Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 77 (2004) 351–359
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Fenfluramine-induced hypothermia is associated with cutaneous dilation in conscious rats

Srividya Subramanian, Regis R. Vollmer*

Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261, USA

Received 28 June 2003; received in revised form 30 October 2003; accepted 10 November 2003

Abstract

The antiobesity agent, fenfluramine, produces hypothermia in rodents by an, as yet, uncharacterized mechanism. The present study was conducted in conscious rats to determine if fenfluramine-induced hypothermia was associated with cutaneous dilation. In animals maintained at 16 °C, core body temperature (T_{CORE}) was measured telemetrically, and tail surface temperature was monitored with thermocouples fixed to the tail (T_{TAIL}). D-Fenfluramine (10 mg/kg ip) produced a rapid increase in T_{TAIL} of 7.7 ± 0.4 °C ($P < .001$) and a decline in T_{CORE} of 4 ± 0.3 °C ($P < .001$). Two findings indicate that the increase in T_{TAIL} was due to the withdrawal of a sympathetic vasoconstrictor tone. First, pretreatment with the ganglionic blocker, pentolinium, prevented fenfluramine-induced changes in T_{TAIL}. Second, when sympathetic tone to the tail was physiologically withdrawn by increasing the environmental temperature to 28 °C, fenfluramine treatment produced no increase in T_{TAIL}. Moreover, the effects of fenfluramine on T_{TAIL} and T_{CORE} depended on the uptake of fenfluramine into serotonergic neurons because these effects were markedly attenuated by pretreatment with the selective serotonin re-uptake inhibitor, fluoxetine. The hypothermic effect of fenfluramine occurred despite the fact that total body oxygen consumption increased by 20%. The results suggest that heat loss due to the dilation of the cutaneous circulation contributes to fenfluramine-induced hypothermia.

Keywords: Cutaneous circulation; Fenfluramine; Hypothermia; Oxygen consumption; Serotonin; Sympathetic nervous system; Thermoregulation

1. Introduction

Fenfluramine, an amphetamine analogue, was widely used as an antiobesity agent before being withdrawn from the market, following reports of cardiac valvular damage and the development of primary pulmonary hypertension (Fishman, 1999; Gross and Lepor, 2000). At doses that effectively reduce body weight in experimental animals, fenfluramine disrupts the normal homeostatic capacity to maintain the constancy of body temperature during fluctuations in environmental temperature. Rodents treated with fenfluramine become hypothermic when ambient temperature is reduced and become hyperthermic when the ambient temperature is increased (Malberg and Seiden, 1997; Cryan et al., 2000; Preston et al., 1990).

The hyperthermia that occurs when fenfluramine is administered to animals in a warm environment appears to be caused by a centrally mediated increase in sympathetic neural activity to brown adipose tissue (Lupien and Bray, 1985; Rothwell and Stock, 1987; Subramanian and Vollmer, 2002). Pharmacologic or surgical block of the sympathetic innervation of brown fat prevents the increases in body temperature and total body oxygen consumption that occur with fenfluramine (Rothwell and Stock, 1987). Since heat loss through radiation, convection and conduction is reduced as ambient temperature rises, the heat that is generated by fenfluramine stimulation of brown fat activation takes a longer duration to be lost to the environment, thereby leading to an increase in T_{CORE}. The hyperthermic effects of fenfluramine are due to its effects on serotonergic neurons because the pretreatment with a selective serotonin uptake inhibitor has been shown to attenuate the hypothermia (Sabol et al., 1992).

In contrast to the hyperthermic response to fenfluramine that occur in animals kept in a warm environment, less is known about the thermoregulatory processes that account for the hypothermic response when animals are

* Corresponding author. Tel.: +1-412-648-8565; fax: +1-412-383-7436.
E-mail address: vollm@pitt.edu (R.R. Vollmer).

0091-3057/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2003.11.009

maintained at a normal laboratory temperature or cooler temperatures. Fenfluramine administered to rats at normal laboratory temperatures reduces body temperature, and the magnitude of the fall is exacerbated by lowering the environmental temperature (Subramanian and Vollmer, 2002; Cryan et al., 2000). Body temperature declines despite the fact that brown adipose tissue is activated (Subramanian and Vollmer, 2002). The hypothermic effect of fenfluramine appears to be mediated by the entry of fenfluramine into serotonin neurons, and the release of serotonin because the fall in body temperature was attenuated by the pretreatment with a serotonin re-uptake blocker, sertraline, a serotonin 5-HT_{1A} receptor antagonist, WAY 100635, or a 5-HT_{2C} receptor antagonist, RO 43-0440 (Cryan et al., 2000).

There is experimental precedent for the concept that serotonin released in the central nervous system can cause a fall in body temperature that is associated with heat loss. Intracerebroventricular administration of serotonin in mice and rats maintained at 22 °C produced a hypothermic response (Brittain and Handley, 1967; Feldberg and Lotti, 1967; Yamada et al., 1988). In addition, the injection of serotonin into the rostral ventrolateral medulla of urethane-anesthetized rats produced a fall in body temperature and a dilation of the cutaneous vasculature (Key and Wigfield, 1992). Similarly, administration of certain serotonin receptor agonists (Bagdy and To, 1997) and the serotonin precursor, L-tryptophan, produced hypothermia and an increase in the tail and foot skin temperature (Lin et al., 1978) of rats kept at 22 °C. Furthermore, at 22 °C, the selective serotonin uptake blocker, fluoxetine, produced cutaneous dilation, which was coincident with a fall in body temperature (Lin, 1978). The finding that cutaneous dilation occurs, during hypothermia, to agents influencing serotonin pathways raised the possibility that fenfluramine-induced hypothermia might also be associated with heat loss through the cutaneous circulation.

Therefore, the experiments of the present study were conducted to test the hypothesis that fenfluramine, by provoking the release of serotonin, elicits hypothermia that is due to augmented heat loss, secondary to the dilation of the cutaneous vasculature. An additional objective of the study was to assess the impact of fenfluramine on heat generation by measuring whole-body oxygen consumption.

