to establish whether 8-OH-DPAT either facilitates transmission along the relevant 5-hydroxytryptamine (5-HT) pathway(s) (by stimulating postsynaptic 5-HT_{1A} receptors) or inhibits transmission by stimulating somatodendritic 5-HT_{1A} autoreceptors (leading to an attenuation of 5-HT release from axonal terminals). In this context, somatodendritic autoreceptors should be clearly distinguished from inhibitory terminal autoreceptors (mediating a local negative feedback on 5-HT release into the synapse) which are of the 5-HT_{1B} or 5-HT_{1D} subtype, according to species (Engel *et al.*, 1986; Schlicker *et al.*, 1989).

In the mouse, there is good evidence that the 5-HT_{1A} receptors mediating 8-OH-DPAT-induced hypothermia are presynaptic, since presynaptic 5-HT nerve terminal lesions (produced by the selective 5-HT neurotoxin, 5,7-dihydroxytryptamine; 5,7-DHT) or 5-HT depletion (following synthesis inhibition by *p*-chlorophenylalanine; pCPA) virtually abolished the hypothermic response to 8-OH-DPAT (Goodwin *et al.*, 1985). In the rat, there is some controversy as

to whether thermoregulatory 5-HT_{1A} receptors are presynaptic (Goodwin *et al.*, 1987) or postsynaptic (Hjorth, 1985; Hutson *et al.*, 1987). The latter authors found that 5-HT depletion or 5,7-DHT lesions, rather than attenuating 8-OH-DPAT-induced hypothermia, tended to enhance the response. These results indicate that the relevant 5-HT_{1A} receptors are located postsynaptically in the rat: the observed enhancement of 8-OH-DPAT-induced hypothermia would be consistent with the development of postsynaptic 5-HT_{1A} receptor supersensitivity following 5-HT depletion. However, Goodwin *et al.* (1987) found that PCPA-induced 5-HT depletion prevented 8-OH-DPAT-induced hypothermia in the rat, concluding that the 5-HT_{1A} receptors mediating this response are presynaptic.

In an attempt to resolve this controversy, we have carried out two parallel series of experiments in the mouse and rat, in which we examined the effects of facilitation or attenuation of 5-HT release on 8-OH-DPAT-induced hypothermia. In addition to the 5-HT depletion techniques (5,7-DHT; PCPA) used previously, we have examined the effects of facilitation of 5-HT release (using selective 5-HT reuptake inhibitors, precursor loading, and a 5-HT releasing agent) on 8-OH-DPAT-induced hypothermia in both species. Some of these data have been published in preliminary form (Bill & Fletcher, 1988; Bill *et al.*, 1989).

Methods

Animals

Female albino Tuck (T/O strain) mice weighing 20–27 g, and male Sprague-Dawley rats (150–250 g; Charles River) were maintained on a 12 h light:dark cycle (lights on at 08 h 00 min), and supplied with food (mice: CRM pellets; rats: 41B pellets, Labsure) and water *ad libitum*. The animals were transferred to the experimental laboratory between 11 h 00 min–

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12 h 00 min where they were housed at an ambient temperature of $20 \pm 0.5^\circ\text{C}$ in groups of eight (mice) or four (rats) for at least 2 h before body temperature measurement and drug administration. All experiments were performed between 13 h 00 min and 17 h 00 min.

8-OH-DPAT-induced hypothermia

Body temperatures were measured with a thermistor probe (connected to an electronic digital 2001 thermometer; Comark Electronics Limited, Sussex) inserted 2 cm into the rectum (mice) or 4 cm into the oesophagus (rat). Temperatures were measured immediately before the administration of test compounds, again immediately before 8-OH-DPAT administration, and at 15 and 30 min following 8-OH-DPAT administration. The hypothermic response to 8-OH-DPAT was measured as the maximum decrease in body temperature recorded in this latter period.

Behavioural observations

In addition to hypothermia, the overt behavioural response to 8-OH-DPAT (the 5-HT syndrome) was measured in control rats and in rats pretreated with 5,7-DHT. The intensity of the syndrome was measured 10 min after the s.c. administration of 8-OH-DPAT by assigning an arbitrary score for each component of the syndrome (see Tricklebank *et al.*, 1985), i.e. forepaw treading, headweaving, flat body posture and tremor. A score of 0–3 (corresponding to arbitrary ratings of 0 = absent, 1 = equivocal, 2 = present or 3 = severe) was assigned to each component, except tremor, which was scored as absent (0) or present (1). Therefore the maximum possible score was 10 per rat.

5,7-DHT lesions and PCPA-induced depletion

For 5,7-DHT lesioning, rats (weighing 280–320 g) were anaesthetized on day 1 with sodium pentobarbitone (Sagatal) (75 mg kg^{-1} , i.p.) and mounted in a stereotaxic frame. The skull was exposed and a hole drilled to allow a $10 \mu\text{l}$ Hamilton fixed needle syringe to be positioned for vehicle or 5,7-DHT solution ($150 \mu\text{g}$ in $2.5 \mu\text{l}$) to be infused into the third ventricle over a period of 1 min. The needle was left in position for a further minute. Coordinates from the stereotaxic brain atlas of Paxinos & Watson (1986) were used to position the tip of the needle on the midline, 2 mm posterior to the bregma, and 9.5 mm below the skull surface. On day 10, 8-OH-DPAT-induced hypothermia and syndrome were measured.

