Chapter V. Evaluation of the Effects of d-Fenfluramine on the Cutaneous Vasculature and Total Metabolic Heat Production

Experiments presented in this chapter were designed to investigate the possible mechanisms involved in the hypothermia produced by fenfluramine treatment. The results of the previous chapter, chapter IV, clearly demonstrate that fenfluramine produced dose-related decreases in body temperature and the decreases were exacerbated at 4^0C. Moreover, the results indicated that the hypothermia occurred despite stimulation of heat generation through BAT activation. Thus, the mechanisms that account for the hypothermia to fenfluramine treatment remain undefined. Body temperature maintenance depends on the balance between heat generation and heat loss. Therefore, experiments were designed to determine if fenfluramine-induced hypothermia was due to either accelerated heat loss or insufficient heat generation.

As discussed in Chapter I, the cutaneous vasculature is an important thermoregulatory system, when constricted it prevents heat loss and when dilated, it promotes heat loss. The primary mechanism for control of heat loss is the sympathetic nervous system control of cutaneous vascular tone. At normal ambient temperatures, the cutaneous vasculature is constricted and the constriction is sustained as environmental temperature is reduced. Previous studies have shown that agents that interfere with the sympathetic tone to the cutaneous vasculature produce a decline in body temperature. For example, drugs that block alpha-adrenoceptor mediated vasoconstriction such as phentolamine and delequamine and ganglionic blocking agents such as pentolinium and chlorisondamine produce hypothermia (Kobayashi et al., 1998; Redfern et al., 1995; Lin et al., 1979; Brittain et al., 1967). Therefore, we hypothesized that fenfluramine could interfere

with sympathetic nervous system control of cutaneous vascular tone and prevent cutaneous vasoconstriction and produce heat loss and consequently the body temperature falls. To our knowledge, no studies have been conducted to explore the possibility that fenfluramine interferes with the sympathetic nervous system tone to the cutaneous vasculature and produces heat loss.

It is most likely, based on observations discussed in chapter I ("Role of serotonin in thermoregulation"), that fenfluramine influences sympathetic activity through its effects on the serotonergic neuronal systems since serotonin and certain serotonin agonists have been shown to produce heat loss. Serotonin, when administered intracerebroventricularly, produces heat loss in rabbits and rats (Bligh et al., 1971; Key et al., 1992). Additional proof that serotonergic systems are involved in producing heat loss through cutaneous vasodilation is that fluoxetine, which increases synaptic serotonin levels and serotonin-1A receptor agonist 8-OHDPAT produce heat loss as indicated by increases in rat tail skin temperature (Lin, 1978; Oerther, 2000).

Rats are particularly suited for the study of cutaneous thermoregulation because of the important role of the tail vasculature in thermoregulation. The tail of the rat has been commonly used to study peripheral vascular tone (Dawson et al., 1979; Rand et al., 1965). Tail surface temperature is generally measured in order to obtain an indication of changes in tail blood flow. Increases in tail surface temperature were interpreted as increased blood flow to the tail due to cutaneous vascular dilation and decreases in tail skin temperature were interpreted as decreased blood flow to the tail due to cutaneous vascular constriction.

The experiments were conducted at a cool ambient temperature of 16-17^{0}C. The selection of the ambient temperature was based on two criteria. First, at 16^{0}C, the magnitude

of d-fenfluramine-induced hypothermia was equivalent to that observed at 4^0C. 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).

The first experiment was conducted to determine the effect of d-fenfluramine on tail skin temperature. The results indicated that d-fenfluramine administration increased tail skin temperature (Experiment 1) supporting the supposition that d-fenfluramine caused cutaneous vasodilation. This observation led to the development of two additional experiments. The second experiment was designed to determine if d-fenfluramine altered tail skin temperature by producing a withdrawal of the sympathetic vasoconstrictor tone. To test this possibility, the effect of d-fenfluramine on tail skin temperature was studied in animals treated with and without the ganglionic blocker pentolinium, which blocks the sympathetic tone to the cutaneous vasculature. Another approach was also used to determine if d-fenfluramine caused changes in cutaneous blood flow that were independent of sympathetic tone. In this third experiment, animals were placed in the test chamber that had been warmed to 28^0C, ambient temperature at which the cutaneous vasculature of the rat would be dilated due to withdrawal of sympathetic tone (Hellstorm, 1975a). Thus, once the tail vasculature is dilated, subsequently administered d-fenfluramine should not be able to affect cutaneous blood vessels by removing sympathetic tone. The fourth experiment was designed to determine if d-fenfluramine-induced increase in tail skin temperature was secondary to release of serotonin. This was achieved by evaluating the effect of d-fenfluramine on tail skin temperature in animals treated with and without fluoxetine, a selective serotonin reuptake

inhibitor, which blocks d-fenfluramine-induced serotonin release (Berger et al., 1992; Sabol et al., 1992; Cheetam et al., 2000).

The fifth experiment of this chapter was designed to assess the effect of d-fenfluramine on total metabolic heat production. As stated earlier, the hypothermia to fenfluramine could be due to insufficient heat generation. It was considered that fenfluramine could produce a fall in body core temperature by reducing total metabolic heat production. The total heat generated by an animal is the sum of shivering and nonshivering thermogenesis. Although fenfluramine activates nonshivering thermogenesis (BAT), it might interfere with shivering thermogenesis and thus reduce total heat production. Therefore the effect of d-fenfluramine on total heat generation would indicate if d-fenfluramine produces hypothermia by interfering with heat generation by non-BAT thermogenesis. Whole body oxygen consumption (VO_2) was measured as an index of total metabolic heat generation. This experiment was also conducted at an ambient temperature of 16-17^{0}C.

A. Protocols

1. Experiment 1. Effects of d-fenfluramine on tail skin and body temperature of rats kept at 16^{0}C.

This experiment was conducted to determine if d-fenfluramine treatment would affect tail skin temperature. All animals were implanted with temperature transmitters. On the day of the experiment, a thermocouple was attached to the surface of the tail. The animals were then moved to the walk-in cold room maintained at 16^{0}C and baseline tail and body temperature were measured for an hour. The animals were then treated with d-fenfluramine, 10 mg/kg, i.p., or saline (n=7/group) and observed for another 90 minutes. The dose of d-

fenfluramine, 10 mg/kg, was selected since this dose produces a pronounced hypothermia and BAT thermogenesis.

2. **Experiment 2. Effects of pentolinium pretreatment on** d-**fenfluramine-induced changes in tail skin and body temperature.**

This experiment was conducted to determine if d-fenfluramine altered tail skin temperature by producing a withdrawal of the sympathetic vasoconstrictor tone.
All animals were implanted with temperature transmitters. On the day of the experiment, a thermocouple was attached to the surface of the tail. Four groups (n=7/group) of animals were studied, each receiving a different sequence of three treatments. Baseline tail skin and body temperature was measured in all animals for an hour before the first treatment was administered. After 30 minutes the second treatment was administered. The animals were then observed for 90 minutes. All animals received a third treatment of pentolinium and were observed for an additional 30-minute period. The first group of animals received saline as treatment one and saline as treatment two. The second group of animals received saline as treatment one and d-fenfluramine as treatment two. The third group received pentolinium, 10 mg/kg, i.p., as treatment one and saline as treatment two. The fourth group received pentolinium as treatment one and d-fenfluramine as treatment two. The dose of pentolinium used was 10 mg/kg. This dose was previously shown by other investigators to totally block sympathetic vasoconstrictor tone to the tail (Redfern et al., 1995).