2. Methods

2.1. General

Male Sprague–Dawley rats (Hilltop Labs, Scottsdale, PA), weighing 250–275 g on delivery, were housed in a room maintained at 22 ± 1 °C with a 12:12-h light–dark cycle. Food and water were available ad libitum. All experimental procedures were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

2.2. Measurement of T_{CORE}

The animals were anesthetized with pentobarbital sodium (60 mg/kg ip), and surgery was performed utilizing aseptic procedures. Temperature transmitters (Minimitter VMFH, Bend, OR) were inserted into the abdominal cavity, via a ventral midline incision, for continuous measurement of body core temperature (T_{CORE}). After surgery, the animals were housed individually and were allowed to recover for a minimum of 5 days before being utilized for an experiment.

2.3. Measurement of T_{TAIL}

On the morning of the experimental day, the animals were placed in a tethering device (Harvard Apparatus, Holliston, MA) attached to a swivel mounted above the observation container. A thermocouple (K-Type, Omega, Stamford, CT) was fixed 7 cm from the tip of the tail using surgical tape, as described by Redfern et al. (1995). The thermocouple leads were passed along the tail to its base and were fixed in place by surgical tape at 2-cm intervals. The electrical leads were passed along the spring connecting the tethering harness to the swivel. The animals were allowed a 1-h period to adjust to the experimental conditions. A thermocouple placed inside the animal observation container was used to measure the temperature of the air surrounding the animal (environmental temperature). The thermocouples were attached to a printing and logging thermometer (Model H666PL4C, Omega).

All testing was done within a thermostatically controlled walk-in test chamber housed in our laboratory. The test chamber was maintained at a cool ambient temperature of 16–17 °C. The selection of the ambient temperature was based on two criteria. First, in initial experiments, we found that at 16 °C, the magnitude of fenfluramine-induced hypothermia was maximal, equivalent to what we previously reported at 4 °C (Subramanian and Vollmer, 2002). Second, other investigators have demonstrated that robust cutaneous vasodilation can be observed at this temperature with agents that interfere with sympathetic vasoconstrictor tone (Redfern et al., 1995).

2.4. Experiment 1: The effect of fenfluramine on T_{TAIL} and T_{CORE}

This experiment was conducted to determine if fenfluramine treatment would affect cutaneous heat loss. After placement of the animals in the test chamber maintained at 16 °C, baseline T_{TAIL} and T_{CORE} were measured for 1 h before the administration of fenfluramine (10 mg/kg ip, $n = 7$) or saline ($n = 7$). The D-isomer of fenfluramine was used for all studies. The dose of fenfluramine was selected based on prior experiments, which demonstrated that this dose produces a pronounced hypothermia (Subramanian and Vollmer, 2002). The effects of the treatments were observed for 90 min.

2.5. Experiment 2: The effect of pentolinium pretreatment on fenfluramine-induced changes in T_{TAIL} and T_{CORE}

This experiment was conducted to determine if the fenfluramine-induced increase in T_{TAIL} observed in Experiment 1 was due to the withdrawal of the sympathetic vasoconstrictor tone. After placement of the animals in the test chamber maintained at 16 °C, baseline T_{TAIL} and T_{CORE} were measured for 1 h. Four groups ($n=7$ per group) of animals were studied. The first group served as control and received saline intraperitoneally as pretreatment followed 30 min later by a second saline treatment. The second group received saline as pretreatment, followed 30 min later with fenfluramine (10 mg/kg ip). The third group received pentolinium (10 mg/kg ip) as a pretreatment, followed 30 min later by saline intraperitoneally. The fourth group received pentolinium as a pretreatment, followed 30 min later by fenfluramine (10 mg/kg ip). The dose of pentolinium (10 mg/kg ip) was previously shown to block sympathetic vasoconstrictor tone to the tail, for a duration sufficient to cover the length of the present experiments (Redfern et al., 1995). The animals were observed for 90 min after the second treatment. To assess the level of sympathetic tone to the cutaneous vasculature, pentolinium (10 mg/kg ip) was administered at the end of the 90-min observation period, and the animals were observed for an additional 30 min.

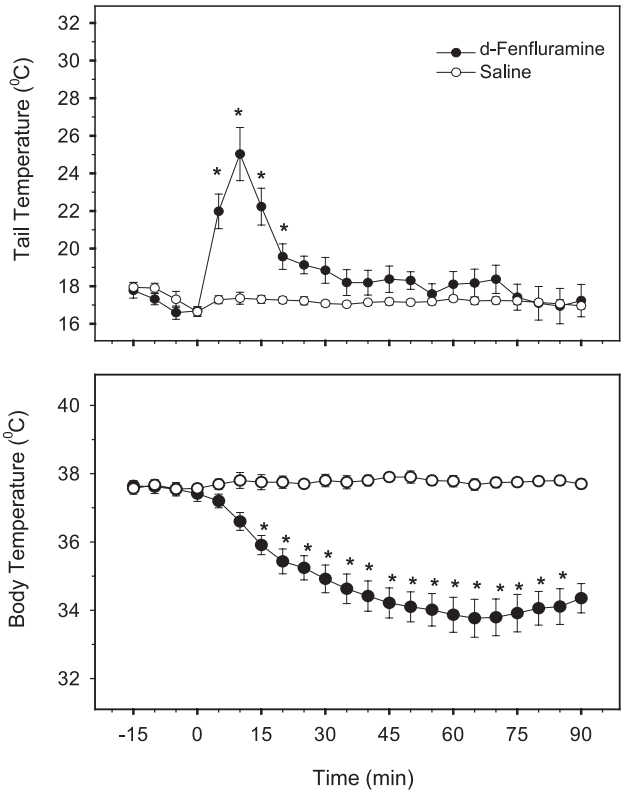

Fig. 1. Effects of fenfluramine (10 mg/kg ip) on T_{TAIL} and T_{CORE} of rats at an ambient temperature of 16 °C. Fenfluramine or saline was administered at time 0 ($n=7$ per group). The T_{TAIL} of the fenfluramine-treated group increased, and the T_{CORE} decreased significantly when contrasted to the saline-treated group ($P<.01$, repeated-measures ANOVA). Asterisks indicate a significant difference between T_{TAIL} and T_{CORE} between the two groups at specific time points (Bonferoni t test).