5,7-DHT ($50 \mu\text{g}$ per animal) was administered intracerebroventricularly (i.c.v.) to mice in a dose volume of $5 \mu\text{l}$ /mouse by the method of Brittain (1966). The site of injection was within 1 mm of a point on the midline 2 mm rostral to a line joining the anterior bases of the ear pinnae. Injections were made with a $25 \mu\text{l}$ Hamilton syringe attached to a 26 gauge/10 mm microlance hypodermic needle (Luer type) inserted perpendicularly through the skull, and penetrating the brain to a depth of 2.5 mm below the upper surface of the skull. 8-OH-DPAT-induced hypothermia was measured on day 5 following i.c.v. administration of 5,7-DHT.

PCPA (200 mg kg^{-1} , p.o.) or vehicle were administered to rats on day 1 and day 2. On day 5, 8-OH-DPAT-induced hypothermia was measured. The same dosing schedule was used for mice, except that each dose of PCPA was 750 mg kg^{-1} , p.o.

Biochemical measurements

The dosing schedules described above were used to determine the effects of 5,7-DHT lesioning and PCPA pretreatment on mouse and rat brain levels of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline (NA) and dopamine. These materials were assayed by high performance liquid chromatography (h.p.l.c.) with electrochemical detection by a modifi-

cation of the method of Wagner *et al.* (1982). Animals were decapitated and the brains rapidly removed. The cerebella were discarded and the remaining brains were weighed, frozen on dry ice and stored at -30°C until homogenization in 2.0 or 3.0 ml of 0.4 M perchloric acid containing 0.15% sodium metabisulphite, 0.05% disodium EDTA and 400 ng ml^{-1} of isoprenaline (internal standard). Tissue samples were then centrifuged at $20,000 g$ for 20 min at 4°C and the supernatants removed and stored at -20°C until analysis (no longer than 2 to 3 days). Aliquots (10 or $25 \mu\text{l}$) of the supernatants were analysed by an h.p.l.c./electrochemical detection system consisting of an LKB 2150 pump, refrigerated WISP710B automatic injector, ESA Coulochem 5100A electrochemical detector and Spectra-Physics SP4200 integrator (Waters Associates, Harrow).

Drugs

Drugs were administered in a volume of 10 ml kg^{-1} (mice) or 2.5 ml kg^{-1} (rats), routinely as mg kg^{-1} of salt. PCPA and 5,7-DHT doses are expressed as mg kg^{-1} or μg of active base. Drugs administered orally were dissolved or suspended in 0.3% hydroxypropylmethylcellulose (HMPC) in distilled water, whereas parenteral injections comprised solutions in isotonic saline except for carbidopa (sonicated in saline containing 0.1 mg ml^{-1} ascorbate and a trace of Tween 80). 5,7-DHT was dissolved in sterile isotonic saline containing 0.1 mg ml^{-1} ascorbic acid.

The drugs used (and their sources) were as follows: 8-OH-DPAT hydrobromide (Wyeth Research UK), fenfluramine hydrochloride (Sigma), 5-hydroxy-L-tryptophan base (Sigma), carbidopa base (Merck, Sharp and Dohme), fluvoxamine maleate (Duphar), citalopram hydrobromide (Lundbeck), panuramine hydrochloride (Wyeth Research UK), Wy 27587 maleate (N-[[[1-[(6-fluoro-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3-pyridine carboxamide (Wyeth Research UK)), PCPA ethyl ester hydrochloride (Sigma), 5,7-DHT creatinine sulphate (Sigma), desipramine hydrochloride (Ciba-Geigy), nomifensin hydrochloride (Wyeth Research UK).

Experimental design and statistical analysis

Animals were weighed and numbered before being allocated randomly to treatment groups, by use of random number tables (Fisher & Yates, 1957). Appropriate vehicle control groups were included in all experiments, which were performed with coded drug or vehicle solutions such that the operator was 'blind' to pretreatments. Body temperature data were analysed by the parametric one-way analysis of variance (ANOVA) before comparing pairs of treatment groups by Student's *t* test. In calculating *t* values and assessing their significance, the error mean square value and the error degrees of freedom from the ANOVA were used. Non-parametric syndrome data were analysed by the Mann-Whitney U-test. ED_{50} values (doses of 5-HT uptake inhibitors attenuating the hypothermic response to 8-OH-DPAT by 50%) and 95% confidence limits were calculated by regression analysis.

Results

8-OH-DPAT-induced hypothermia in the mouse and rat

Following subcutaneous administration, 8-OH-DPAT (0.03 – 3.0 mg kg^{-1}) dose-dependently reduced body temperature in the mouse and rat (Figure 1). In both species, maximal decreases in body temperature were recorded at either 15 or 30 min following 8-OH-DPAT administration (Figure 2a and b). In subsequent drug interaction studies, submaximal doses of 8-OH-DPAT were employed, i.e. 0.8 mg kg^{-1} s.c. for mice and 0.3 mg kg^{-1} s.c. for rats. Throughout the experiments described in this paper, the hypothermic responses to these

Figure 1 Dose-response relationships for 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)-induced hypothermia in the mouse (■) and rat (●). Animals ($n = 8$) were injected s.c. with 8-OH-DPAT (0.03–3.0 mg kg⁻¹) or saline. Body temperatures were measured immediately before, and at 15, 30 and 60 min post-drug administration. The hypothermic response to 8-OH-DPAT was measured as the maximum decrease in body temperature occurring in the 30 min period post-administration of 8-OH-DPAT. Open symbols show the mean change in body temperature after s.c. injection of saline in mice (□) and rats (○). Error bars denote $\pm$ s.e.mean. * $P < 0.05$; ** $P < 0.01$ (one-way ANOVA followed by Student's t test cf. the saline-treated control groups).