3. **Experiment 3. Effects of d-fenfluramine on tail skin and body temperature of rats maintained at 28^0C.**

This experiment was also conducted to determine if d-fenfluramine caused changes in cutaneous blood flow that were independent of sympathetic tone. All animals were implanted

with temperature transmitters. On the day of the experiment, a thermocouple was attached to the surface of the tail. Two groups (n=7/group) of animals with indwelling temperature transmitters and tail thermocouples were transferred to the test chamber maintained at 22^0C and the temperature was slowly increased to 28^0C. Baseline tail skin and body temperature measurements were then made for 30 minutes. d-Fenfluramine, 10 mg/kg, i.p., or saline was then administered and the animals were observed for another 90 minutes.

4. Experiment 4. Effects of fluoxetine pretreatment on d-fenfluramine-induced changes in tail skin and body temperature.

This experiment was conducted to determine if d-fenfluramine-induced changes in tail skin and body temperature were mediated via release of serotonin. All animals were implanted with temperature transmitters. On the day of the experiment, a thermocouple was attached to the surface of the tail. Two groups (n=7/group) of animals were studied. Baseline tail skin and body temperature measurements were made for 30 minutes. Fluoxetine, 10 mg/kg, i.p., was then administered to both groups. After 40 minutes, one group received d-fenfluramine, 10 mg/kg, i.p. and the other group received saline. The animals were observed for 90 minutes. At the end of the 90-minute observation period, the two groups received pentolinium and were observed for an additional 30 minutes. The dose of fluoxetine, 10 mg/kg, has been shown by others to have blocked fenfluramine-induced serotonin release (Gundlah et al., 1997).

5. Experiment 5. Effects of d-fenfluramine and pentolinium on whole body oxygen consumption (VO_2).

This experiment was conducted to assess the effects of d-fenfluramine and pentolinium on total metabolic heat generation. All animals were implanted with temperature

transmitters. On the day of the experiment, the animals were transferred to a metabolic chamber in the walk-in cold room maintained at 16^0C. Baseline body temperature and oxygen consumption measurements were made for a period of one hour. The animals were then separated in to three treatment groups (n=7/group). Group one received d-fenfluramine, 10 mg/kg, i.p., group two received pentolinium, 10 mg/kg, i.p. and group three received saline. The animals were observed for 90 minutes. At the end of the 90-minute observation period the d-fenfluramine treated group was treated with pentolinium and observed for an additional hour. The group initially treated with pentolinium was treated with d-fenfluramine at the end of the 90-minute period and observed for another hour.

Statistical Analyses.

Results are presented as mean $\pm$ S.E. The differences in tail skin and body temperature 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 $p < 0.05$.

B. Results.

1. Experiment 1. Effects of d-fenfluramine, 10 mg/kg, i.p., on tail skin and body temperature of rats kept at 16^0C.

d-Fenfluramine produced a rapid increase in tail skin temperature when compared to the saline-treated controls, $p < 0.01$ (Repeated measures ANOVA), Fig. 24, top panel. The tail skin temperature reached a maximum approximately 10 minutes after injection, but

returned to pretreatment levels by the end of the 90-minute observation period (Bonferoni t-test).

d-Fenfluramine treatment produced a significant decrease in body temperature when compared to the saline-treated controls, $p < 0.01$ (Repeated measures ANOVA), Fig. 24, bottom panel.

Note that the magnitude of hypothermia to d-fenfluramine at 16^0C is similar to the magnitude of hypothermia to d-fenfluramine treatment at 4^0C.

2. Experiment 2. Effects of pentolinium pretreatment on d-fenfluramine-induced increases in tail skin temperature and hypothermia.

Pentolinium treatment produced a significant increase in tail skin temperature, $p < 0.01$ (Repeated measures ANOVA), Fig. 25, top panel. The tail skin temperature did not return to control levels by the end of the 90-minute observation period. In the group of animals pretreated with pentolinium, subsequent d-fenfluramine treatment decreased tail skin temperature when compared to the pentolinium-pretreated group that received saline treatment and the tail skin temperature returned to control levels by the end of the observation period (Bonferoni t-test), Fig. 26, top panel. Pentolinium administered at the end of the 90-minute observation period did not affect tail skin temperature, Fig. 26, top panel.

Pentolinium treatment produced a decrease in body temperature when compared to the saline controls, $p < 0.01$(Repeated measures ANOVA), Fig. 25, bottom panel. However, the body temperature returned to control levels by the end of the observation period. In the group of animals pretreated with pentolinium, subsequent administration of d-fenfluramine produced an additional, more pronounced hypothermia (Bonferoni t-test), Fig. 26, bottom panel.

3. Experiment 3. Effects of d-fenfluramine, 10 mg/kg, i.p., on tail skin and body temperature of rats kept at 28^0C (thermoneutrality).

In the range of ambient temperatures from 21.9^0 to 27.7^0C, tail temperature increased parallel to ambient temperature. However when the ambient temperature reached 27.9 ± 0.1^0C, tail temperature rose more rapidly than the ambient temperature, Fig. 27.

d-Fenfluramine was administered when tail ambient temperature was above the temperature point at which the tail surface temperature was no longer changing, i.e. 28^0C. d-Fenfluramine administration at 28^0C produced a significant decrease in tail skin temperature when compared to the saline controls, $p < 0.01$ (Repeated measures ANOVA), Fig. 28, top panel. The decrease in tail skin temperature became maximal approximately 20 minutes after treatment (Bonferoni t-test).

d-Fenfluramine produced a significant hyperthermic response when compared to the saline treated controls, $p < 0.01$ (Repeated measures ANOVA), Fig. 28, bottom panel. The hyperthermia lasted the entire 90-minute observation period (Bonferoni t-test).

4. Experiment 4. Effects of fluoxetine pretreatment on d-fenfluramine induced increases in tail skin temperature and hypothermia.

Fluoxetine treatment resulted in increases in tail skin temperature when compared to the saline controls, $p < 0.01$ (Repeated measures ANOVA), Fig. 29, top panel. However, the tail skin temperature returned to baseline levels at the end of 45 minutes (Bonferoni t-test). In the group of animals pretreated with fluoxetine, subsequent d-fenfluramine treatment did not produce an increase in tail temperature (Bonferoni t-test), Fig. 30, top panel.

Pentolinium administered to the fluoxetine-saline group at the end of the 90 - minute observation period caused a significant increase in tail temperature (Bonferoni t-test).