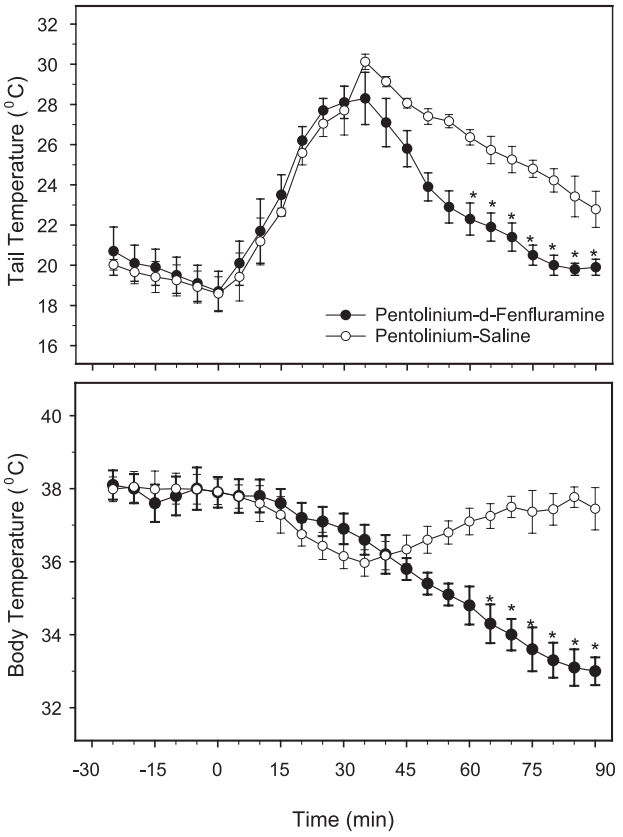

Fig. 2. Effects fenfluramine (10 mg/kg ip) on T_{TAIL} and T_{CORE} following pretreatment with pentolinium (10 mg/kg ip) of rats at an ambient temperature of 16 °C. Animals were treated with pentolinium at time 0, and at 30 min, half of the animals ($n=7$) were treated with fenfluramine and half ($n=7$) were treated with saline. Fenfluramine decreased the T_{TAIL} and T_{CORE} of pentolinium-treated animals in contrast to saline treatment ($P<.01$, repeated-measures ANOVA). Asterisks indicate that there is a significant difference between the two groups at specific time points (Bonferoni t test).

2.6. Experiment 3: Effect of fenfluramine on T_{TAIL} and T_{CORE} in animals maintained at 28 °C

This experiment, like Experiment 2, was conducted to determine if the cutaneous vasodilation observed in animals treated with fenfluramine was dependent on the presence of sympathetic vasoconstrictor tone before the administration of fenfluramine. However, instead of pharmacologically blocking sympathetic activity, sympathetic vasoconstrictor tone was physiologically withdrawn by maintaining the animals in a warm environment before the administration of fenfluramine. Animals were first placed in the test chamber set at 22 °C, and baseline T_{CORE} and T_{TAIL} measurements were made. The temperature of the test chamber was slowly increased to 28 °C. The test chamber temperature at which the T_{TAIL}

abruptly increased was recorded as the temperature at which sympathetic tone to cutaneous vasculature was withdrawn. When the test chamber reached 28 °C, the temperature was kept constant for the remainder of the experiment. Baseline T_{CORE} and T_{TAIL} were monitored for 30 min. The animals were randomly split into two groups ($n=7$ per group). One group was treated with saline, and the other with fenfluramine (10 mg/kg ip), and the animals were observed for 90 min.

2.7. Experiment 4: Effect of fluoxetine pretreatment on fenfluramine-induced changes in T_{TAIL} and T_{CORE}

This experiment was conducted to determine if the increase in T_{TAIL} observed in Experiment 1 required the uptake of fenfluramine into serotonergic neurons. Baseline T_{CORE} and T_{TAIL} measurements were obtained for 30 min, and the animals were randomly assigned to two groups ($n=7$ per group). In Group 1, the serotonin uptake transporter was intact (saline treated) and, in Group 2, the transporter was blocked with fluoxetine (10 mg/kg ip). The dose of fluoxetine was selected based on previous studies demonstrating that fenfluramine-induced serotonin release was blocked (Rothwell and Stock, 1987; Cryan et al., 2000; Yamada et al., 1988). After 40 min, fenfluramine (10 mg/kg ip) was administered to both groups, and the animals were observed for 90 min. After 90 min, pentolinium (10 mg/kg ip) was administered to both groups, and the animals were observed for an additional 30 min to assess the sympathetic vascular tone to the tail.

2.8. Experiment 5: Effect of fenfluramine on whole-body oxygen consumption (VO_2)

This experiment was conducted to assess the effect of fenfluramine on total heat generation due to oxidative metabolism. All animals were implanted with temperature transmitters. On the day of the experiment, the animals were transferred to a metabolic chamber (Columbus Instruments, Oxymax, Columbus, OH) located within the temperature-controlled observation room maintained at 16 °C. A thermometer was attached to the metabolic chamber, and the ambient temperature inside the chamber was 16 °C throughout the experimental period. Baseline T_{CORE} and VO_2 were monitored for 1 h, after which, the animals were randomly divided into three groups ($n=7$ per group). Group 1 received fenfluramine (10 mg/kg ip), Group 2 received pentolinium (10 mg/kg ip) and Group 3 received saline. The animals were observed for 90 min.