Figure 2 Time-dependent effects of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) on the body temperature of (a) mice and (b) rats. Animals ($n = 8$) were injected s.c. with either saline (○) or 0.03 (▲), 0.1 (■), 0.3 (●), 1.0 (◆) or 3.0 (▼) mg kg⁻¹ of 8-OH-DPAT. Body temperatures were measured immediately before, and at 15, 30 and 60 min post-administration of 8-OH-DPAT. Maximal decreases in body temperature were recorded either 15 or 30 min post-drug injection. Error bars denote $\pm$ s.e.mean.

doses ranged from -2.0 ± 0.3 to $-3.0 \pm 0.4^\circ\text{C}$ for mice, and from -1.6 ± 0.3 to $-2.3 \pm 0.3^\circ\text{C}$ for rats (mean values $\pm$ s.e.mean; $n = 8-10$).

Effects of selective 5-HT and catecholamine uptake inhibitors on 8-OH-DPAT-induced hypothermia

In the mouse, the selective 5-HT uptake inhibitors citalopram, fluvoxamine, panuramine (Blurton *et al.*, 1984) and Wy 27587 (Fletcher *et al.*, 1988; Billingsley *et al.*, 1989), administered orally 60 min before 8-OH-DPAT, dose-dependently attenuated 8-OH-DPAT-induced hypothermia (Figure 3). The ED₅₀ values (doses required to attenuate hypothermia by 50%) for citalopram, fluvoxamine, panuramine and Wy 27587 were 2.9 (2.3–3.6), 8.0 (5.5–11.6), 8.7 (5.0–15.2) and 1.7 (1.2–2.4) mg kg⁻¹, orally, respectively. Figures in parentheses are 95% confidence limits determined by linear regression analysis. The top doses of citalopram and fluvoxamine (30 mg kg⁻¹) completely abolished hypothermia, whereas panuramine induced a maximum attenuation of 62% ($P < 0.001$) at 15 mg kg⁻¹, and Wy 27587 a 63% attenuation ($P < 0.001$) at 2.0 mg kg⁻¹. The 5-HT uptake inhibitors did not modify body temperatures significantly in their own right. In marked contrast to the mouse data, the 5-HT uptake inhibitors had no significant effect on 8-OH-DPAT-induced hypothermia in the rat up to doses (mg kg⁻¹) of 50 (fluvoxamine), 100 (panuramine) or 10 (Wy 27587). The selective noradrenaline uptake inhibitor, desipramine (up to a dose of 30 mg kg⁻¹), had no significant effect on 8-OH-DPAT-induced hypothermia in either species. The selective dopamine uptake inhibitor, nomifensin, slightly (by 33%) and significantly ($P < 0.01$) attenuated hypothermia at the top dose of 30 mg kg⁻¹, orally in the rat (Table 1B). In contrast, in the mouse, nomifensin (3 mg kg⁻¹, p.o.) significantly ($P < 0.05$) enhanced (by 52%) 8-OH-DPAT-induced hypothermia (Table 1A).

Effects of fenfluramine and 5-HTP/carbidopa on 8-OH-DPAT-induced hypothermia

In the mouse, fenfluramine (20 mg kg⁻¹ i.p., administered 20 min before 8-OH-DPAT) markedly attenuated 8-OH-DPAT-induced hypothermia by 76% ($P < 0.001$). In this species, carbidopa (25 mg kg⁻¹, i.p., 60 min before 5-HTP) plus 5-HTP (50 mg kg⁻¹, s.c., 30 min before 8-OH-DPAT) virtually abolished the hypothermia induced by 8-OH-DPAT (93% attenuation; $P < 0.001$). These data are displayed in Figure 4.

Carbidopa/5-HTP (25 mg kg⁻¹, i.p./12.5–50 mg kg⁻¹, s.c.) had no significant effect on 8-OH-DPAT-induced hypothermia in the rat whereas fenfluramine, at the top dose of 5 mg kg⁻¹, i.p., slightly attenuated (by 26%; $P < 0.05$) the hypothermia (see Table 2). This dose of fenfluramine alone, however, significantly ($P < 0.01$) reduced body temperature prior to 8-OH-DPAT administration (Table 2).

Effects of lesioning and 5-HT depletion on 8-OH-DPAT-induced hypothermia and syndrome

By day 5 after i.c.v. administration to mice, 5,7-DHT had produced a 61% decrease in brain 5-HT content, and a 56% decrease in 5-HIAA levels (Table 3A). This pretreatment totally abolished the hypothermic response to 8-OH-DPAT (0.1–1.0 mg kg⁻¹, s.c.; Figure 5a). PCPA administration to mice reduced brain 5-HT levels by 54% and 5-HIAA levels by 65% (Table 3A). This pretreatment attenuated 8-OH-DPAT-induced hypothermia; an effect which was significant ($P < 0.05$) against 0.3 mg kg⁻¹ of 8-OH-DPAT (Figure 6a).

