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Characterization of D-fenfluramine-induced hypothermia: evidence for multiple sites of action

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Abstract

The effects of D-fenfluramine on core body temperature has been largely investigated under conditions of either high or low ambient temperature, whereas little research has focused on this response under normal environmental conditions. Moreover, there has been neglect in research on the mechanisms underlying changes in body temperature. In this study, we demonstrate that D-fenfluramine (5 and 10 mg/kg) induces a sustained decrease in body temperature in the rat under normal ambient temperatures. Pre-treatment with the selective serotonin reuptake inhibitor sertraline (5 mg/kg), the full 5-HT_{1A} receptor antagonist 4-fluoro-*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-2-pyridinyl benzamide], WAY 100635 (0.15 mg/kg) and the 5-HT_{2C} receptor antagonist benzofuran-2-carboxamidine, RO 43-0440 (2.5 mg/kg) blocked D-fenfluramine-induced hypothermia. Depletion of 5-hydroxytryptamine (5-HT) stores following treatment with the serotonergic neurotoxin parachlorophenylalanine reversed the initial hypothermic effects of D-fenfluramine but not the later effects, as D-fenfluramine produced a delayed hypothermia ($>$ 120 min post-challenge) in animals pre-treated with parachlorophenylalanine. Such findings are consistent with a requirement for D-fenfluramine uptake into 5-HT neurons followed by release of 5-HT from intracellular stores and stimulation of post-synaptic 5-HT receptors to reduce body temperature. The hypothermic response to D-fenfluramine was potentiated by ketanserin pre-treatment 30 min post-challenge but then antagonized at later time intervals. Pre-treatment with the dopamine, D_2 antagonist, haloperidol (1 mg/kg) and sulpiride (30 mg/kg) had a similar effect in blocking the hypothermia as WAY 100635, suggesting a role for dopamine D_2 receptors in the response. Pre-treatment with the α_2-adrenoceptor antagonist yohimbine failed to block the hypothermic response. These results suggest multiple sites of action mediating D-fenfluramine-induced hypothermia and may be the result of a combined effect of D-fenfluramine and its active metabolite norfenfluramine affecting not only the release of 5-HT but also stimulation of post-synaptic receptors.

Keywords: D-Fenfluramine; Hypothermia; 5-HT_{1A} receptor; Sertraline; WAY 100635; RO 43-0440; Ketanserin; Parachlorophenylalanine; Haloperidol; Sulpiride; Yohimbine

1. Introduction

D-Fenfluramine is the potent isomer of the substituted amphetamine fenfluramine, which stimulates 5-hydroxytryptamine (5-HT) release and increases extracellular 5-HT levels in many brain regions (Auerbach et al., 1989; Schwartz et al., 1989; Kreiss et al., 1993; Series et al., 1994). The D-isomer of fenfluramine is a more specific 5-HT probe, being free of the catecholamine effects of the racemic D,L-fenfluramine (Garattini et al., 1987). A plethora of 5-HT-mediated behavioural and physiological responses (including feeding and thermoregulation) have also been assessed using D-fenfluramine or its racemate (Rose et al., 1997; Baumann et al., 1998). It has been used extensively as a neuroendocrine probe of serotonergic function in both psychiatric patients and healthy individuals (O'Keane and Dinan, 1991; O'Keane et al., 1992; Feeney et al., 1993; Cleare et al., 1996).

Rodents that are exposed to D- or D,L-fenfluramine have been shown to have impaired thermoregulatory ability and alterations in core body temperature (Jespersen et al., 1969; Preston et al., 1990; Malberg and Seiden, 1997; Stewart et al., 1997). Under elevated environmental tem-

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perature conditions (> 25°C) D- and D,L-fenfluramine induce a hyperthermic response in the rat (Frey, 1975; Sulpizio et al., 1978; Sugrue, 1984) in a similar fashion as other substituted amphetamines, such as 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine. In contrast, under lower ambient temperature conditions (< 10°C) a distinct hypothermic response to fenfluramine has been observed (Malberg and Seiden, 1997). While many studies have focused on the effects of fenfluramine-induced temperature changes under extreme environmental conditions, there has been a relative neglect in the characterization of its effects on core body temperature under normal laboratory conditions. In addition, the receptor subtypes involved in mediating these temperature responses at any environmental temperature remain uncertain. Recently, it has been demonstrated that D- or D,L-fenfluramine, induces a reduction in core body temperature in both rats and mice, kept under normal laboratory temperature (20–24°C) (O'Callaghan and Miller, 1994; Miller and O'Callaghan, 1995; Malberg and Seiden, 1997). This is in contrast to the traditional view that D-, or D,L-fenfluramine evokes little change in core temperature under these conditions (Sugrue, 1984; Stewart et al., 1997).

Both noradrenergic and dopaminergic neurotransmitter systems have been implicated in the mediation of the hypothermic response in many species, with both α_2-adrenoceptors (Myers et al., 1987; Minor et al., 1989; Menon et al., 1990) and dopamine receptors (Nunes et al., 1991; Zarrindast and Tabataba, 1992; Millan et al., 1994; Parada et al., 1995) thought to play a major role. In addition, the relationship between the serotonergic system and hypothermic mechanism has long been associated (Lin et al., 1983, 1998; Won and Lin, 1988) with hypothermia as a consequence of 5-HT_{1A} receptor activation being the best characterized (see Millan et al., 1993; De Vry, 1995; Cryan et al., 1999a). Agonists at this receptor subtype such as 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), flesinoxan and ipsapirone show a dose-dependent hypothermic response following administration and this response has been used as an in vivo marker of 5-HT_{1A} receptor activation in rodents and man (Goodwin et al., 1985; Hjorth, 1985; Lesch et al., 1990; De Vry, 1995; Pitchot et al., 1995; Cryan et al., 1999b).