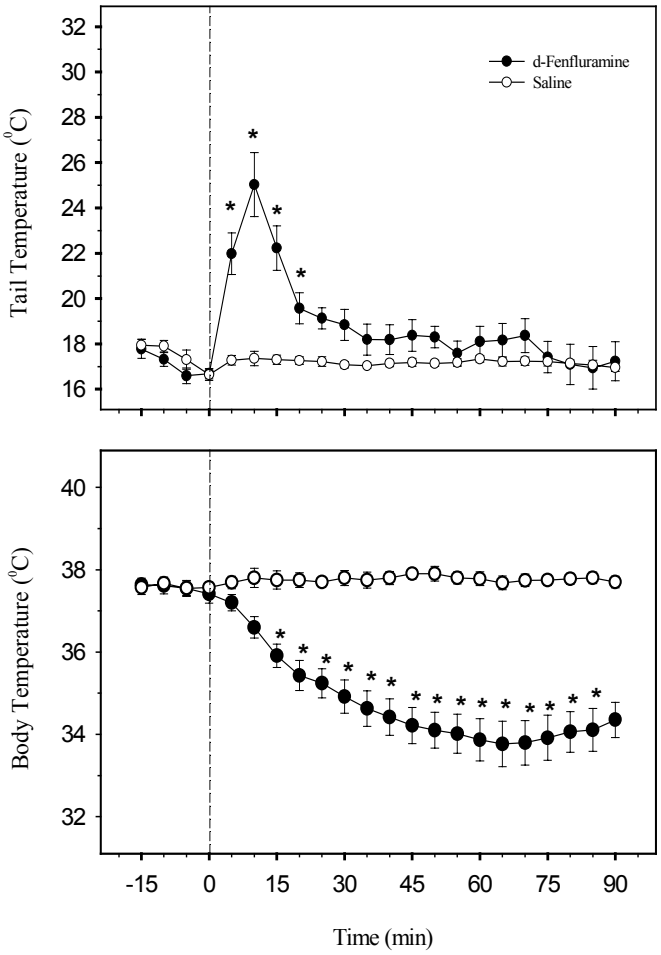

Fig. 24. Effects of saline or d-fenfluramine, 10 mg/kg, i.p., on tail skin and body temperature of rats kept at 16^0C (n=7/group). D-Fenfluramine or saline was administered at time "0". The tail skin temperature responses of the d-fenfluramine and saline treated groups were significantly different, $p < 0.01$ (Repeated measures ANOVA). The body temperature responses of the d-fenfluramine and saline treated groups were significantly different, $p < 0.01$ (Repeated measures ANOVA). Asterisks indicate that there is a significant difference in tail skin and body temperature between the two groups at specific time points, Bonferoni t-test.

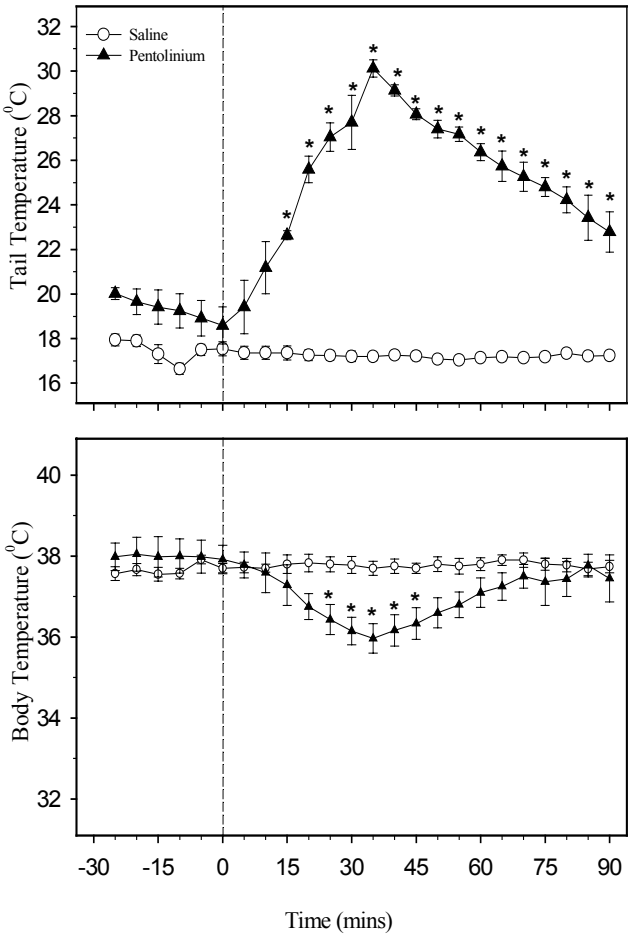

Fig. 25. Effects of saline or pentolinium, 10 mg/kg, i.p., on tail skin and body temperature of rats kept at 16^{0}C (n=7/group). Pentolinium or saline was administered at time "0"and saline was administered to both groups after 30 minutes. Pentolinium produced a significant increase in tail skin temperature. The tail skin temperature responses of the pentolinium and saline treated groups were significantly different, $p < 0.01$ (Repeated measures ANOVA). Pentolinium also produced a mild hypothermia. The body temperature responses of the pentolinium and saline treated groups were significantly different, $p < 0.01$ (Repeated measures ANOVA). Asterisks indicate that there is a significant difference in tail skin and body temperature between the two groups at specific time points, Bonferoni t-test.