5,7-DHT lesioning in the rat reduced brain 5-HT levels by 70% and 5-HIAA levels by 83% (Table 3B). This pretreatment attenuated 8-OH-DPAT-induced hypothermia only at the top dose of 8-OH-DPAT (1.0 mg kg⁻¹ by 52%; $P < 0.01$) and had no effect on the hypothermic responses to lower doses (Figure 5b). 5,7-DHT-lesioned rats were irritable, hyperreactive and aggressive, and had a slightly (non-significant) higher initial

Figure 3 Effects of selective 5-hydroxytryptamine (5-HT) uptake inhibitors on 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT)-induced hypothermia in the mouse. (a) Fluvoxamine, (b) citalopram, (c) Wy 27587 and (d) panuramine were administered orally 60 min before the s.c. injection of 8-OH-DPAT (0.8 mg kg⁻¹) or saline. Body temperatures were measured immediately before each drug injection, and at 15 and 30 min post-injection of 8-OH-DPAT. Data shown are the mean maximum decreases in body temperature occurring in this latter period. Error bars denote $\pm$ s.e.mean ($n = 8$). Open columns show hypothermic response to 8-OH-DPAT in animals pretreated with vehicle. Stippled columns show hypothermic responses to 8-OH-DPAT in animals pretreated with 5-HT uptake inhibitors. The 5-HT uptake inhibitors alone and the s.c. injection of saline had no significant effect on body temperature. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; (one-way ANOVA followed by Student's unpaired t test cf. vehicle/8-OH-DPAT-treated group).

body temperature than controls. The 8-OH-DPAT behavioural syndrome was significantly ($P < 0.05$) enhanced in 5,7-DHT-lesioned rats (Figure 7). PCPA pretreatment in rats reduced brain levels of 5-HT and 5-HIAA by 90% and 80%

Figure 4 Effects of (a) fenfluramine and (b) carbidopa/L-5-hydroxytryptophan (L-5-HTP) on 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)-induced hypothermia in the mouse. Fenfluramine (5–20 mg kg⁻¹) or vehicle were administered i.p. 20 min prior to the s.c. injection of 8-OH-DPAT (0.8 mg kg⁻¹) or saline. Carbidopa (25 mg kg⁻¹, i.p.) was administered 60 min before the s.c. injection of L-5-HTP (25–100 mg kg⁻¹). 8-OH-DPAT (0.8 mg kg⁻¹) or saline were injected s.c. a further 30 min after L-5-HTP. Body temperatures were recorded immediately before each drug injection, and at 15 and 30 min after injection of 8-OH-DPAT. Data shown are the mean maximum decreases in body temperature occurring after injection of 8-OH-DPAT. Error bars denote $\pm$ s.e.mean ($n = 8$). Open columns show hypothermic response to 8-OH-DPAT in animals pretreated with vehicle. Stippled columns show hypothermic responses to 8-OH-DPAT in animals pretreated with fenfluramine or L-5-HTP. *** $P < 0.001$ (one-way ANOVA followed by Student's unpaired t test cf. vehicle/8-OH-DPAT-treated group).

respectively (Table 3B), and significantly ($P < 0.05$) enhanced the hypothermic responses to 0.125 and 0.5 mg kg⁻¹ of 8-OH-DPAT (Figure 6b).

Discussion

The results of our experiments on 8-OH-DPAT-induced hypothermia in the mouse are clearly defined, in that both prior depletion of neuronal 5-HT and lesioning of presynaptic function (with PCPA or 5,7-DHT respectively) markedly attenuated the hypothermic response. These observations are consistent with previous work (Goodwin *et al.*, 1985) which indicated that the 5-HT_{1A} receptors mediating 8-OH-DPAT-induced hypothermia in the mouse are located presynaptically on 5-HT neurones. Our finding that agents which facilitate 5-HT release acutely, also attenuated 8-OH-DPAT-induced hypothermia in the mouse, suggests that an autoreceptor-mediated inhibition of 5-HT release, on to an (as yet) unidentified postsynaptic 5-HT receptor, is primarily responsible for this hypothermic response. However, in the rat, the pharmacological profile of 8-OH-DPAT-induced hypothermia differs markedly from that in the mouse. In the rat, selective 5-HT uptake inhibitors had no significant effect, even at doses known to inhibit 5-HT re-uptake maximally in this species (our own unpublished data; see also Fletcher *et al.*, 1988). Similarly, the administration of 5-HTP, following peripheral decarboxylase inhibition, had no significant effect on the hypothermic response in rats, but virtually abolished the response to 8-OH-DPAT in the mouse. Also the 5-HT releasing agent, fenfluramine, markedly inhibited hypothermia in the mouse, but only slightly attenuated the response to 8-OH-DPAT in the rat, at a dose which itself reduced body temperature significantly prior to 8-OH-DPAT administration. It is feasible that this modest (26%) apparent attenu-

Table 1 Effect of the dopamine uptake inhibitor, nomifensin, on 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)-induced hypothermia in (A) mouse and (B) rat