The aim of the present study was to characterize the effects of D-fenfluramine on core body temperature under normal laboratory conditions in rats. The contribution of the 5-HT reuptake site, 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors in conjunction with the role of intracellular 5-HT stores were assessed in the D-fenfluramine-induced response. In addition, we further investigated the selectivity of the responses by assessing the interaction of other ligands with affinity for other monoamine receptors implicated in altering core body temperature. The results suggest that D-fenfluramine-induced hypothermia is the result of D-fenfluramine uptake, induction of 5-HT release and stimulation of post-synaptically located receptors.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (300–350 g) were obtained from in-house breeding facilities, National University of Ireland, Galway. The animals were housed four per cage in standard hard bottom polypropylene cages (45 × 28 × 20 cm), containing wood shavings and with stainless steel lids. The animals had ad libitum access to food and water. The animals were maintained at a constant temperature (20 ± 1°C) and at standard lighting conditions (12:12 h light–dark, lights on from 0800 to 2000 h). All challenge studies were conducted between 0900 and 1530 h, this being the timeframe in which many temperature challenge studies have been successfully carried out in our laboratory (Cryan et al., 1997, 1998, 1999a,b; Harkin et al., 1999, 2000). The experimental protocol was carried out in accordance with the guidelines of the Animal Welfare Committee, National University of Ireland, Galway and was in compliance with the European Communities Council directive 1986.

2.2. Measurement of core body temperature

Core body temperatures were taken by inserting a digital rectal thermometer (Omron digital thermometer, MC-63B) 3 cm into the rectum. The rats were lightly restrained by hand during the procedure. A steady read-out of its temperature was obtained usually approximately 30 s after insertion of the probe.

2.3. Determination of biogenic amines in brain tissue by high performance liquid chromatography (HPLC)

Concentrations of 5-HT, noradrenaline, dopamine, dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) were measured by HPLC with electrochemical detection (Seyfried et al., 1986). The tissue was homogenized by sonication in 1 ml of mobile phase (pH 2.8) that was spiked with *N*-methyl-dopamine (Sigma, Poole, Dorset, UK) as an internal standard. The mobile phase contained 0.1 M citric acid, 0.1 M sodium dihydrogen phosphate, 1.4 mM octane-1-sulphonic acid, 0.1 mM ethylenediaminetetra-acetic acid and 9% v/v methanol. The pH of the mobile phase was adjusted to 2.8 with concentrated NaOH. The retention times of biogenic amines varied between 5 and 40 min on an LI Chrosorb RP-18 column. The flow rate of the mobile phase through the column was 1 ml/min at a pressure of approximately 200 bar. The column oven was maintained at 30°C. An electrochemical detector (Shimadzu) was coupled to the HPLC system and was set at a potential of +0.8 V for the detection of the amines. All standards were purchased from Sigma. The neurotransmitters were quantified using a Merck-Hitachi D-2000 integrator. Neurotransmitter con-

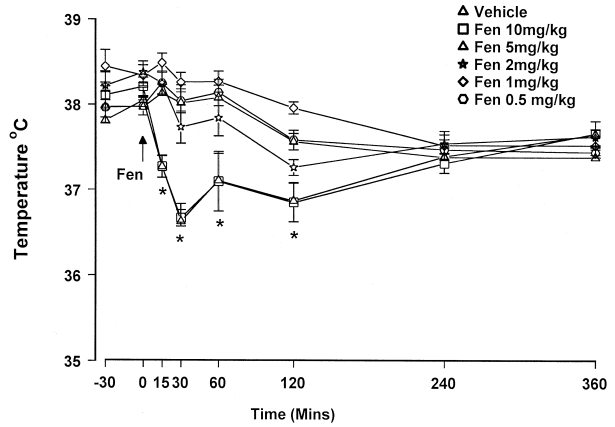

Fig. 1. The effects of D-fenfluramine on core body temperature. Values represent mean with standard error of 7 animals. $^{*}P < 0.01$ vs. control.

centrations were expressed as nanogram of neurotransmitter per gram fresh weight of brain tissue.

2.4. Drugs

D-Fenfluramine HCl (Sigma) and WAY 100635 maleate (Wyeth, Taplow, UK) were dissolved in 0.89% NaCl and administered in an injection volume of 1 ml/kg. Sulpiride (Sigma), haloperidol (Sigma), yohimbine HCl (Sigma), R0-43-0440 (Hoffmann-La Roche, Basle, Switzerland) and Ketanserin tartrate (Sigma) were all dissolved in 0.5% Tween saline and administered in an injection volume of 1 ml/kg. All drugs were administered subcutaneously (s.c.). PCPA methyl ester HCl (RBI, Natick, USA) was dissolved in 0.89% NaCl and administered i.p. in an injection volume of 1 ml/kg. Sertraline HCl was obtained from Pfizer UK. Sertraline was prepared in saline to a concentration of 2.5 mg/ml. This was administered at 2 ml/kg i.p., giving a dose of 5 mg/kg. Controls received injections of vehicle alone. All doses of drugs were calculated as salt.

2.5. D-Fenfluramine-induced hypothermia: dose–response study

Doses of 0.5, 1, 2, 5 and 10 mg/kg or vehicle was administered s.c. to the animals ($n = 7$) and their core body temperatures were taken 30 min and immediately prior to challenge. Temperatures were taken 15, 30, 60, 120, 240 and 360 min subsequent to the D-fenfluramine challenge.

2.6. The effect of sertraline on D-fenfluramine-induced hypothermia

Rats received sertraline (5 mg/kg i.p.) or vehicle and 2 h later were challenged with D-fenfluramine (5 mg/kg s.c.). The dose of sertraline selected was based on the ability of sertraline to block D-fenfluramine-induced depletion of 5-HT concentrations in rat cortex and hypothalamic tissue (data not shown). Rectal temperatures were taken before treatment with D-fenfluramine and 30, 90, and 210 min post-challenge.