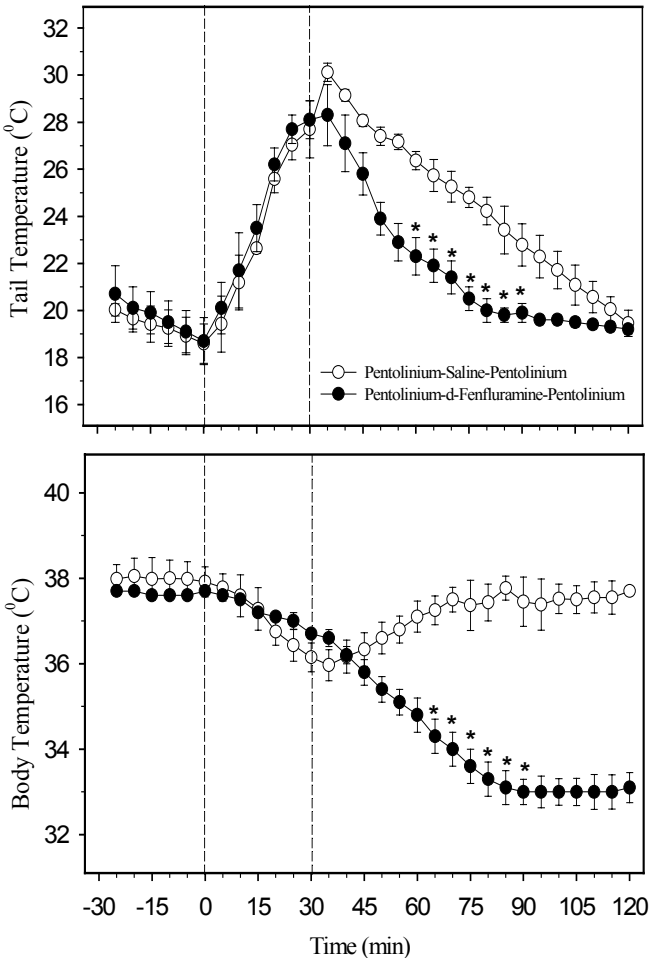

Fig. 26. Effects of pentolinium, 10 mg/kg, i.p., pretreatment on d-fenfluramine, 10 mg/kg, induced increases in tail skin temperature and hypothermia (n= 7/group). Pentolinium was administered to both groups at time "0" and after 30 minutes, one group received saline and the other fenfluramine. After 90 minutes, pentolinium was administered to both groups. Pentolinium produced a significant increase in tail skin temperature, subsequent fenfluramine treatment did not produce any additional increase, instead produced a decrease in tail skin temperature. Pentolinium administration at the end of 90 minutes did not affect tail skin temperature. Pentolinium produced a mild hypothermia. Subsequent d-fenfluramine treatment produced an additional more severe hypothermia. The tail skin and body temperature responses of the groups are significantly different, $p < 0.01$ (Repeated measures ANOVA). Asterisks indicate that there is a significant difference between the two groups at specific time points, Bonferoni t-test.

Also, pentolinium caused an increase in tail temperature in the fluoxetine-fenfluramine group, Fig. 30, top panel.

Fluoxetine treatment produced a significant hypothermia, $p < 0.01$ (Repeated measures ANOVA) when compared to the saline controls, Fig. 29, bottom panel. Core temperature recovered to baseline by the end of the 90-minute observation period (Bonferoni t-test). In the group of animals pretreated with fluoxetine, subsequent d-fenfluramine treatment also produced a hypothermia, $p < 0.01$ (Repeated measures ANOVA), Fig. 30, bottom panel. But the hypothermia to d-fenfluramine was attenuated.

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

d-fenfluramine increased whole body oxygen consumption as compared to the saline controls, $p < 0.01$ (Repeated measures ANOVA). The increase in oxygen consumption develops slowly, Fig. 31, top panel. d-fenfluramine treatment produced a significant hypothermia as compared to the saline controls, $p < 0.01$ (Repeated measures ANOVA), Fig. 31, bottom panel. The increase in oxygen consumption occurs while the body temperature was still declining.

Pentolinium treatment also increases oxygen consumption when compared to the saline controls, $p < 0.01$ (Repeated measures ANOVA), Fig. 32, top panel. The increase peaks at approximately 30 minutes after pentolinium administration. Pentolinium treatment also produces a mild hypothermia when compared to the saline controls, $p < 0.01$ (Repeated measures ANOVA), Fig. 32, bottom panel. The hypothermia to d-fenfluramine was greater than the hypothermia to pentolinium treatment, (Bonferoni t-test).

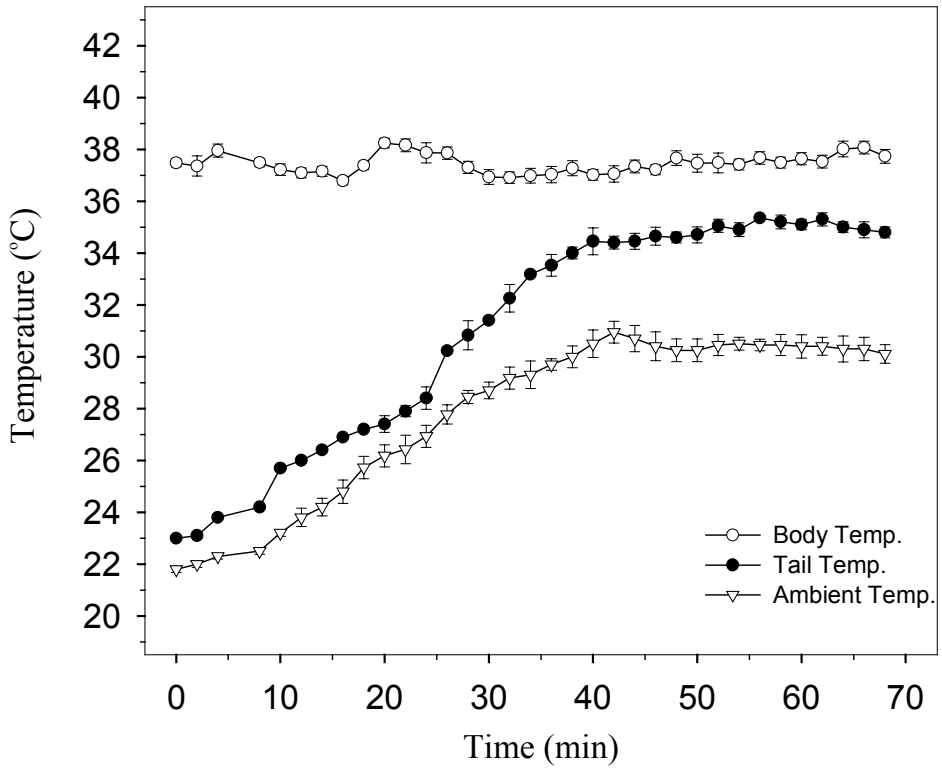

Fig. 27. Effects of increasing ambient temperature on body and tail skin temperature of rats (n=7). The rats were placed in an observation chamber initially maintained at 22^0C. The ambient temperature was gradually increased to 28^0C. There was an abrupt increase in tail skin temperature at 28^0C.

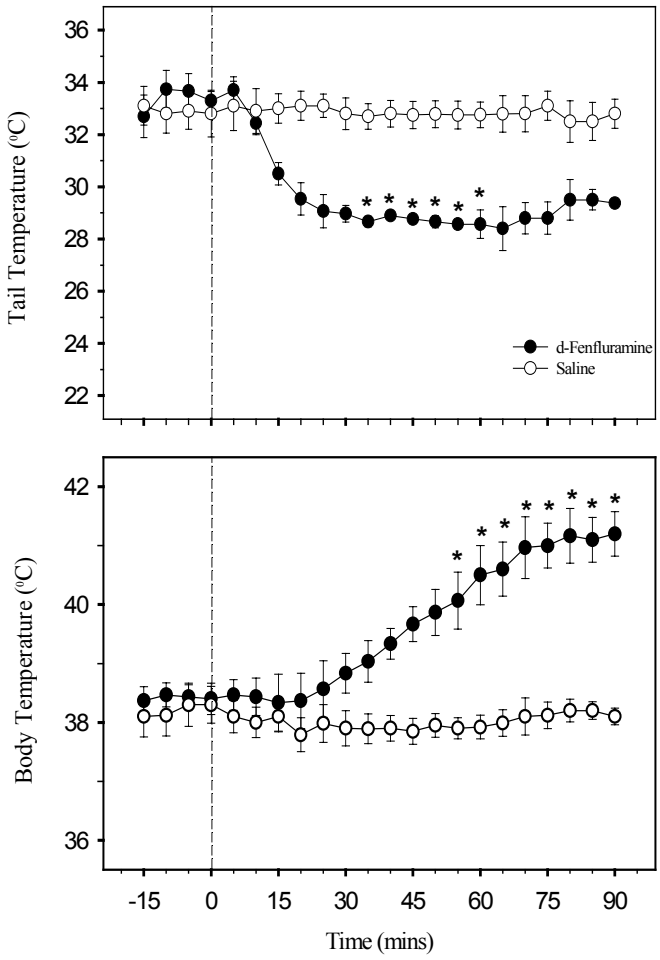

Fig. 28. Effects of saline or d-fenfluramine, 10 mg/kg, i.p., on tail skin and body temperature of rats kept at 28^0C (n=7/group). D-Fenfluramine or saline was administered at time "0". D-Fenfluramine produced a significant decrease in tail skin temperature. D-Fenfluramine also produced a significant hyperthermia. The tail skin and body temperature responses of the d-fenfluramine and saline groups are significantly different, $p < 0.01$ (Repeated measures ANOVA). Asterisks indicate that there is a significant difference in tail skin and body temperature between the two groups at specific time points, Bonferoni t-test.