	Treatment 1 (mg kg ⁻¹ , p.o.)	Treatment 2 (mg kg ⁻¹ , s.c.)	Δt (°C) (0–30 min post— 8-OH-DPAT)	% change
A Mouse	Vehicle	Saline	+0.2 ± 0.1	—
	Vehicle	8-OH-DPAT (0.8)	-2.0 ± 0.3	—
	Nomifensin (0.3)	8-OH-DPAT (0.8)	-1.9 ± 0.4	-5
	Nomifensin (1.0)	8-OH-DPAT (0.8)	-2.3 ± 0.3	+13
	Nomifensin (3.0)	8-OH-DPAT (0.8)	-3.1* ± 0.3	+52
	Nomifensin (10.0)	8-OH-DPAT (0.8)	-2.8* ± 0.2	+40
	Nomifensin (10.0)	Saline	-0.1 ± 0.1	—
B Rat	Vehicle	Saline	+0.5 ± 0.1	—
	Vehicle	8-OH-DPAT (0.3)	-1.6 ± 0.2	—
	Nomifensin (3.0)	8-OH-DPAT (0.3)	-1.8 ± 0.2	+8
	Nomifensin (10.0)	8-OH-DPAT (0.3)	-1.4 ± 0.1	-8
	Nomifensin (30.0)	8-OH-DPAT (0.3)	-0.8** ± 0.1	-33
	Nomifensin (30.0)	Saline	+0.3 ± 0.1	—

Mice or rats ($n = 8$) were treated orally with nomifensin or vehicle 60 min prior to the s.c. injection of 8-OH-DPAT (0.8 mg kg⁻¹ for mice; 0.3 mg kg⁻¹ for rats) or saline. Body temperatures were recorded immediately before each drug treatment, and at 15 and 30 min post-injection of 8-OH-DPAT or saline.

Δt shows the decrease in body temperature (mean ± s.e.mean) occurring in this latter period. Nomifensin alone had no significant effect on the body temperature of mice or rats prior to injection of 8-OH-DPAT or saline. Nomifensin potentiated 8-OH-DPAT-induced hypothermia in the mouse, but at a high dose attenuated it in the rat. * $P < 0.05$, ** $P < 0.01$ (one-way ANOVA followed by Student's unpaired t test cf. vehicle/8-OH-DPAT-treated groups).

Table 2 Effect of (A) fenfluramine, or (B) carbidopa/L-5-hydroxytryptophan (L-5-HTP) on 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)-induced hypothermia in the rat

	Treatment 1 (mg kg ⁻¹)	Treatment 2 (mg kg ⁻¹ s.c.)	Δt (°C) (0–30 min post— 8-OH-DPAT)
A	Vehicle (i.p.)	Saline	+0.1 ± 0.1
	Vehicle (i.p.)	8-OH-DPAT (0.3)	-1.8 ± 0.2
	Fenfluramine (1.25 i.p.)	8-OH-DPAT (0.3)	-1.7 ± 0.1
	Fenfluramine (2.50 i.p.)	8-OH-DPAT (0.3)	-2.0 ± 0.2
	Fenfluramine (5.00 i.p.)	8-OH-DPAT (0.3)	-1.3 ± 0.1*
	Fenfluramine (5.00 i.p.)	Saline	0.0 ± 0.1
B	Vehicle (s.c.)	Saline	0.0 ± 0.1
	Vehicle (s.c.)	8-OH-DPAT (0.3)	-1.6 ± 0.3
	L-5-HTP (12.5 s.c.)	8-OH-DPAT (0.3)	-1.6 ± 0.3
	L-5-HTP (25.0 s.c.)	8-OH-DPAT (0.3)	-1.4 ± 0.2
	L-5-HTP (50.0 s.c.)	8-OH-DPAT (0.3)	-2.0 ± 0.3
	L-5-HTP (50.0 s.c.)	Saline	0.0 ± 0.1

(A) Fenfluramine (1.25–5.0 mg kg⁻¹) or vehicle were administered i.p. to rats ($n = 8$) 20 min before the s.c. injection of 8-OH-DPAT (0.3 mg kg⁻¹) or saline. Administration of fenfluramine alone (5.0 mg kg⁻¹, i.p.) significantly ($P < 0.01$) reduced body temperature prior to 8-OH-DPAT administration (i.e. changes in body temperatures 20 min following treatment 1 were: vehicle + 0.2 ± 0.1; fenfluramine (5) -0.8 ± 0.1).

(B) Carbidopa (25 mg kg⁻¹, i.p.) was administered to rats 60 min before the s.c. injection of L-5-HTP (12.5–50 mg kg⁻¹) or saline. 8-OH-DPAT (0.3 mg kg⁻¹) or saline were injected s.c. a further 30 min later. Body temperatures were recorded immediately before each drug treatment, and at 15 and 30 min post-injection of 8-OH-DPAT or saline. Δt shows the change in body temperature (mean ± s.e.mean; $n = 8$) occurring in this latter period.

* $P < 0.05$ (one-way ANOVA followed by Student's unpaired t test cf. vehicle/8-OH-DPAT treated group).

Table 3 Effects of pretreatment with 5,7-dihydroxytryptamine (5,7-DHT) or *p*-chlorophenylalanine (PCPA) on 5-hydroxytryptamine (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) levels in (A) the mouse and (B) the rat

		Brain monoamine and metabolite levels (ng g ⁻¹ wet weight)			
	Treatments	5-HT	5-HIAA	NA	Dopamine
A Mouse	Saline (5 μ l/mouse i.c.v.)	506 ± 15	259 ± 13	290 ± 27	715 ± 45
	5,7-DHT (50 μ g/mouse i.c.v.)	199 ± 23*** (-61%)	114 ± 14*** (-56%)	255 ± 42 (-16%)	733 ± 39 (+3%)
	Vehicle (10 ml kg ⁻¹ p.o. × 2)	401 ± 24	239 ± 20	249 ± 10	797 ± 25
	PCPA (750 mg kg ⁻¹ p.o. × 2)	191 ± 36*** (-54%)	85 ± 25*** (-65%)	239 ± 10 (-4%)	713 ± 35 (-11%)
	Saline (2.5 μ l/rat i.t.v.)	491 ± 25	224 ± 11	392 ± 16	956 ± 21
B Rat	5,7-DHT (150 μ g/rat i.t.v.)	125 ± 19*** (-75%)	49 ± 8*** (-78%)	275 ± 16** (-30%)	954 ± 17 (0%)
	Vehicle (2.5 ml kg ⁻¹ p.o. × 2)	407 ± 15	241 ± 7	366 ± 15	769 ± 18
	PCPA (200 mg kg ⁻¹ p.o. × 2)	36 ± 4*** (-91%)	20 ± 1*** (-92%)	247 ± 10 (-5%)	730 ± 26 (-5%)