2.7. The effects of parachlorophenylalanine treatment on D-fenfluramine-induced hypothermia

Parachlorophenylalanine (150 mg/kg, i.p.) or vehicle was injected once daily for 3 consecutive days. Seventy-two hours following the last injection, the animals were challenged with D-fenfluramine (5 mg/kg s.c.) or vehicle. The core body temperatures of each animal were taken 20 min and immediately prior to the D-fenfluramine challenge, and 30, 60, 120 and 240 min subsequent to challenge. Six hours following D-fenfluramine administration, the rats were killed by decapitation, and their brains rapidly removed and the left frontal cortex and hypothalamus dissected on a cold plate as described by Popov et al. (1967). Concentrations of noradrenaline, dopamine, di-hydroxyphenylacetic acid, 5-HT and 5-HIAA were measured by HPLC.

2.8. The effect of WAY 100635 on D-fenfluramine-induced hypothermia

Animals were pretreated with WAY 100635 [4-fluoro-*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-2-pyridinyl benzamide]or vehicle 20 min prior to a D-fenfluramine (5 mg/kg s.c.) challenge. A dose of 0.15 mg/kg s.c. was selected, as previous studies show that this dose is effective in reversing the hypothermic effect of the potent and selective 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (Cryan et al., 1999a). The core body temperatures of each animal were taken immediately prior to pre-treatment, immediately prior to the D-fenfluramine challenge and 15, 30, 60, 120 and 240 min subsequent to challenge.

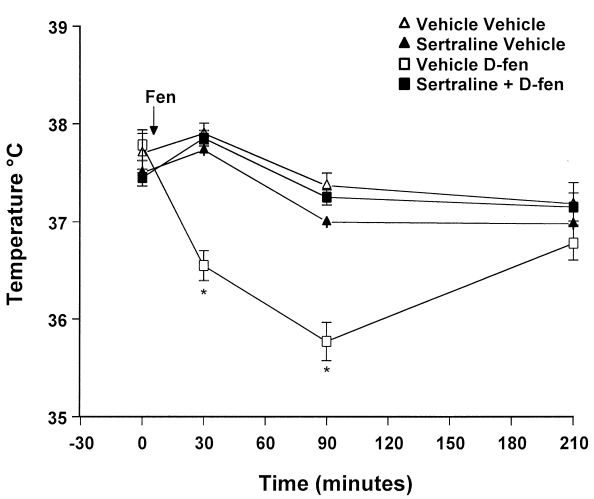

Fig. 2. The effects of pretreatment with sertraline (5 mg/kg) on D-fenfluramine (5 mg/kg)-induced hypothermia. Values represent mean with standard error of 6 animals. $^{*}P < 0.01$ vs. Veh Fen.

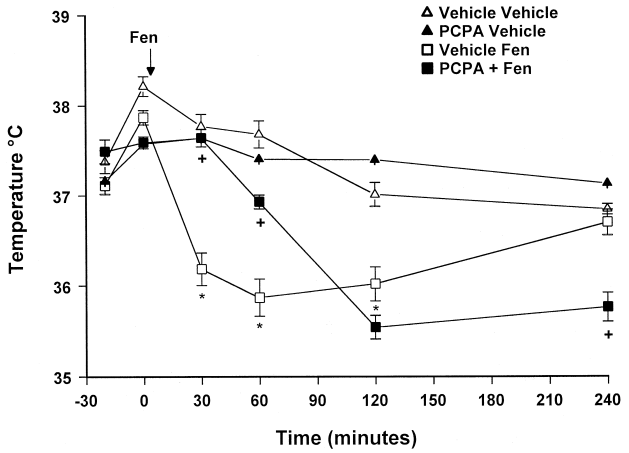

Fig. 3. The effect of pre-treatment with PCPA (150 mg/kg for 3 days) on D-fenfluramine (5 mg/kg)-induced hypothermia. Values represent mean with standard error of 5–6 animals. $^{*}P < 0.01$ vs. Veh. Veh; $+ P < 0.01$ vs. Veh Fen.

2.9. The effects of Ro 43-0440, haloperidol, sulpiride and yohimbine on D-fenfluramine-induced hypothermia

Animals were pre-treated with the benzofuryl-derivative, benzofuran-2-carboxamidine, R0 43-0440 (2.5 mg/kg, s.c.), haloperidol (1 mg/kg, s.c.), sulpiride (30 mg/kg, s.c.), yohimbine (2 mg/kg s.c.), or vehicle 20 min prior to the D-fenfluramine challenge. The dose of RO 43-0440 was selected based on our observations that pre-treatment with RO 43-0440 at doses not less than 2.5 mg/kg s.c. can block D-fenfluramine (5 mg/kg s.c.)-induced hypophagia (Harkin, A., unpublished observations), a response that has previously been reported to be mediated via stimulation of 5-HT_{2C} receptors (Gibson et al., 1993). RO 43-0440 has recently been shown to have a selectivity for 5-HT_{2C} ($pK_i = 7.1$) over 5-HT_{2A} ($pK_i = 5.3$) receptors (International application published under the Patent Cooperation Treaty, Benzofuryl derivatives and their use WO 97/42183). Doses of haloperidol and sulpiride were chosen based on the ability of these compounds to antagonize the hypothermic effects of the dopamine agonist, apomorphine (Nsimba, 1995). The dose of yohimbine was chosen based on its ability to antagonize the hypothermic response to the α_2-adrenoceptor agonist, clonidine (O'Donnell et al., 1996). The core body temperatures of each animal were taken immediately prior to pre-treatment, and immediately prior to the D-fenfluramine challenge and 30, 90 and 210 min subsequent to challenge.