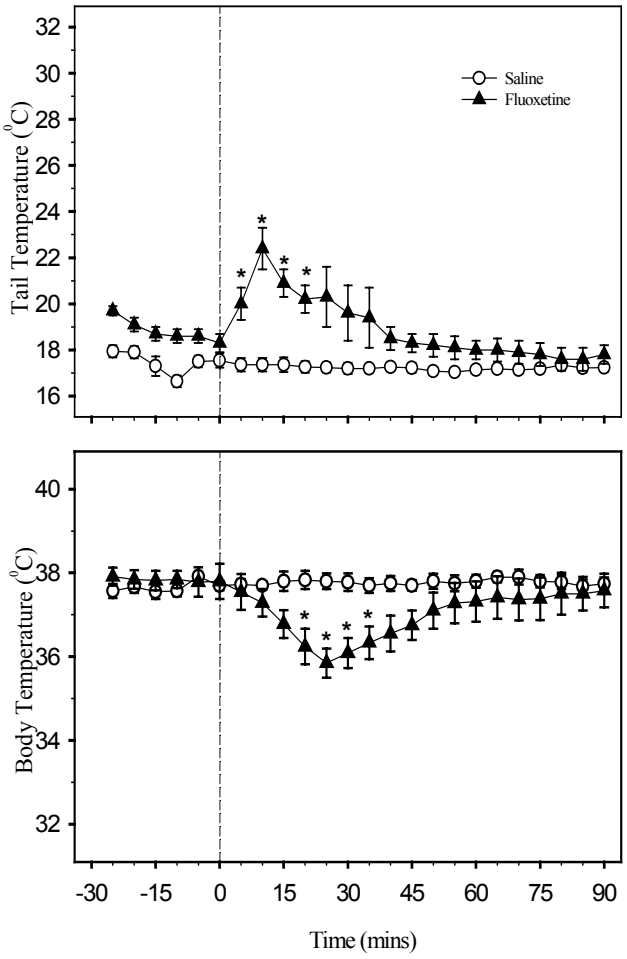

Fig. 29. Effects of saline or fluoxetine, 10 mg/kg, i.p., on tail skin and body temperature of rats kept at 16^0C (n=7/group). Fluoxetine or saline was administered at time "0" and saline was administered to both groups after 30 minutes. Fluoxetine produced an increase in tail skin temperature. Fluoxetine also produced a mild hypothermia. The tail skin and body temperature responses of the groups are significantly different, $p < 0.01$ (Repeated measures ANOVA). Asterisks indicate that there is a significant difference in tail skin and body temperature between the two groups at specific time points, Bonferoni t-test.

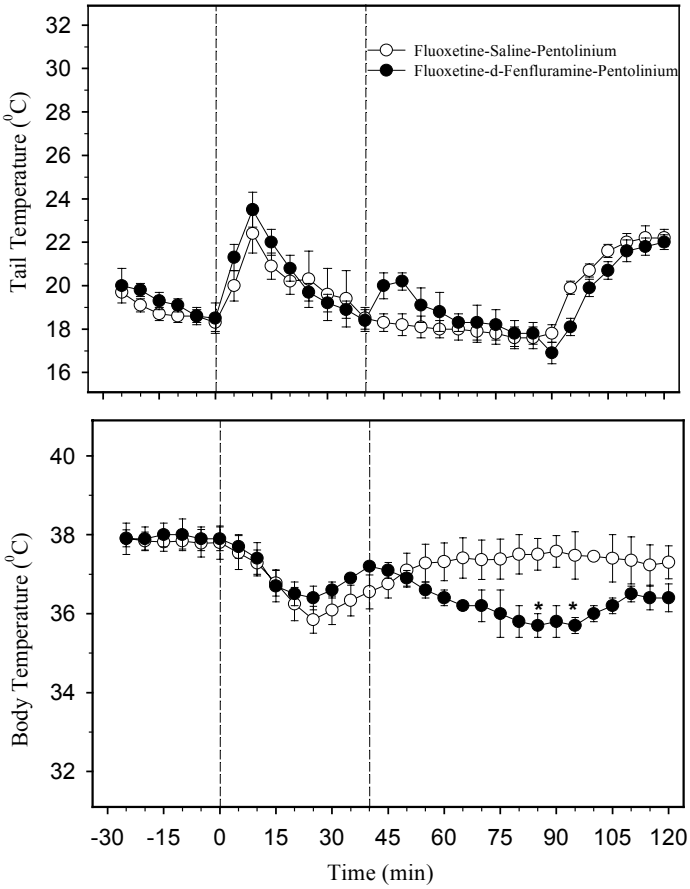

Fig. 30. Effects of fluoxetine, 10 mg/kg, i.p., pretreatment on d-fenfluramine, 10 mg/kg, induced increases in tail skin temperature and hypothermia (n= 7/group). Fluoxetine was administered to both groups at time "0" and after 40 minutes, one group received saline and the other d-fenfluramine. After 90 minutes, pentolinium was administered to both groups. Fluoxetine produced a significant increase in tail skin temperature; subsequent d-fenfluramine treatment did not produce an increase in tail skin temperature. Pentolinium administration at the end of 90 minutes produced an increase in the tail skin temperature in both the groups. Fluoxetine produced a mild hypothermia. The hypothermia to subsequent d-fenfluramine treatment was attenuated. Repeated measures ANOVA indicated that the tail skin and body temperature responses of the two groups are significantly different, $p < 0.01$. Asterisks indicate that there is a significant difference in tail skin and body temperature between the two groups at specific time points, Bonferoni t-test.

The increase in oxygen consumption produced by pentolinium was greater than the oxygen consumption to d-fenfluramine treatment, (Bonferoni t-test).

Pentolinium treatment at the end of 90 minutes, in the d-fenfluramine-treated group did not produce any increase in oxygen consumption, $p < 0.01$ (One way ANOVA, Bonferoni t-test), Fig. 33. Similarly, d-fenfluramine treatment at the end of 90 minutes, in the pentolinium treated group did not increase oxygen consumption, $p < 0.01$ (One way ANOVA, Bonferoni t-test), Fig. 34.