Mice were injected i.c.v. with ascorbate/saline (5 μ l) or 5,7-DHT (50 μ g base) 5 days before determination of whole brain (minus cerebellum) monoamine levels. Rats were injected i.t.v. (intra-third ventricle) with ascorbate/saline (2.5 μ l) or 5,7-DHT (150 μ g base) 10 days before determination of brain monoamine levels. PCPA or vehicle were administered p.o. to both mice and rats on days 1 and 2, with brain monoamine levels determined by h.p.l.c. with electrochemical detection on day 5. Values are mean ± s.e.mean levels from 6–8 animals. ** $P < 0.01$; *** $P < 0.001$ (Student's unpaired t test cf. saline or vehicle-pretreated control groups).

Figure 5 Effects of 5,7-dihydroxytryptamine (5,7-DHT) lesions on 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT)-induced hypothermia in (a) the mouse and (b) the rat. Mice were injected i.c.v. with 5 μ l of ascorbate/saline (●) or 50 μ g base of 5,7-DHT (■) 5 days before the s.c. administration of 8-OH-DPAT (0.1–1.0 mg kg⁻¹) or saline. Rats were injected into the third ventricle (i.t.v.) with 2.5 μ l of ascorbate/saline (●) or 150 μ g base of 5,7-DHT (■) 10 days before the s.c. administration of 8-OH-DPAT or saline. Body temperatures were measured immediately before, and at 15 and 30 min after 8-OH-DPAT. The hypothermic response to 8-OH-DPAT was measured as the maximum decrease in body temperature occurring in this latter period. Open symbols show changes in body temperature after s.c. injection of saline in animals pretreated with ascorbate/saline (○) or 5,7-DHT (□). Data points are mean $\pm$ s.e.mean ($n = 8$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, (ANOVA followed by Student's unpaired t test cf. ascorbate/saline pretreated groups).

ation in the rat could result from stimulation of postsynaptic 5-HT_{1A} receptors following fenfluramine-induced 5-HT release, which alone might induce a slight hypothermia prior to administration of 8-OH-DPAT. Fenfluramine alone, had no significant effects on body temperature in the mouse. The present experiments were performed at a near normal ambient temperature of 20°C. In rats housed at ambient temperatures of 28°C or greater, fenfluramine elicits hyperthermia, an effect thought to be mediated via 5-HT release onto postsynaptic 5-HT₂ receptors (Blackburn *et al.*, 1985; 1990). However, previous work also indicates that fenfluramine has little effect on or actually decreases the body temperature of rats maintained at ambient temperatures of 20 or 23°C (Sulpizio *et al.*, 1978; Preston *et al.*, 1990). The different dose-ranges of fenfluramine used for rats or mice in the present study reflect the fact that these two species appear to differ markedly in their sensitivity to the 5-HT-releasing action of this drug. For example, we, and many other workers, find that fenfluramine exerts a marked anorectic action in the rat over the dose-range employed in this study (Blundell & Latham, 1978; Garattini *et al.*, 1978), whereas higher doses are required to inhibit food consumption in the mouse (our unpublished data).

Figure 6 Effect of *p*-chlorophenylalanine (PCPA) pretreatment on 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)-induced hypothermia in (a) the mouse and (b) the rat. PCPA (■) (750 mg kg⁻¹ for mice; 200 mg kg⁻¹ for rats) or vehicle (●) were administered orally on days 1 and 2. On day 5 groups of 8 animals were dosed s.c. with saline or 8-OH-DPAT (0.1–1.0 mg kg⁻¹ for mice; 0.125–0.5 mg kg⁻¹ for rats) or saline. Body temperatures were measured immediately before, and at 15 and 30 min post-injection of 8-OH-DPAT. The hypothermic response to 8-OH-DPAT was measured as the maximum decrease in body temperature occurring in this latter period. Open symbols show changes in body temperature after s.c. injection of saline in animals pretreated with vehicle (○) or PCPA (□). Data points are mean $\pm$ s.e.mean ($n = 8$). * $P < 0.05$ (ANOVA followed by Student's unpaired t test).