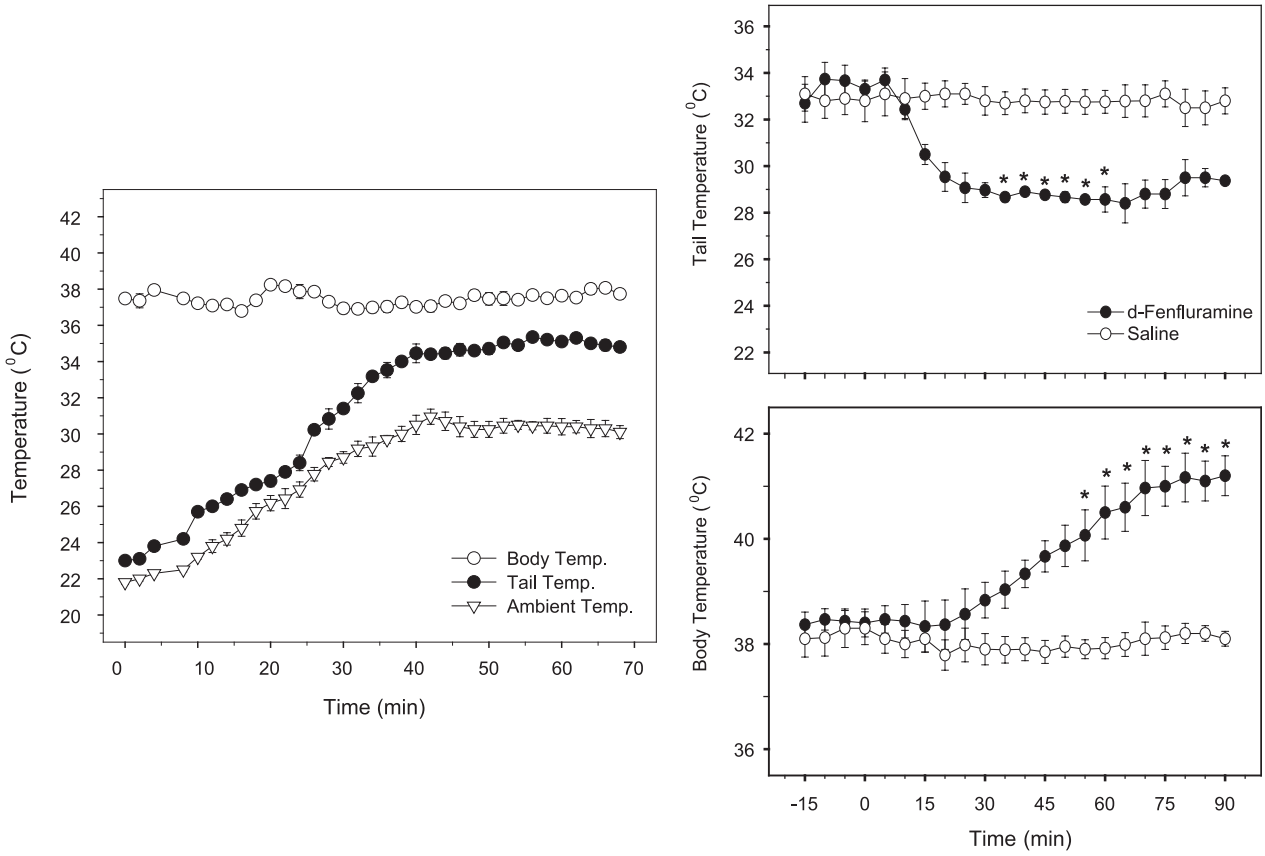

Fig. 3. Effects of fenfluramine (10 mg/kg ip) on T_{TAIL} and T_{CORE} of rats maintained at 28 °C. The effects of gradually raising the temperature of the observation chamber to 28 °C on T_{TAIL} and T_{CORE} is shown in the left panel. In the right panel, fenfluramine, administered at time 0, produced a significant decrease in T_{TAIL} and a significant increase in T_{CORE} when contrasted to saline-treated animals ($P<.01$, repeated-measures ANOVA). Asterisks indicate that there is a significant difference between the two groups at specific time points (Bonferoni t test).

2.9. Statistical analysis

Results are presented as mean ± S.E. The differences in T_{CORE}, T_{TAIL} and whole-body oxygen consumption between multiple groups and between multiple measurements taken over a period of time were assessed using repeated-measures analysis of variance. Post hoc pairwise comparisons were done using Bonferoni *t* test. A statistically significant effect was accepted when $P < .05$.

3. Results

3.1. Experiment 1: Effects of fenfluramine (10 mg/kg ip) on T_{TAIL} and T_{CORE}

Fenfluramine produced a rapid increase in T_{TAIL}, which reached a maximum approximately 10 min after injection ($P < .01$, repeated-measures ANOVA; Fig. 1). The T_{TAIL} returned to pretreatment levels by the end of the 90-min observation period. Fenfluramine also produced a significant sustained decrease in T_{CORE} ($P < .01$, repeated-measures ANOVA; Fig. 1).

3.2. Experiment 2: Effects of pentolinium pretreatment on fenfluramine-induced increases in T_{TAIL} and T_{CORE}

Pentolinium treatment produced a significant increase in T_{TAIL} ($P < .01$ repeated-measures ANOVA; Fig. 2), which recovered partially during the 90-min observation period. When fenfluramine was administered after pentolinium, the T_{TAIL} decreased significantly lower than pentolinium-treated animals that received saline instead of fenfluramine ($P < .01$, repeated-measures ANOVA). As a test of the adequacy of the pentolinium blockade of sympathetic tone, pentolinium was administered at the end of the 90-min observation period, and no change in T_{TAIL} was observed.

Pentolinium significantly decreased T_{CORE} (Dunnet's *t* test, $P < .01$; Fig. 2), but the magnitude of the decrease was significantly less than what was observed with fenfluramine alone (Fig. 1). However, T_{CORE} returned to control levels by the end of the observation period. In the group of pentolinium-treated animals that received fenfluramine, an additional significant hypothermic response was observed (Fig. 2).