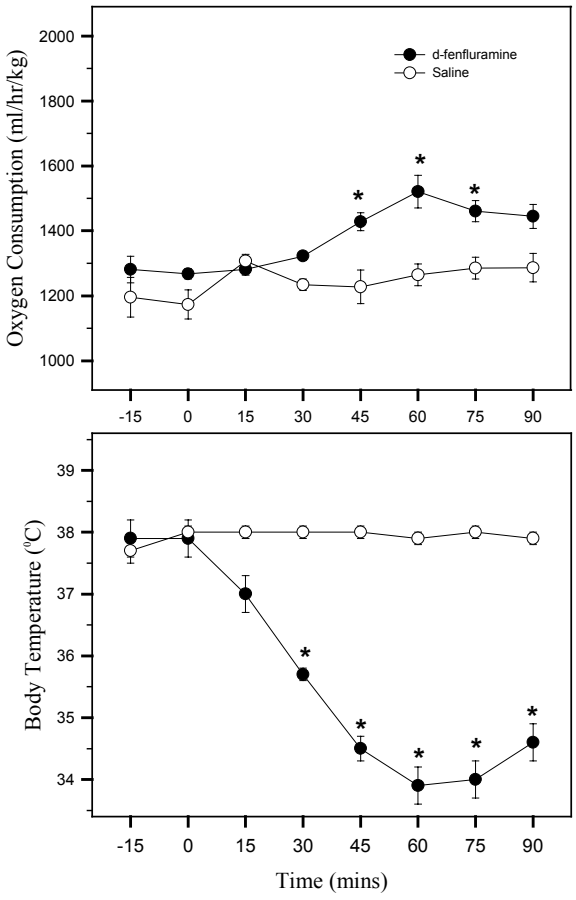

Fig. 31. Effects of saline or d-fenfluramine, 10 mg/kg, i.p., on whole body oxygen consumption (VO_2) and body temperature of rats kept at 16^0C (n=7/group). D-Fenfluramine or saline was administered at time "0". D-Fenfluramine produced a significant increase in VO_2. Repeated measures ANOVA indicated that the groups are significantly different, $p < 0.01$. Asterisks indicate a significant difference between the d-fenfluramine and the saline group at specific time-points, Bonferoni t-test. However, d-fenfluramine produced a significant hypothermia. Repeated measures ANOVA indicated that the groups are significantly different, $p < 0.01$. Asterisks indicate a significant difference between the d-fenfluramine-treated group and the saline control group at specific time-points, Bonferoni t-test.

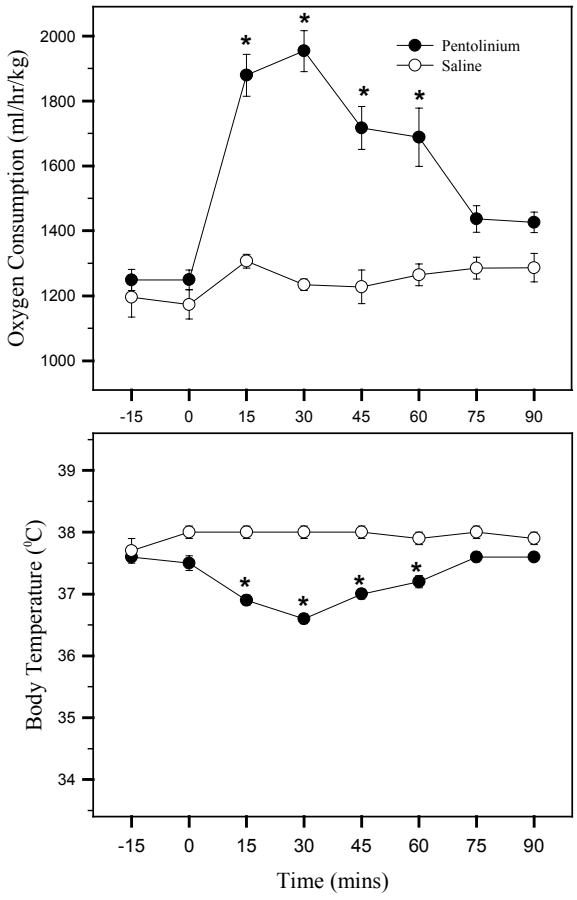

Fig. 32. Effects of saline or pentolinium, 10 mg/kg, i.p., on whole body oxygen consumption (VO_2) and body temperature of rats kept at 16^0C (n=7/group). Pentolinium or saline was administered at time "0". Pentolinium produced a significant increase in VO_2. Repeated measures ANOVA indicated that the groups are significantly different, $p < 0.01$. Asterisks indicate a significant difference between the pentolinium and the saline group at specific time-points, Bonferoni t-test. However, pentolinium produced a significant hypothermia. Repeated measures ANOVA indicated that the groups are significantly different, $p < 0.01$. Asterisks indicate a significant difference between the pentolinium-treated group and the saline control group at specific time-points, Bonferoni t-test.

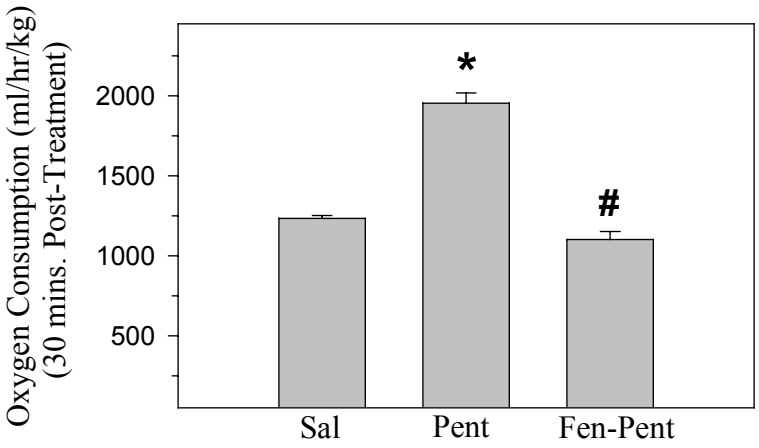

Fig. 33. Effect of d-fenfluramine (Fen), 10 mg/kg, i.p., pretreatment on pentolinium-induced increases in whole body oxygen consumption (VO_2), measured 30 minutes after treatment, in rats kept at 16^0C (n=7/group). Pentolinium (Pent) increased VO_2 significantly, 30 minutes post-treatment. Fen, when administered 90 minutes before Pent, blocked Pent-induced increases in VO_2. One-way ANOVA indicated that the groups are significantly different, $p < 0.01$. Asterisks indicate that the VO_2 to Pent treatment is significantly different from the VO_2 to the saline (Sal) treated control group. The # sign indicates that the VO_2 to Fen-Pent treatment is different from the VO_2 to Pent treatment, Bonferoni t-test.