A 90% depletion of brain 5-HT levels in the rat, by prior administration of PCPA, did not attenuate 8-OH-DPAT-induced hypothermia in the rat. On the contrary, PCPA pretreatment significantly increased the hypothermic response to the lowest and highest doses of 8-OH-DPAT, although the response to the intermediate dose was unchanged. Lesioning of 5-HT neurones with 5,7-DHT had no effect on the hypothermic responses to the lower doses of 8-OH-DPAT, but significantly attenuated the response to the top dose (1.0 mg kg⁻¹). However, it should be noted that 5,7-DHT pretreatment also significantly increased the behavioural stimulation (5-HT syndrome) induced by 8-OH-DPAT at a dose of 0.3 mg kg⁻¹ (Figure 7). Therefore, enhanced motor responses to higher doses of 8-OH-DPAT in lesioned animals may raise body temperature non-specifically, thereby attenuating hypothermia. The stereotypy induced by the top dose of 8-OH-DPAT was not increased significantly in lesioned animals. This may reflect the fact that the behavioural syndrome response was near maximal in sham-operated control rats at this dose. 8-OH-DPAT-induced motor stimulation *per se* was not measured in these experiments, but may have been enhanced in lesioned animals. This point should be investigated in subsequent experiments.

A possible explanation for the discrepancy between our data and those of Goodwin *et al.* (1987) could be related to

Figure 7 Effects of 5,7-dihydroxytryptamine (5,7-DHT) lesioning on the 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)-induced behavioural syndrome in the rat. Rats were sham-treated with ascorbate/saline (2.5 µl) (open columns) or treated with 5,7-DHT (150 µg base) (hatched columns) injected into the 3rd ventricle (i.t.v.) by stereotaxic techniques. On day 10 post-lesioning, animals were injected s.c. with saline or 8-OH-DPAT (0.1–1.0 mg kg⁻¹); 10 min later the severity of the behavioural syndrome induced by 8-OH-DPAT was assessed on a scale 0–10 for each rat. Syndrome scores for each treatment group (n = 6) were summed (max. possible score = 60). * $P < 0.05$ (Mann-Whitney U-test cf. sham-operated animals).

the fact that, as these authors reported, the dose-response curve for 8-OH-DPAT-induced hypothermia in the rat is biphasic. This appears to be a result of the general motor stimulation and behavioural syndrome which occurs at higher doses of 8-OH-DPAT. Since Goodwin *et al.* (1987) used a high challenge dose of 8-OH-DPAT (0.75 mg kg⁻¹, s.c.) relative to the dose inducing a maximum hypothermic response (0.25 mg kg⁻¹), it is feasible that an enhanced postsynaptic receptor sensitivity induced by 5-HT depletion resulted in a more severe syndrome, thereby shifting the hypothermic response further along the down-slope of the dose-response curve. An apparent attenuation of 8-OH-DPAT-induced hypothermia would be observed under these circumstances.

The selective noradrenaline uptake inhibitor, desipramine, had no effect on 8-OH-DPAT-induced hypothermia in either

species, suggesting that the release of noradrenaline is not involved in this response. However, the selective dopamine re-uptake inhibitor, nomifensin, significantly enhanced the hypothermic response to 8-OH-DPAT in the mouse, possibly suggesting that dopamine release is involved secondarily in this response. Such an involvement is also consistent with the original observation of Goodwin *et al.* (1985), that the dopamine antagonist, haloperidol, attenuated 8-OH-DPAT-induced hypothermia in the mouse. We have found that other dopamine antagonists (e.g. fluphenazine and sulpiride) also inhibit 8-OH-DPAT-induced hypothermia in the mouse (unpublished observations). However, the lack of activity of flupenthixol (Goodwin *et al.*, 1985; and our own unpublished observations) in this model is an anomalous observation which is not consistent with an involvement of dopamine release in the expression of 8-OH-DPAT-induced hypothermia. In the rat, haloperidol did not significantly modify the hypothermic response to 8-OH-DPAT (unpublished observation), whereas nomifensin attenuated it slightly at high doses. This latter effect may have been due to a motor stimulant action of nomifensin, inducing a slight rise in body temperature which non-specifically attenuated the hypothermia. This suggestion is supported by the results of our own experiments demonstrating significant increases in open field activity of rats at these doses of nomifensin (unpublished data). It appears, therefore, that the mechanism underlying 8-OH-DPAT-induced hypothermia in the mouse and the rat, may also differ in terms of dopamine involvement.

In conclusion, our data indicate a fundamental difference in the mechanisms by which 8-OH-DPAT induces hypothermia in the mouse and rat. All data are consistent with a presynaptic 5-HT_{1A} receptor agonist action in the mouse, leading to an attenuation of 5-HT release whereas the 5-HT_{1A} receptor mediating hypothermia in the rat appears to be located postsynaptically with respect to 5-HT neurones.

Other differences are also apparent, in that there is no clear evidence for an involvement of dopamine release in the rat, whereas, in the mouse, opposing effects of a dopamine uptake inhibitor and several dopamine D₂ receptor antagonists, may indicate an involvement of dopamine in the mechanism of 8-OH-DPAT-induced hypothermia in this species.