3.3. Experiment 3: Effects of fenfluramine on T_{TAIL} and T_{CORE} in rats kept at 28 °C

Animals in this experiment were first placed in the test chamber maintained at 22 °C and the temperature was gradually increased (Fig. 3). Initially, T_{TAIL} increased parallel to ambient temperature. However, when the ambient temperature reached 27.9 ± 0.1 °C, T_{TAIL} rose significantly more rapidly than the ambient temperature.

After reaching 28 °C, the test chamber temperature was held constant. Fenfluramine administered at 28 °C produced a significant decrease in T_{TAIL} ($P < .01$) compared with the saline controls (repeated-measures ANOVA; Fig. 3). The decrease in T_{TAIL} became maximal approximately 20 min after treatment.

Fenfluramine produced a significant increase in T_{CORE} compared with the saline-treated controls ($P < .01$, repeated-measures ANOVA; Fig. 3). The hyperthermia persisted throughout the 90-min observation period. We also observed that the rats were constantly grooming as an attempt to increase heat loss through evaporation.

3.4. Experiment 4: Effects of fluoxetine pretreatment on fenfluramine-induced changes in T_{TAIL} and T_{CORE}

Fluoxetine increased T_{TAIL} ($P < .01$) compared with the saline-treated control group (repeated-measures ANOVA; Fig. 4). However, T_{TAIL} returned to baseline levels by 45 min. Fenfluramine administered after fluoxetine produced no change in T_{TAIL} in contrast with the increase in T_{TAIL} that was observed when fenfluramine was given without pre-

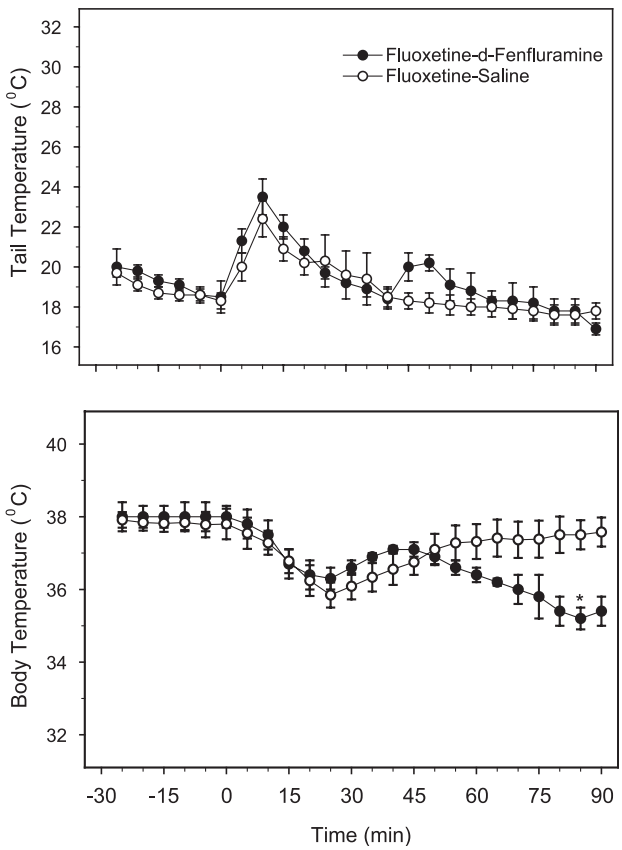

Fig. 4. Effects of fenfluramine (10 mg/kg ip) on T_{TAIL} and T_{CORE} following pretreatment with fluoxetine (10 mg/kg ip) of rats at an ambient temperature of 16 °C. Fluoxetine was administered (10 mg/kg ip) at time 0 and, after 40 min, half of the animals were treated with saline ($n = 7$) and half with fenfluramine (10 mg/kg ip). Fenfluramine treatment did not increase the T_{TAIL} of fluoxetine-treated animals. Repeated measures ANOVA indicated that the T_{CORE} of the two groups are significantly different ($P < .01$). Asterisks indicate that there is a significant difference between the two groups at specific time points (Bonferoni *t* test).

treatment (Fig. 1). At the end of the 90-min observation period, pentolinium was administered to determine the extent of sympathetic vasoconstrictor tone. Pentolinium caused a significant increase in T_{TAIL}, in both the fenfluramine and the saline treatment groups.

Fluoxetine produced a significant hypothermia ($P < .01$) compared with the saline controls (repeated-measures ANOVA). T_{CORE} recovered to baseline by the end of the 90-min observation period. In the fluoxetine-pretreated animals, fenfluramine produced an additional hypothermia ($P < .01$, repeated-measures ANOVA). However, the magnitude of the hypothermia was reduced compared with the hypothermia when fenfluramine was given with no pretreatment (Fig. 1).

3.5. Experiment 5: Effects of fenfluramine and pentolinium on whole-body oxygen consumption

Fenfluramine produced a significant slowly developing increase in whole-body oxygen consumption ($P < .01$) compared with the saline-treated control animals (repeated-measures ANOVA; Fig. 5). As was the case in Experiment 1, fenfluramine treatment produced a significant hypothermia ($P < .01$) compared with the saline-treated control animals (repeated-measures ANOVA; Fig. 5).

Pentolinium treatment resulted in a large rapidly developing increase in oxygen consumption ($P < .01$, repeated-measures ANOVA) compared with the saline-treated controls (Fig. 5). Pentolinium produced a significant hypothermia ($P < .01$) compared with the saline controls (repeated-measures ANOVA), which was of lesser magnitude than the fenfluramine-induced hypothermia. The increase in oxygen consumption produced by pentolinium was greater than the oxygen consumption to fenfluramine treatment.