2.10. The effects of ketanserin on D-fenfluramine-induced hypothermia

Rats received ketanserin s.c. or vehicle and 30 min later were challenged with D-fenfluramine (5 mg/kg s.c.). The time interval and dose of ketanserin were selected based on previous studies. Many experimenters have used ketanserin as a selective 5-HT_{2A} receptor antagonist and report that it retains selectivity for the 5-HT_{2A} receptor subtype (Hoyer, 1989; Kennett et al., 1994; Ninan and Kalkman, 1998). Rectal temperatures were taken before treatment and 30, 90, 220 and 350 min post-challenge.

2.11. Statistical analysis

Initially, a one-way analysis of variance (ANOVA) was performed in the dose–response study. All other data was analyzed using a two-way ANOVA where pre-treatment and challenge were the first and second factors. If any statistically significant changes were found in any of the studies, the data was further analyzed using post-hoc Fisher's least significant difference tests (LSD). All data were deemed significant at $P < 0.01$.

3. Results

3.1. D-Fenfluramine-induced hypothermia: dose–response

There was no difference in temperature between groups prior to the D-fenfluramine challenge. D-fenfluramine induced a significant effect on temperature 15 ($F(5,36) = 18.85$, $P < 0.0001$), 30 ($F(5,36) = 19.05$, $P < 0.0001$), 60 ($F(5,36) = 5.09$, $P < 0.0012$) and 120 min ($F(5,36) = 8.57$, $P < 0.0001$) following the challenge. There was no significant difference between the groups 240 and 360 min following challenge. Post-hoc analysis revealed that both 5 and 10 mg/kg doses significantly induced this change in

Table 1
Effects of pre-treatment with parachlorophenylalanine on the D-fenfluramine-induced changes to biogenic amines in the rat frontal cortex
Parachlorophenylalanine (150 mg/kg i.p.) was administered once daily for 3 days. 72 h later the animals were challenged with D-fenfluramine. Each value is the mean and standard error of 5–6 animals.

	NA	DA	DOPAC	5-HT	5-HIAA
Vehicle vehicle	355 ± 29	182 ± 77	56 ± 7	427 ± 38	217 ± 22
Parachlorophenylalanine vehicle	355 ± 41	298 ± 157	59 ± 20	20 ± 7[a]	0[a]
Vehicle D-fenfluramine	390 ± 31	306 ± 170	78 ± 27	208 ± 38[a]	166 ± 18[a]
Parachlorophenylalanine D-fenfluramine	275 ± 20	116 ± 34	46 ± 7	0	0

[a] $P < 0.01$ vs. Veh. Veh. NA: noradrenaline; DA: Dopamine; DOPAC: Dihydroxyphenylacetic acid; 5-HT: 5-hydroxytryptamine; 5-HIAA: 5-hydroxyindoleacetic acid.

Table 2
Effects of pre-treatment with parachlorophenylalanine on the D-fenfluramine-induced changes to biogenic amines in the rat hypothalamus
Parachlorophenylalanine (150 mg/kg i.p.) was administered once daily for 3 days. 72 h later the animals were challenged with D-fenfluramine. Each value is the mean and standard error of 5–6 animals.

	NA	DA	DOPAC	5-HT	5-HIAA
Vehicle vehicle	4064 ± 441	569 ± 45	nd	1210 ± 129	398 ± 40
Parachlorophenylalanine vehicle	3191 ± 368	362 ± 60	nd	79 ± 27[a]	8 ± 8[a]
Vehicle D-fenfluramine	2775 ± 209	468 ± 32	nd	748 ± 53[a]	234 ± 18[a]
Parachlorophenylalanine D-fenfluramine	250 ± 271	357 ± 81	nd	28 ± 18	12 ± 7

[a] $P < 0.01$ vs. Veh. Veh. nd = not detected. NA: noradrenaline; DA: Dopamine; DOPAC: Dihydroxyphenylacetic acid; 5-HT: 5-hydroxytryptamine; 5-HIAA: 5-hydroxyindoleacetic acid.

temperature at all time points up to and including 60 min post-challenge (see Fig. 1).

3.2. The effect of sertraline on D-fenfluramine-induced hypothermia

Following administration of sertraline there was no difference in temperature prior to D-fenfluramine administration. D-fenfluramine reduced core temperatures 30 [$F(1,20) = 26.47$, $P < 0.001$] and 90 min [$F(1,20) = 20.85$, $P < 0.001$] post-challenge ($P < 0.01$). This effect was blocked by pre-treatment with sertraline 30 [$F(1,20) = 37.44$, $P < 0.001$] and 90 min [$F(1,20) = 39.16$, $P < 0.001$] post-challenge ($P < 0.01$) (see Fig. 2).

3.3. The effects of parachlorophenylalanine treatment on D-fenfluramine-induced hypothermia

There was no difference in temperature between groups prior to D-fenfluramine challenge. There was an effect of pre-treatment 30 [$F(1,20) = 19.29$, $P < 0.001$], 60 [$F(1,20) = 7.36$, $P = 0.013$] and 240 min [$F(1,20) = 7.31$, $P = 0.014$] following D-fenfluramine challenge. There was also an effect of D-fenfluramine 30 [$F(1,20) = 27.58$, $P < 0.001$], 60 [$F(1,20) = 21.01$, $P < 0.001$], 120 [$F(1,20) = 111.29$, $P < 0.001$] and 240 min [$F(1,20) = 39.36$, $P < 0.001$] following challenge. There was an interaction between parachlorophenylalanine pre-treatment and D-fenfluramine challenge 30 [$F(1,20) = 27.35$, $P < 0.001$], 120 [$F(1,20) = 10.29$, $P = 0.004$] and 240 min [$F(1,20) = 25.80$, $P < 0.001$] following D-fenfluramine administration. Post-hoc comparisons revealed that D-fenfluramine reduced body temperature 30, 60 and 120 min post-challenge. Parachlorophenylalanine pre-treatment blocked this response 30 and 60 min following D-fenfluramine administration but potentiated the response to D-fenfluramine 240 min post-challenge (see Fig. 3).