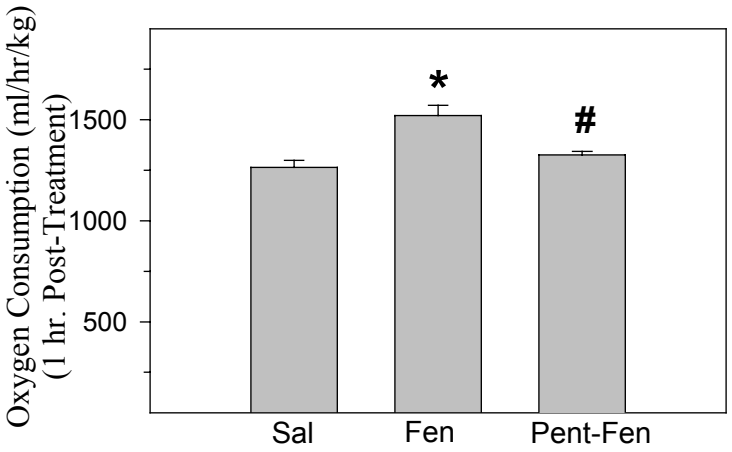

Fig. 34. Effect of pentolinium (Pent), 10 mg/kg, i.p., pretreatment on d-fenfluramine-induced increases in whole body oxygen consumption (VO_2), measured one hour after treatment, in rats kept at 16^0C (n=7/group). D-Fenfluramine (Fen) increased VO_2 significantly, one hour post-treatment. Pent, when administered 90 minutes before Fen, blocked Fen-induced increases in VO_2. One-way ANOVA indicated that the groups are significantly different, $p < 0.01$. Asterisks indicate that the VO_2 to Fen treatment is significantly different from the VO_2 to the saline (Sal) treated control group. The # sign indicates that the VO_2 to Pent-Fen treatment is different from the VO_2 to Fen treatment, Bonferoni t-test.

C. Discussion

The results of the experiments indicate that when animals were maintained at a cool ambient temperature of 16^0C, d-fenfluramine treatment produced an increase in tail skin temperature. This observation suggests that d-fenfluramine produced a dilation of the tail cutaneous vasculature and provides support for the conclusion that d-fenfluramine produces heat loss that may contribute to its hypothermic effect. Furthermore, the results support the conclusion that d-fenfluramine produces a withdrawal of the sympathetic vasoconstrictor tone due to central release of serotonin. Also, the increase in metabolic heat production to d-fenfluramine treatment was insufficient to overcome its hypothermic effect.

An important finding of these experiments is that d-fenfluramine produces heat loss by dilating the cutaneous vasculature in animals maintained at 16^0C. At 16^0C, dilation of the cutaneous vasculature is physiologically inappropriate. d-fenfluramine produced a significant increase in tail skin temperature within minutes of intraperitoneal injection. The increase lasted for about 30 minutes and the tail skin temperature returned to control levels by the end of the 90-minute observation period. Also, d-fenfluramine produced a significant drop in body temperature consistent with the results of the experiments in chapter IV.

The importance of the presence of sympathetic vasoconstrictor tone to the expression of d-fenfluramine-induced increases in tail skin temperature was explored by contrasting the effects of d-fenfluramine on tail skin temperature in animals treated with and without ganglionic blocker pentolinium. Pentolinium treatment resulted in a marked increase in tail skin temperature. The effect lasted the full duration of the 90-minute observation period. This observation is consistent with the reports of others that pentolinium produces an increase in tail skin temperature (Redfern et al., 1995). Disruption of sympathetic cutaneous

vasoconstrictor tone by pentolinium leads to vasodilation as indicated by increases in tail skin temperature. Although there was some recovery of tail temperature towards control levels at the end of the 90-minute observation period, the recovery could not be explained by a waning of the ganglionic blocking activity of pentolinium, since an additional dose of pentolinium had no effect on tail temperature. Thus the dose of pentolinium we selected for the study produced a complete blockade of sympathetic vasoconstrictor tone for the duration of the experiments.

In animals pretreated with pentolinium, subsequent d-fenfluramine treatment did not result in additional increases in tail skin temperature, instead the tail skin temperature decreased and returned to control levels by the end of the 90-minute observation period. This observation indicates that d-fenfluramine cannot cause vasodilation if the sympathetic tone is withdrawn and also that d-fenfluramine may have a vasoconstrictive effect. The fact that d-fenfluramine may have a direct vasoconstrictive effect is also demonstrated by administering pentolinium at the end of the 90 minute observation period. The additional dose of pentolinium did not result in an increase in tail skin temperature. The reduction in tail temperature in the presence of sympathetic blockade suggests that d-fenfluramine produced vasoconstriction of the cutaneous vasculature that was completely independent of sympathetic innervation.

Pentolinium treatment also produced a distinct hypothermia. However, the body temperature returned to control levels by the end of the observation period. In pentolinium pretreated animals, although d-fenfluramine did not produce additional cutaneous vasodilation, d-fenfluramine treatment produced an additional more severe hypothermia.

In addition to the pharmacologic blockade of sympathetic tone, d-fenfluramine was also administered to animals maintained at a warm environmental temperature of 28^0C, an ambient temperature at which the sympathetic tone to the cutaneous vasculature is absent (Hellstorm, 1975a). To assure that the cutaneous vasculature was dilated, animals were placed in the test chamber that was initially at 22^0C. The chamber was gradually warmed toward thermoneutrality and an abrupt dilation of the tail that occurred at approximately 27-28^0C was observed. This confirms previous reports that the physiologic removal of sympathetic tone is an abrupt on/off response (Young et al., 1982; Hellstorm, 1975b). In these animals the withdrawal of sympathetic tone was sustained when the chamber was kept at 28^0C. Tonic sympathetic activity normally keeps the vasculature constricted until high ambient temperatures are reached. This is due to the fact that the animal is able to maintain a normal body core temperature throughout a wide range of temperatures before the ambient temperature reaches the point at which vasodilation is needed to help dissipate heat. The results are consistent with this concept, since no evidence of cutaneous dilation was found until the ambient temperature was increased to about 28^0C. At that temperature there was a dramatic increase in tail temperature that is consistent with vasodilation. Administration of d-fenfluramine in the warm environment did not cause vasodilation, a finding that supports the concept that d-fenfluramine causes vasodilation by withdrawing sympathetic tone. Thus, when sympathetic tone is removed physiologically in a warm environment or pharmacologically through administration of pentolinium, d-fenfluramine does not produce additional vasodilation. Also in the absence of sympathetic tone at 28^0C, d-fenfluramine produced a vasoconstriction of the cutaneous vasculature, which is physiologically inappropriate at this temperature. The vasoconstriction was similar in time course and

magnitude to the vasoconstriction observed in animals pretreated with pentolinium at an environmental temperature of 16^0C.

d-fenfluramine treatment of animals kept at a warm environmental temperature, 28^0C, produced an increase in body core temperature. While the finding that d-fenfluramine produced hyperthermia at 28^0C confirms previous reports that the environmental temperature influences the effects of d-fenfluramine on body temperature (Malberg et al., 1997; Preston et al., 1990), this study provides new insights that relate the changes in body temperature to the effect of d-fenfluramine on the tone of the cutaneous vasculature.