4. Discussion

The experiments presented provide the first evidence that fenfluramine interferes with sympathetic tone to the cutane-

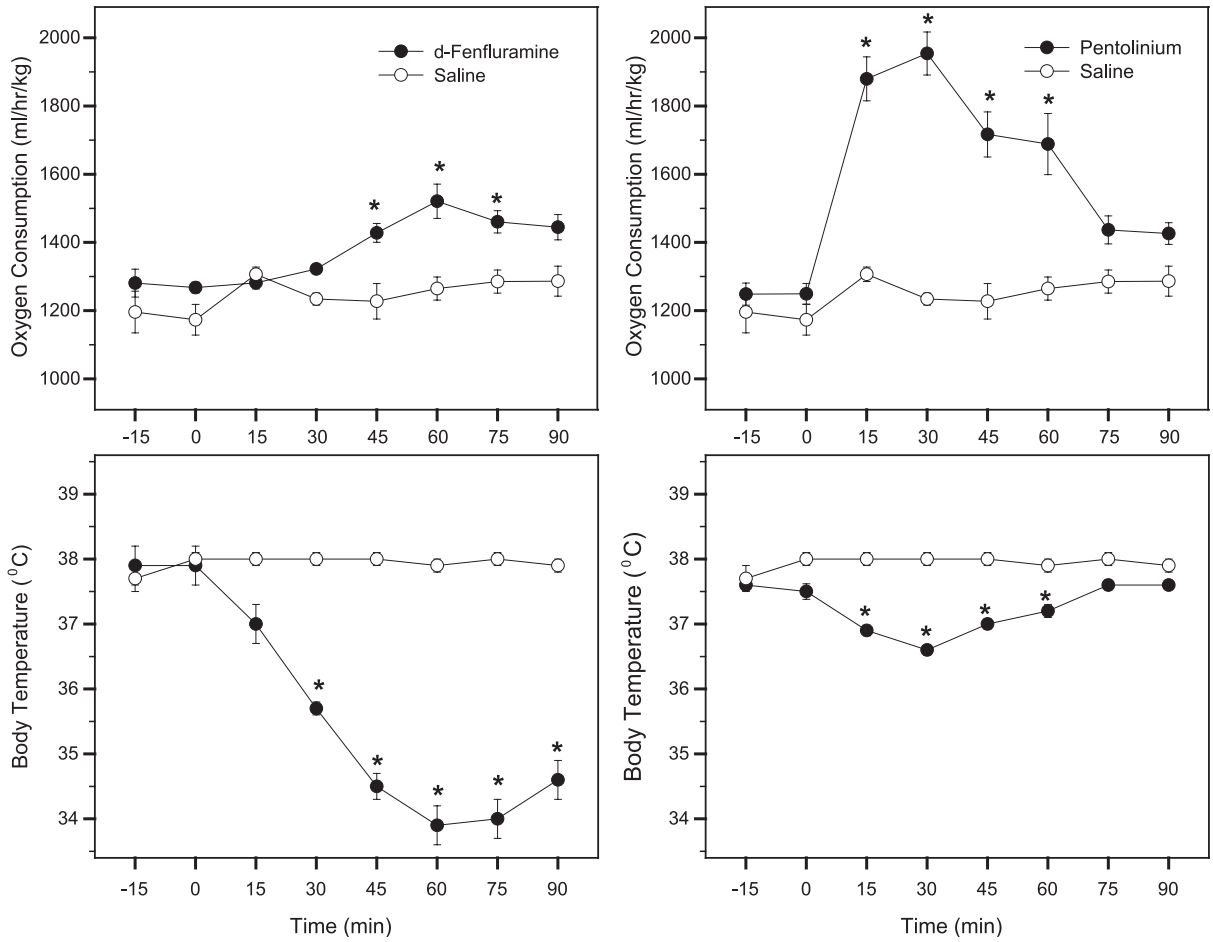

Fig. 5. Effects of fenfluramine (10 mg/kg ip) and pentolinium (10 mg/kg ip) on the whole-body oxygen consumption (VO_2) and T_{CORE} of rats kept at 16 °C ($n = 7$ per group). Fenfluramine or saline was administered at time 0. Fenfluramine and pentolinium produced significant increases in VO_2 and hypothermia when contrasted with the saline-treated group ($P < .01$, repeated-measures ANOVA). The increase in VO_2 was significantly greater in the pentolinium-treated group than in the fenfluramine-treated group ($P < .01$), and the decrease in T_{CORE} was greater in the fenfluramine-treated group than in the pentolinium-treated group ($P < .01$). Asterisks indicate a significant difference between the fenfluramine or pentolinium and the saline group at specific time points (Bonferoni t test).

ous vasculature, resulting in heat loss that may contribute to fenfluramine-induced hypothermia. The findings support the conclusion that fenfluramine must enter into serotonergic neurons to produce its effects since the cutaneous dilation and the hypothermia were antagonized by pretreatment with a selective serotonin re-uptake inhibitor, fluoxetine.

Administration of fenfluramine to conscious rats, studied at an ambient temperature of 16 °C, was consistently followed by a rapid increase in T_{TAIL}, indicating cutaneous vasodilation. This finding is consistent with the interpretation of other investigators that an increase in T_{TAIL} is a reliable indicator of increased blood flow through the cutaneous circulation (Lin, 1978; Redfern et al., 1995). The studies were conducted at a cool ambient temperature of 16 °C to ensure that the cutaneous vasculature was constricted by the activity of sympathetic neurons. The magnitude of the fall in T_{CORE} to fenfluramine, at 16 °C, was more pronounced than what we had previously reported for animals studied at a laboratory temperature of 22 °C (Subramanian and Vollmer, 2002). In fact, the decline in T_{CORE} at 16 °C may be the maximal drop that can be produced by fenfluramine since it was equal to the fall observed at 4 °C (Subramanian and Vollmer, 2002).

The dilation of the cutaneous vasculature produced by fenfluramine appears to be produced by the withdrawal of sympathetic vasoconstrictor tone. This conclusion is supported by the fact that if sympathetic vasoconstrictor tone is absent prior to the administration of fenfluramine, then, fenfluramine is unable to cause vasodilation. In the present study, two different approaches were used to remove vasoconstriction, pharmacologic blockade or physiologic removal of tone.