3.4. The effect of pre-treatment with parachlorophenylalanine on 5-HIAA and 5-HT concentrations in rat cortex and hypothalamus

In the cortex, ANOVA of 5-HIAA concentrations showed an effect of pre-treatment (parachlorophenylalanine) [$F(1,20) = 181.15$, $P < 0.001$]. ANOVA of 5-HT concentrations showed an effect of pre-treatment [$F(1,20) = 127.27$, $P < 0.001$] and of D-fenfluramine [$F(1,20) = 19.21$, $P < 0.001$]. Post-hoc comparisons revealed that parachlorophenylalanine and D-fenfluramine reduced 5-HIAA and 5-HT concentrations in the cortex (Table 1). In the hypothalamus, ANOVA of 5-HIAA concentrations showed an effect of pre-treatment [$F(1,19) = 159.23$, $P < 0.001$] and of D-fenfluramine [$F(1,19) = 11.05$, $P = 0.004$]. ANOVA of 5-HT concentrations showed an effect of pre-treatment [$F(1,19) = 150.45$, $P < 0.001$] and D-fenfluramine [$F(1,19) = 11.58$, $P = 0.003$]. Post-hoc comparisons revealed that parachlorophenylalanine and D-fenfluramine reduced 5-HIAA and 5-HT concentrations in the hypothalamus (see Table 2).

3.5. The effect of WAY 100635 on D-fenfluramine-induced hypothermia

There was no difference in temperature between groups prior to challenges. There was a significant effect of pre-treatment with WAY 100635 15 [($F(1,10) = 19.08$, $P = 0.0014$)], 30 [($F(1,10) = 9.68$, $P = 0.011$], 60 [($F(1,10) = 16.47$, $P = 0.0023$)] and 120 min [($F(1,10) =$

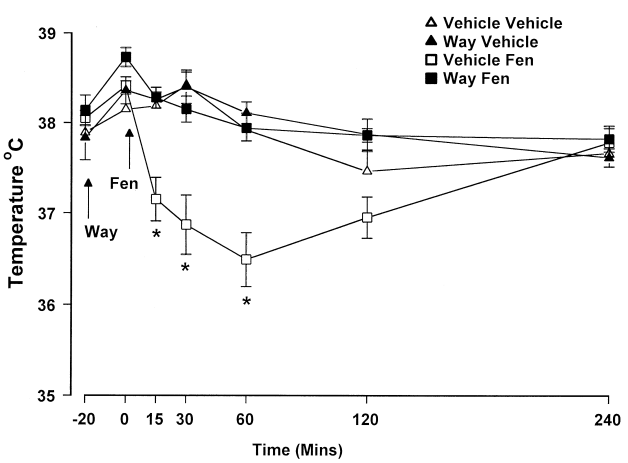

Fig. 4. The effects of pretreatment with WAY 100635 (0.15 mg/kg) on D-fenfluramine (5 mg/kg)-induced hypothermia. Values represent mean with standard error of 6 animals. *$P < 0.01$ vs. control (Fisher's LSD).

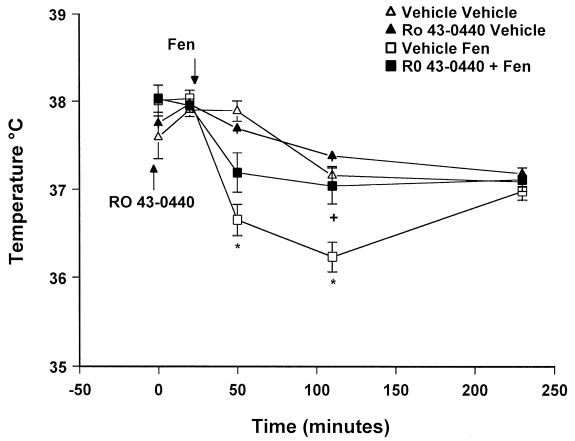

Fig. 5. The effect of RO 43-0440 (2.5 mg/kg) on D-fenfluramine (5 mg/kg)-induced hypothermia. Each value is the mean and standard error of 5–6 animals. $^{*}P < 0.01$ vs. Veh. Veh; $+ P <$ 0.01 vs. Veh Fen.

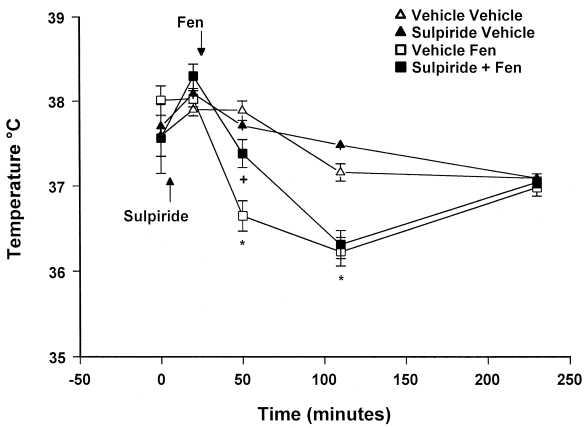

Fig. 7. Effects of pre-treatment with sulpiride (30 mg/kg) on D-fenfluramine (5 mg/kg)-induced hypothermia. Each value is the mean and standard error of 5–6 animals. $^{*}P < 0.01$ vs. Veh. Veh; $+ P < 0.01$ vs. Veh Fen.