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References

- BILL, D.J. & FLETCHER, A. (1988). The attenuation of 8-OH-DPAT-induced hypothermia in the mouse by selective inhibitors of 5-HT uptake. *Br. J. Pharmacol.*, **93**, 198P.
- BILL, D.J., KNIGHT, M., SUTHERLAND, H., FORSTER, E.A. & FLETCHER, A. (1989). Evidence for an important species difference in the mechanism of 8-OH-DPAT-induced hypothermia. *Br. J. Pharmacol.*, **98**, 920P.
- BILLINGSLEY, A.J., BRAMMER, N.T., WOOD, M.D. & MINCHIN, M.C.W. (1989). A comparison of the effects of the selective 5-HT uptake inhibitor Wy 27587 on 5-HT uptake into platelets and synaptosomes in the rat. *Neuropharmacology*, **28**, 33–36.
- BLACKBURN, T.P., COX, B. & HEYWOOD, B. (1985). Fenfluramine-induced hypothermia – an *in vivo* model for the evaluation of drugs active at 5-HT₂ receptors. *Br. J. Pharmacol.*, **85**, 381P.
- BLACKBURN, T.P., COX, B., THORNER, C.W. & PEARCE, R.J. (1990). Pharmacological studies *in vivo* with ICI 169,369, a chemically novel 5-HT₂/5-HT_{1C} receptor antagonist. *Eur. J. Pharmacol.*, **180**, 229–237.
- BLUNDELL, J.E. & LATHAM, C.J. (1978). Pharmacological manipulation of feeding behaviour: possible influences of serotonin and dopamine on food intake. In *Central Mechanisms of Anorectic Drugs*, ed. Garattini, S. & Samanin, R. pp. 83–109. New York: Raven Press.
- BLURTON, P., BROADHURST, A.M., CROSS, J.A., ENNIS, C., WOOD, M.D. & WYLLIE, M.G. (1984). Panuramine, a selective inhibitor of uptake of 5-hydroxytryptamine in the brain of the rat. *Neuropharmacology*, **23**, 1049–1052.
- BRITAIN, R.T. (1966). The intracerebral effects of noradrenaline and its modification of drugs in the mouse. *J. Pharm. Pharmacol.*, **18**, 621–623.
- DOURISH, C.T., HUTSON, P.H. & CURZON, G. (1986). Parachlorophenylalanine prevents feeding induced by the serotonin agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). *Psychopharmacology*, **89**, 467–471.
- ENGEL, G., GOTHERT, M., HOYER, D., SCHLICKER, E. & HILLENBRAND, K. (1986). Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites. *Naunyn-Schmiedeberg Arch. Pharmacol.*, **332**, 1–7.
- FISHER, R.A. & YATES, F. (1957). *Statistical Tables for Biological, Agricultural and Medical Research*. Edinburgh: Oliver and Boyd Ltd.
- FLETCHER, A., FORSTER, E.A., BILL, D.J. & BILLINGSLEY, A.J. (1988). Wy 27587: a novel and highly selective inhibitor of 5-HT uptake *in vitro* and *in vivo*. *Br. J. Pharmacol.*, **94**, 384P.
- GARATTINI, S., BORRONI, E., MENNINI, T. & SAMANIN, R. (1978). Differences and similarities among anorectic agents. Pharmacological manipulation of feeding behaviour: possible influences of serotonin and dopamine on food intake. In *Central Mechanisms of Anorectic Drugs*, ed. Garattini, S. & Samanin, R. pp. 127–143. New York: Raven Press.

- GOODWIN, G.M., DE SOUZA, R.J. & GREEN, A.R. (1985). 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., HEAL, D.J. & GREEN, A.R. (1987). The pharmacology of the behavioural and hypothermic responses of rats to 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). *Psychopharmacology*, **91**, 506–511.
- GUDELSKY, G.A., KOENIG, J.I. & MELTZER, H.Y. (1986). Thermoregulatory responses to serotonin (5-HT) receptor stimulation in the rat. *Neuropharmacology*, **25**, 1307–1313.
- 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., LINDBERG, P., SANCHEZ, D., WIKSTROM, H., ARVIDSSON, L.-E., HACKSELL, U. & NILSSON, J.L.G. (1982). 8-Hydroxy-2-(di-n-propylamino)tetralin, 8-OH-DPAT, a potent and selective simplified ergot congener with central 5-HT-receptor stimulating activity. *J. Neural Transm.*, **55**, 169–188.
- 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.
- MIDDLEMISS, D.N. & FOZARD, J.R. (1983). 8-hydroxy-2-(di-n-propylamino)tetralin discriminates between subtypes of the 5-HT₁ recognition site. *Eur. J. Pharmacol.*, **90**, 151–153.
- PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in Stereotaxic Coordinates*. London: Academic Press Inc. Ltd.
- PRESTON, E., MA, S. & HAAS, N. (1990). Ambient temperature modulation of fenfluramine-induced thermogenesis in the rat. *Neuropharmacology*, **29**, 277–283.
- SCHLICKER, E., FINK, K., GOTHERT, M., HOYER, D., MOLDERINGS, G., ROSCHKE, I. & SCHOEFFTER, P. (1989). The pharmacological properties of the presynaptic serotonin autoreceptor in the pig brain cortex conform to the 5-HT_{1D} receptor subtype. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **340**, 45–51.
- SULPIZO, A., FOWLER, P.J. & MACKO, E. (1978). Antagonism of fenfluramine-induced hyperthermia: a measure of central serotonin inhibition. *Life Sci.*, **22**, 1439–1446.
- TRICKLEBANK, M.D., FORLER, C. & FOZARD, J.R. (1985). The involvement of subtypes of the 5-HT₁ receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-n-propylamino)tetralin in the rat. *Eur. J. Pharmacol.*, **106**, 271–282.
- WAGNER, J., VITALI, P., PALFREYMAN, M.G., ZRAIKA, M. & HUOT, S. (1982). Simultaneous determination of 3,4-dihydroxyphenylalanine, 5-hydroxytryptophan, dopamine 4-hydroxy-3-methoxyphenylalanine, norepinephrine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, serotonin and 5-hydroxyindolacetic acid in rat cerebrospinal fluid and brain by high-performance liquid chromatography with electrochemical detection. *J. Neurochem.*, **38**, 1241–1254.

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