Pharmacologic blockade of sympathetic tone to the cutaneous vasculature, with the ganglionic blocker pentolinium, produced a marked dilation of the cutaneous vasculature, as indicated by a significant rise in the T_{TAIL}, in agreement with the reports of others (Redfern et al., 1995). Interestingly, when fenfluramine was administered after pentolinium T_{TAIL} did not increase, but instead, T_{TAIL} decreased, indicating a vasoconstrictor effect. These observations indicate that fenfluramine cannot cause vasodilation if sympathetic tone is blocked prior to fenfluramine administration.

The dependency of the cutaneous vasodilator effect of fenfluramine on preexisting sympathetic tone was further substantiated by studying the animals at 28 °C, a temperature at which sympathetic tone to cutaneous vasculature is physiologically withdrawn. To assure that sympathetic tone was minimized as environmental temperature increased, the animals were placed in the test chamber set at 22 °C. The chamber was gradually warmed to 28 °C. In each animal, the withdrawal of sympathetic tone was observed as a rather abrupt increase in T_{TAIL}, which occurred at an environmental temperature of 26–27 °C, confirming previous reports that the physiologic removal of sympathetic tone is an abrupt on/off response that occurs when ambient temperature is increased toward the thermoneutral temperature (O'leary et al., 1985).

The administration of fenfluramine in the warm environment did not cause vasodilation but instead, like the finding in animals treated with pentolinium, fenfluramine caused a constriction of the tail. These observations are consistent with the conclusion that fenfluramine causes vasodilation by withdrawing sympathetic tone.

In addition, it is important to note that fenfluramine treatment in the warm environment resulted in a significant increase in T_{CORE}. Others have determined that fenfluramine-induced hyperthermic response is attributable to sympathetic activation of BAT (Lupien and Bray, 1985; Rothwell and Stock, 1987). The resulting heat generated cannot be dissipated by cutaneous dilation. In fact, the vasoconstriction produced by fenfluramine in a warm environment may exacerbate the hyperthermia.

Cutaneous dilation may play a part in fenfluramine-induced hypothermia, but it does not fully account for the reduction in T_{CORE}. Following fenfluramine administration, T_{TAIL} rapidly increased within 10 min, and the initial fall in T_{CORE} began at the same time that heat was being lost due to the cutaneous dilation. However, T_{CORE} continued to decline while the T_{TAIL} recovered. Furthermore, pentolinium produced a more sustained increase in T_{TAIL} than fenfluramine did; yet, the magnitude and the duration of the hypothermic effect to pentolinium were less than the effect to fenfluramine. In fact, pentolinium-treated animals were able to restore T_{CORE} to control levels despite the fact that T_{TAIL} remained significantly elevated. In addition, fenfluramine further lowered T_{CORE} when sympathetic influences were already removed by pentolinium treatment.

Pentolinium produced a more sustained increase in T_{TAIL} than fenfluramine did; yet, T_{CORE} was reduced to a lesser extent than fenfluramine. Therefore, we considered the possibility that animals treated with fenfluramine were less able to generate heat to offset cutaneous losses. Whole-body oxygen consumption (VO_2) was measured as an index of total metabolic heat generation. Although fenfluramine increased VO_2, the magnitude of the increase was significantly less than that produced by pentolinium. Thus, the increment in VO_2 after fenfluramine was insufficient to offset the loss of body heat due to cutaneous vasodilation. Interestingly, the increase in metabolic activity after pentolinium must be due principally to the heat production from sources other than BAT, since sympathetic activation of BAT is blocked by pentolinium (Subramanian and Vollmer, 2002). Fenfluramine is known to activate sympathetic neural activity to BAT and generate heat via this tissue. However, despite BAT activation, the total VO_2 increment in fenfluramine-treated animals was less than in pentolinium-treated animals. Heat generation by pentolinium from non-BAT sources seems to include increased shivering thermogenesis. While not quantified, pentolinium-treated animals were observed to shiver, whereas, shivering did not occur in the fenfluramine-treated animals despite the hypothermia.

In the present study, the blockade of serotonin uptake with fluoxetine antagonized fenfluramine-induced changes in T_{TAIL} and T_{CORE}. This observation suggests that fenfluramine must be taken up into serotonin neurons for its vasodilator action to be observed. A previous study has also demonstrated that the serotonin re-uptake inhibitor sertraline attenuated the hypothermia to fenfluramine (Cryan et al., 2000). Our findings fit well with previous reports indicating that central serotonergic neurons influence sympathetic outflow to the cutaneous circulation.

Central administration of serotonin into the ventricular spaces has been shown to produce both cutaneous dilation, as indicated by a rise in tail surface temperature, and hypothermia (Key and Wigfield, 1992). Similarly, the serotonin-1A receptor agonist, 8-OH-DPAT, also produced increases in rat T_{TAIL} and hypothermia (Oerther, 2000).

Evidence to support the concept that neuronally released serotonin may cause dilation of the cutaneous vasculature is provided by a previous report that fluoxetine administration was followed by an increase in T_{TAIL} and hypothermia (Lin, 1978). Similarly, in the present study, fluoxetine produced a significant, but transient, increase in T_{TAIL} and a decrease in T_{CORE}. The effects of fluoxetine could be due to an increase in synaptic serotonin, immediately following administration, when neuronal re-uptake of serotonin from discharging neurons is blocked. The increment in serotonin could initiate the withdrawal of cutaneous vascular tone. The transient nature of the response could be because synaptic serotonin concentrations could be brought back toward pretreatment levels by a compensatory reduction in the firing rate of the serotonin neurons as the animal attempts to restore temperature homeostasis. In fact, we saw that the vasoconstrictor tone to the cutaneous vasculature was intact following the recovery of T_{CORE} in fluoxetine-treated animals because subsequent pentolinium treatment resulted in cutaneous vasodilation and hypothermia.