11.92, $P = 0.0062$)] following D-fenfluramine challenge. There also was a significant effect of D-fenfluramine, 15 [($F(1,10) = 6.04$, $P = 0.034$)], 30 [($F(1,10) = 16.39$, $P =$ 0.0023] and 60 min [($F(1,10) = 17.27$, $P = 0.002$] following challenge. There was a significant interaction between pre-treatment with WAY 100635 and challenging with D-fenfluramine 15 [($F(1,10) = 6.47$, $P = 0.0291$)], 30 [($F(1,10) = 8.62$, $P = 0.0149$)] and 60 min [($F(1,10) =$ 10.67, $P = 0.0085$)] following the challenge. Post-hoc analysis revealed that the group pre-treated with vehicle and then challenged with D-fenfluramine showed a significant decrease in core body temperature 15, 30 and 60 min when compared to control group. Pre-treatment with WAY 100635 completely blocked this effect (see Fig. 4).

3.6. The effects of RO 43-0440, haloperidol, sulpiride and yohimbine on D-fenfluramine-induced hypothermia

There was no difference between the groups prior to R0 43-0440 or D-fenfluramine administration. There was an effect of RO 43-0440 90 min following D-fenfluramine administration [$F(1,20) = 10.97$, $P = 0.004$] and of D-fenfluramine 30 [$F(1,20) = 29.22$, $P < 0.001$] and 90 min [$F(1,20) = 16.57$, $P < 0.001$] post-challenge. There was an interaction between D-fenfluramine and R0 43-0440 pre-treatment 30 min post-D-fenfluramine [$F(1,20) = 5.30$, $P = 0.032$]. Post-hoc comparisons revealed that D-fenfluramine reduced body temperature 30 and 90 min following challenge. R0 43-0440 blocked this response 90 min following D-fenfluramine administration (see Fig. 5).

There was no difference between the groups prior to haloperidol or D-fenfluramine administration. There was an effect of pre-treatment 30 [$F(1,20) = 8.11$, $P = 0.01$] and 90 min [$F(1,20) = 11.17$, $P = 0.003$] post-challenge with D-fenfluramine and of D-fenfluramine 30 min post-challenge [$F(1,20) = 21.26$, $P < 0.001$]. There was an interaction between D-fenfluramine and haloperidol pre-treatment 90 min post-D-fenfluramine [$F(1,20) = 12.94$, $P = 0.002$].

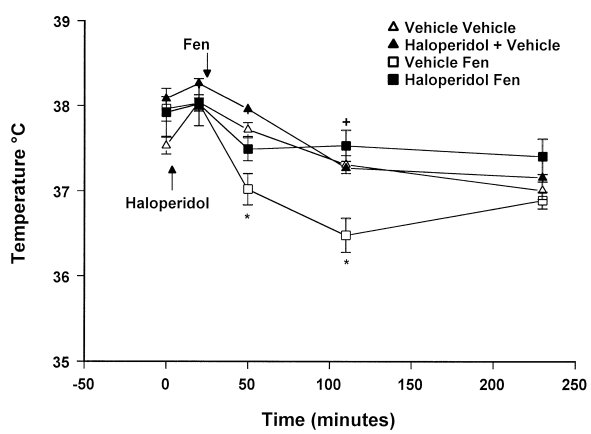

Fig. 6. Effects of pre-treatment with haloperidol (1 mg/kg) on D-fenfluramine (5 mg/kg)-induced hypothermia. Each value is the mean and standard error of 5–6 animals. $^{*}P < 0.01$ vs. Veh. Veh; $+ P < 0.01$ vs. Veh Fen.

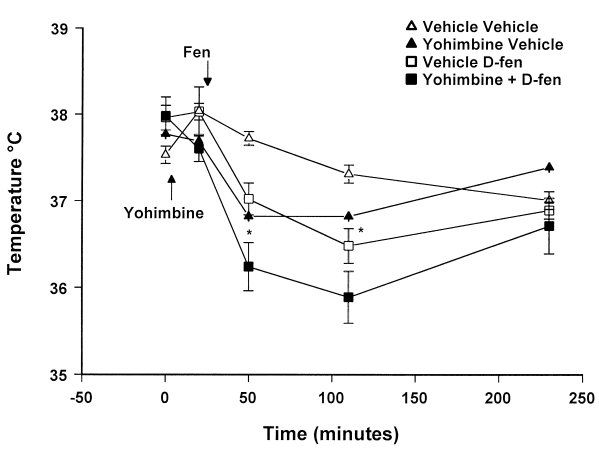

Fig. 8. Effects of pre-treatment with yohimbine (2 mg/kg) on D-fenfluramine (5 mg/kg)-induced hypothermia. Each value is the mean and standard error of 5–6 animals. $^{*}P < 0.01$ vs. Veh Veh.

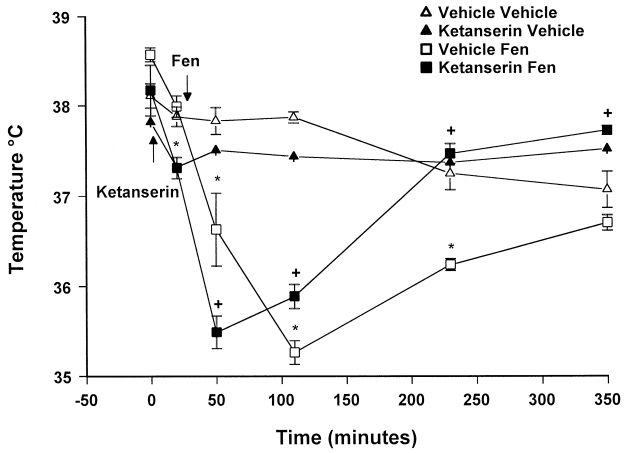

Fig. 9. Effects of pre-treatment with ketanserin (5 mg/kg) on D-fenfluramine (5 mg/kg)-induced hypothermia. Each value is the mean and standard error of 6 animals. $^{*}P < 0.01$ vs. Veh. Veh; $+ P < 0.01$ vs. Veh Fen.

Post-hoc comparisons revealed that D-fenfluramine reduced body temperature 30 and 90 min post-challenge. Haloperidol blocked this response 90 min following D-fenfluramine administration (see Fig. 6).