The anatomy of the sympathetic pathway mediating the thermoregulatory changes in the cutaneous vasculature of the tail has been partially elucidated via the use of electrophysiologic and labeling techniques (Smith and Gilbey, 1998; Rathner and McAllen, 1998, 1999; Smith et al., 1998; Rathner et al., 2001). Preganglionic fibers arise in the lower thoracic and upper lumbar regions of the cord. Premotor neurons arise in the medulla and hypothalamus. In each of these areas, there is evidence for serotonergic neuronal pathways, some medullary, caudal raphe nuclei neurons that were labeled with rabies virus following injection into the tail also stained for serotonin (Smith et al., 1998).

The effect of fenfluramine on the withdrawal of cutaneous vasoconstrictor tone extended beyond the time that T_{TAIL} was elevated. This point was demonstrated by the observation that pentolinium did not produce further dilation 90 min after fenfluramine was administered. This could be due to the fact that fenfluramine produces a vasodilator effect that is due to the blockade of sympathetic vasoconstriction and a vasoconstrictor effect that is independent of the sympathetic nervous system. We speculate that fenfluramine or its metabolites could have a direct vasoconstrictive effect on the cutaneous vasculature or they may act indirectly through release of local serotonin.

In summary, the results of the experiments indicate that when animals were maintained at a cool ambient temperature of 16 °C, fenfluramine treatment produced an increase in T_{TAIL}. This observation suggests that fenfluramine produced a dilation of the tail cutaneous vasculature and provides support for the conclusion that fenfluramine produces heat loss that may contribute to its hypothermic effect. At 16 °C, the dilation of the cutaneous vasculature is physiologically inappropriate. Furthermore, the results support the conclusion that fenfluramine produces a withdrawal of the sympathetic vasoconstrictor tone due to central release of serotonin. In addition, the increase in metabolic heat production to fenfluramine treatment was insufficient to overcome its hypothermic effect.

References

Bagdy G, To CT. Comparison of relative potencies of i.v. and i.c.v. administered 8-OH-DPAT gives evidence of different sites of action for hypothermia, lower lip retraction and tail flicks. Eur J Pharmacol 1997; 323:53–8.

Brittain RT, Handley SL. Temperature changes produced by the injection of catecholamines and 5-hydroxytryptamine into the cerebral ventricles of the conscious mouse. J Physiol 1967;192:805–13.

Cryan JF, Harkin A, Naughton M, Kelly JP, Leonard BE. Characterization of D-fenfluramine-induced hypothermia: evidence for multiple sites of action. Eur J Pharmacol 2000;390:275–85.

Feldberg W, Lotti VJ. Temperature responses to monoamines and an inhibitor of MAO injected into the cerebral ventricles of rats. Br J Pharmacol 1967;31:152–61.

Fishman AP. Aminorex to fen/phen: an epidemic foretold. Circulation 1999;99:156–61.

Gross SB, Lepor NE. Anorexigen-related cardiopulmonary toxicity. Rev Cardiovasc Med 2000;1:80–9.

Key BJ, Wigfield CC. Changes in the tail surface temperature of the rat following injection of 5-hydroxytryptamine into the ventrolateral medulla. Neuropharmacology 1992;31:717–23.

Lin MT. Effect of specific inhibitors of 5-hydroxytryptamine uptake on thermoregulation in rats. J Physiol 1978;284:147–54.

Lin MT, Chow CF, Chern YF, Wu KM. Elevating serotonin levels in brain with 5-hydroxytryptophan produces hypothermia in rats. Pflügers Archiv-Eur J Physiol 1978;377:245–9.

Lupien JR, Bray GA. Effect of fenfluramine on GDP-binding to brown adipose tissue mitochondria. Pharmacol Biochem Behav 1985;23: 509–11.

Malberg JE, Seiden LS. Administration of fenfluramine at different ambient temperatures produces different core temperature and 5-HT neurotoxicity profiles. Brain Res 1997;765:101–7.

Oerther S. Temperature set-point changes induced by DA D2/3 and 5-HT1A receptor agonists in the rat. NeuroReport 2000;11:3949–51.

O'leary DS, Johnson JM, Taylor F. Mode of neural control mediating rat tail vasodilation during heating. J Appl Physiol 1985;59:1533–8.

Preston E, Ma S, Haas N. Ambient temperature modulation of fenfluramine-induced thermogenesis in the rat. Neuropharmacology 1990;29: 277–83.

Rathner JA, McAllen RM. The lumbar preganglionic sympathetic supply to rat tail and hindpaw. J Auton Nerv Sys 1998;69:127–31.

Rathner JA, McAllen RM. Differential control of sympathetic drive to the rat tail artery and kidney by medullary premotor cell groups. Brain Res 1999;834:196–9.

Rathner JA, Owens NC, McAllen RM. Cold-activated raphe-spinal neurons in rats. J Physiol 2001;535:841–54.

Redfern WS, MacLean MR, Clague RU, McGrath JC. The role of alpha 2-adrenoceptors in the vasculature of the rat tail. Br J Pharmacol 1995;114:1724–30.

Rothwell NJ, Stock MJ. Effect of diet and fenfluramine on thermogenesis in the rat: possible involvement of serotonergic mechanisms. Int J Obes 1987;11:319–24.

Sabol KE, Richards JB, Seiden LS. Fluoxetine attenuates the DL-fenfluramine-induced increase in extracellular serotonin as measured by in vivo dialysis. Brain Res 1992;585:421–4.

Smith JE, Gilbey MP. Segmental origin of sympathetic preganglionic neurones regulating the tail circulation in the rat. J Auton Nerv Sys 1998; 68:109–14.

Smith JE, Jansen ASP, Gilbey MP, Loewy AD. CNS cell groups projecting to sympathetic outflow of tail artery: neural circuits involved in heat loss in the rat. Brain Res 1998;786:153–64.

Subramanian S, Vollmer RR. Sympathetic activation by fenfluramine depletes brown adipose tissue norepinephrine content in rats. Pharmacol Biochem Behav 2002;73:639–45.

Yamada J, Sugimoto Y, Wakita H, Horisaka K. The involvement of serotonergic and dopaminergic systems in hypothermia induced in mice by intracerebroventricular injection of serotonin. Jpn J Pharmacol 1988: 145–8.