There was no difference between the groups prior to sulpiride or D-fenfluramine administration. There was an effect of D-fenfluramine 30, $[F(1,20) = 30.93,\ P < 0.001]$ and 90 min $[F(1,20) = 57.33,\ P < 0.001]$ post-challenge and an interaction between D-fenfluramine and sulpiride pre-treatment 30 min post-D-fenfluramine $[F(1,20) = 10.56,\ P = 0.004]$. Post-hoc comparisons revealed that D-fenfluramine reduced body temperature 30 and 90 min post-challenge. Sulpiride blocked this response 30 min following D-fenfluramine administration (see Fig. 7).

There was no difference between the groups prior to yohimbine or D-fenfluramine administration. There was an effect of yohimbine pre-treatment 30 $[F(1,20) = 17.42,\ P < 0.001]$ and 90 min $[F(1,20) = 7.55,\ P = 0.012]$ following D-fenfluramine administration and an effect of D-fenfluramine 30 $[F(1,20) = 10.34,\ P = 0.004]$ and 90 min $[F(1,20) = 20.28,\ P < 0.001]$ post-challenge. Post-hoc comparisons revealed that D-fenfluramine reduced body temperature 90 min post-challenge. Yohimbine failed to significantly interact with this response (see Fig. 8).

3.7. The effect of ketanserin on D-fenfluramine-induced hypothermia

There was no difference in temperatures prior to ketanserin treatment. There was an effect of ketanserin prior to D-fenfluramine administration $[F(1,20) = 25.14,\ P < 0.001]$. Post-hoc comparisons revealed that ketanserin reduced body temperature alone ($P < 0.01$). D-Fenfluramine reduced temperature 30 $[F(1,20) = 46.03,\ P < 0.001]$, 90 $[F(1,20) = 289.43,\ P < 0.001]$ and 220 min $[F(1,20) = 12.78,\ P = 0.002]$ after the D-fenfluramine challenge. There was a potentiation of D-fenfluramine-induced hypothermia by ketanserin 30 min after D-fenfluramine administration ($P < 0.01$). The hypothermic effect of D-fenfluramine was subsequently blocked by ketanserin 90 $[F(1,20) = 18.64,\ P < 0.001]$ and 220 min $[F(1,20) = 19.16,\ P = 0.001]$ post-D-fenfluramine challenge ($P < 0.01$) (see Fig. 9).

4. Discussion

The present study demonstrates that under normal laboratory conditions D-fenfluramine induces a robust and consistent hypothermic response in the rat. This demonstration that D-fenfluramine (at both doses of 5 and 10 mg/kg i.p.) induces a significant hypothermia under normal laboratory temperature conditions is in agreement with some previous studies with both the isomer or its racemate (Preston et al., 1990; O'Callaghan and Miller 1994; Miller and O'Callaghan, 1995; Malberg and Seiden, 1997). However, others have reported that D-, or D,L-fenfluramine evokes little change in core temperature under these conditions (see Sugrue, 1984; Stewart et al., 1997; Clausing et al., 1998). Reasons for these differences are not apparent.

The results of this investigation also suggest that D-fenfluramine-induced hypothermia is dependent upon its uptake via the 5-HT uptake site, release of 5-HT and stimulation of post-synaptic 5-HT receptors. Complete blockade of the hypothermic response to D-fenfluramine was achieved with sertraline pre-treatment consistent with the hypothesis that uptake of D-fenfluramine into 5-HT neurons is necessary to achieve the physiological response. Depletion of central idoleamine concentrations (93–100% in cortex and hypothalamus) following parachlorophenylalanine treatment also blocked the hypothermic effects of D-fenfluramine up to 1 h following challenge, suggesting that release of 5-HT from presynaptic stores is essential for mediating the maximal (15–60 min post-challenge) hypothermic effects as seen in the dose–response curve. Previous investigators have shown that parachlorophenylalanine treatment itself alters thermoregulatory ability (Satinoff et al., 1991). However, following the current injection regime and washout period before challenge, no difference in baseline temperatures between parachlorophenylalanine-treated animals and controls is seen. Notably, a similar regime was utilized by Hutson et al. (1987) to investigate the post-synaptic nature of the hypothermic effects of 5-HT_{1A} receptor agonists.

Of interest, however, is the rebound hypothermic effect prominent after 2 h subsequent to D-fenfluramine administration. This suggests that either fenfluramine or more likely its metabolite, norfenfluramine, is acting through post-synaptic receptors to generate this effect. Callaway et al. (1993) showed that the hypolocomotory effects of D-fenfluramine and norfenfluramine were not altered by parachlorophenylalanine treatment. Similarly, Gibson et al.

(1993) found that severe depletion of 5-HT by parachlorophenylalanine did not prevent D-fenfluramine- or D-norfenfluramine-induced hypophagias, and the latter hypophagia had characteristics that resulted from the direct activation of post-synaptic 5-HT_{2C} receptors, as previously shown for the hypophagic effect of 1-(3-chlorophenyl) piperazine (Kennett and Curzon, 1991). It has been suggested by Curzon et al. (1997) that some of 5-HT-like reactive fibres survive parachlorophenylalanine treatment in the hypothalamus. However, given the extent of the 5-HT depletion in the current study the relevance of this fact is probably negligible. The present study provides evidence for mediation of the hypothermic effects of D-fenfluramine by increased release and availability of 5-HT to receptors but only in the initial phase of the response. The later hypothermic phase of the response may be the result of a combined effect of D-fenfluramine and its active metabolite norfenfluramine with direct stimulation of post-synaptically located receptors. When considered together with the ability of sertraline to produce a complete blockade of the response, a complex profile is apparent whereby intact stores of 5-HT are not necessarily required for D-fenfluramine's hypothermic action, whereas the blockade of uptake through the 5-HT transporter appears critical.

In the dose–response study, the degree of hypothermia obtained was similar following both the 5 and 10 mg/kg doses of D-fenfluramine. This is in contrast to the effects of 8 OH-DPAT, which dose-dependently evokes a hypothermic response, until a physiological ceiling is reached (Hjorth, 1985). Moreover, the hypothermia induced by D-fenfluramine is more prolonged than that of direct 5-HT_{1A} receptor agonists such as 8-OH-DPAT (Cryan et al., 1999a). Thus, D-fenfluramine evokes a hypothermic response qualitatively different to that induced by 5-HT_{1A} receptor agonists but is nevertheless blocked following pre-treatment with a 5-HT_{1A} receptor antagonist. The demonstration that WAY 100635, a potent and selective pre- and post-synaptic 5-HT_{1A} receptor antagonist (Fletcher et al., 1996), completely reversed this hypothermic effect suggests that the action of D-fenfluramine is indeed a 5-HT_{1A} receptor-mediated response. This is hardly surprising given that hypothermia is one of the primary in vivo indices of 5-HT_{1A} receptor function both in rodents (Millan et al., 1993; Cryan et al., 1999a,b) and in man (Lesch et al., 1990). However, this is the first demonstration that the hypothermic response to D-fenfluramine is directly attributable to this receptor subtype. The present results suggest that D-fenfluramine-induced hypothermia may be an indirect measure of 5-HT_{1A} receptor function.

In addition, we further investigated the selectivity of the response by assessing the interaction of other ligands with affinity for other monoamine receptors implicated in altering core body temperatures. In conjunction with the role of the 5-HT_{1A} receptor, other receptors play a significant role in the response, most notably the 5-HT_{2C} receptor. In the present study we demonstrate that RO 43-0440, a novel 5-HT_{2C} receptor antagonist (International application published under the Patent Cooperation Treaty, Benzofuryl derivatives and their use WO 97/42183), attenuates the effects of D-fenfluramine, particularly at later time points, whereas it caused only a slight attenuation at the earlier time points. This suggests that the prolonged hypothermic effects of fenfluramine may be due to stimulation of the 5-HT_{2C} receptor by norfenfluramine as norfenfluramine, but not D-fenfluramine, has been shown to have direct affinity for the 5-HT_{2C} receptor (Caccia et al., 1993; Spedding et al., 1996; Curzon et al., 1997). Potentiation of the hypothermia induced by D-fenfluramine following ketanserin treatment may be accounted for through blockade of 5-HT_{2A} receptors (Hoyer, 1989; Kennett et al., 1994; Ninan and Kalkman., 1998), which have been shown previously to mediate hyperthermic responses (Aulakh et al., 1994). It should be noted that ketanserin has weak α_1-adrenoceptor antagonistic properties (Brogden and Sorkin, 1990), although the impact that this has on the present study is difficult to ascertain. Blockade of the response at later intervals by ketanserin is consistent with the effects of RO 43-0440. However, this is in conflict with the generally accepted view that activation of 5-HT_2 receptors in both rodents and humans induces a hyperthermic response (Maj and Moryl, 1992; Mazzolo-Pomieto et al., 1996; Quested et al., 1997). Reasons for this discrepancy warrant further investigation. As most previous data have relied on interaction studies with nonselective ligands, the availability of newer agents such as RO 43-0440 as used in the present study, M100970 *R*(+)-*a*-(2,3-dimethoxyphenyl)-1-[2-(4-fluoro-phenylethyl)]-4- piperidine-ethanol) and SB 242084 (6-chloro-5-methyl-1-[[2-[2(4-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-indoline) will inevitably aid in the dissection of the functional role of the various 5-HT_2 receptor subtypes in thermoregulatory function.

Treatment with the dopamine D_2 antagonists haloperidol and, to a lesser extent, sulpiride blocked the response to D-fenfluramine. It has recently been shown that norfenfluramine induces 5-HT and dopamine release from vesicular storage pools (Gobbi et al., 1998). D-Fenfluramine has also been shown to increase extracellular dopamine concentrations via a serotonergic mechanism independent of dopamine uptake sites (see De Deurwardere et al., 1995; Balciogu and Wurtman, 1998). Blockade with dopamine D_2 receptor antagonists suggests that stimulation of the dopamine D_2 receptor subsequent to dopamine release plays some role in mediating the hypothermic response to D-fenfluramine. By contrast, the α_2-adrenoceptor antagonist yohimbine had no effect on D-fenfluramine-induced hypothermia and caused a significant hypothermia in its own right. Studies originating from our laboratory have shown that this dose of yohimbine counteracts the hypothermic effects of clonidine (0.05–2 mg/kg) in a similar fashion as another α_2-adrenoceptor antagonist, ida-

zoxan (O'Donnell et al., 1996). Therefore, we may rule out a role for this receptor in mediating D-fenfluramine-induced hypothermia.

In conclusion, we demonstrate that D-fenfluramine induces a consistent reduction in core body temperature that is antagonised by pretreatment with sertraline, WAY 100635, RO 43-0440 and haloperidol. Pre-treatment with parachlorophenylalanine and sulpiride reversed the early part of the hypothermic response but not the later profile, whereas pre-treatment with the 5-HT_{2A} receptor antagonist ketanserin had the opposite effect. Yohimbine had little effect on D-fenfluramine's hypothermic response. We suggest that a complex interaction between the 5-HT_{1A}, 5-HT_{2C} and D_2 receptors are involved in mediating the hypothermic effects of D-fenfluramine at ambient temperatures. The receptors accountable for fenfluramine's effects on core body temperatures under other environmental conditions await further investigation. The results suggest that D-fenfluramine-induced hypothermia may be the result of a combined effect of D-fenfluramine and its active metabolite norfenfluramine involving uptake of D-fenfluramine and consequent 5-HT release in conjunction with direct stimulation of post-synaptically located receptors.

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