The dilation of the cutaneous vasculature by d-fenfluramine also appears to depend on d-fenfluramine-mediated release of serotonin. This point was demonstrated by pretreating animals with the serotonin reuptake inhibitor fluoxetine, which blocks d-fenfluramine-induced serotonin release. In fluoxetine treated animals, d-fenfluramine produced no vasodilation. This observation suggests that d-fenfluramine must release serotonin in order for its vasodilator action to be observed. Also, in fluoxetine treated animals, d-fenfluramine produced no vasoconstriction. This observation suggests that d-fenfluramine must release serotonin to produce vasoconstriction. From the other experiments conducted using pentolinium, we know that the vasoconstrictor effect of fenfluramine is not mediated via the sympathetic nervous system but appears to be a direct effect. Therefore it appears that fenfluramine releases serotonin, possibly from the platelets and produces vasoconstriction.

Central administration of serotonin to urethane anesthetized rats produced tail vasodilation as determined by measurement of tail surface temperature (Key et al., 1992). Interestingly, our results indicate that d-fenfluramine administration releases sufficient serotonin centrally to produce an increase in tail skin temperature. And also, fluoxetine,

which increases synaptic serotonin concentrations, produces tail dilation as shown by others (Lin, 1978) and us. However, the effect lasted only for about 30 minutes.

Fluoxetine treatment also produced a mild hypothermia, which lasted for about 30 minutes. Fluoxetine-mediated hypothermia could be attributable to its effects on tail vascular tone. The hypothermia to d-fenfluramine also appears to depend on its ability to release serotonin because blockade of d-fenfluramine-mediated serotonin release by fluoxetine attenuates the hypothermic response to d-fenfluramine. A previous study has also demonstrated that serotonin reuptake inhibitor sertraline-pretreatment attenuates the hypothermia to fenfluramine (Cryan et al., 2000).

Body temperature of the animals started to fall after d-fenfluramine treatment. The hypothermia lasted the entire 90-minute observation period. In fact, the hypothermia to d-fenfluramine at 16^0C was similar in magnitude to the hypothermia to d-fenfluramine at 4^0C. The vasodilation and consequent heat loss to d-fenfluramine treatment may contribute to the hypothermia that was observed. However cutaneous heat loss cannot be the full explanation for the fall in core temperature after d-fenfluramine because pentolinium produced an even greater dilation of the cutaneous vasculature but the fall in core temperature to pentolinium was significantly less than the fall in core temperature to d-fenfluramine. The animals treated with pentolinium recovered from the hypothermia and their body temperature returned to control levels by the end of the 90-minute observation period. Also, despite the fact that d-fenfluramine did not produce additional cutaneous dilation after pentolinium pretreatment, the treatment resulted in an additional more severe hypothermia.

Thus, cutaneous vasodilation and consequent heat loss could account, in part, for the hypothermia to d-fenfluramine. However, for reasons mentioned in the above paragraph,

cutaneous vasodilation alone could not account for the hypothermia to d-fenfluramine treatment. The other possible mechanism that could account for the hypothermia was insufficient heat generation. The second major objective of this study was to evaluate the effect of d-fenfluramine on total metabolic heat production. Whole body oxygen consumption was measured as an index of metabolic rate.

The results of the experiments clearly demonstrate that d-fenfluramine increases whole body oxygen consumption in animals maintained at 16^0C. However, the increment in oxygen consumption was not sufficient to offset the fall in body temperature as indicated by the marked hypothermia. Interestingly, pentolinium increased oxygen consumption to a much greater extent than the increase produced by d-fenfluramine treatment. Taken with the results of the other experiments in this chapter, which demonstrated the tremendous cutaneous vasodilation produced by pentolinium, it appears that the heat generated by increased metabolic activity offset the heat loss to pentolinium treatment and explains why pentolinium treated animals became only slightly hypothermic when compared to d-fenfluramine treated animals.

Clearly, pentolinium produces a marked increase in oxygen consumption in order to offset the heat being lost due to cutaneous vasodilation. The increased metabolic activity is likely not explainable by increased activity of the sympathetic or parasympathetic nervous systems, since pentolinium is a ganglionic blocker. Of particular importance is that BAT thermogenesis produced by sympathetic stimulation would be blocked by pentolinium. Therefore, the increase in metabolic activity would have to be mediated by the nonautonomic system. Nonautonomic mechanisms involved in heat generation have classically been referred to as shivering thermogenesis whereas BAT thermogenesis has been referred to as

nonshivering-thermogenesis. Thus despite the fact that pentolinium cannot activate sympathetically mediated thermogenesis, pentolinium mediated increases in whole body oxygen consumption is greater than that of d-fenfluramine. One potential explanation for this could be the fact that pentolinium must activate other nonsympathetic thermogenic mechanisms like shivering to generate heat to compensate for the heat loss mediated through cutaneous vasodilation. Alternatively, d-fenfluramine, activates BAT thermogenesis, yet produces a lesser increase in oxygen consumption probably due to the impairment of nonsympathetically mediated mechanisms of heat generation i.e., shivering thermogenesis. This finding is also supported by the fact that d-fenfluramine pretreatment blocks the increase in oxygen consumption to pentolinium. This indicates that d-fenfluramine blocks shivering mechanisms of heat generation and thereby blocks the increase in oxygen consumption to subsequent pentolinium treatment. Similarly, pentolinium pretreatment blocks d-fenfluramine-induced increases in oxygen consumption implying that pentolinium blocks nonshivering thermogenesis (which was also indicated by the fact that pentolinium blocks d-fenfluramine-induced BAT NE depletion) and therefore subsequent d-fenfluramine treatment cannot produce an increase in oxygen consumption.

Thus, the results of the experiments indicate that even though d-fenfluramine increases metabolic heat production through BAT thermogenesis, the increase is insufficient to offset the heat loss it produces and the animal becomes hypothermic.

Chapter VI. Conclusions

The experimental findings presented in this thesis provide insights into the mechanisms by which d-fenfluramine affects body temperature regulation. In the course of conduct of the experiments in this thesis, we found that d-fenfluramine treatment altered body temperature regulation over a range of ambient temperatures as shown in Fig. 35. From the figure, it is clear that the effect of d-fenfluramine on body temperature is dependent upon the ambient temperature at which the animal is maintained, at warm ambient temperatures, d-fenfluramine produces hyperthermia whereas at normal and cooler ambient temperatures, d-fenfluramine produces varying degrees of hypothermia. It is important to note that animals not treated (saline treated) with d-fenfluramine were able to maintain body temperature through the different ambient temperatures (Fig. 35). The effects of d-fenfluramine on body temperature regulation at each of the ambient temperatures shown in Fig. 35 can be explained by the findings of the present study.

As shown in Fig. 35, at an ambient temperature of 28^0C, d-fenfluramine treatment resulted in a distinct hyperthermia. We found that the hyperthermia to d-fenfluramine treatment is due to increased heat generation through BAT activation and the subsequent inability of the animal to lose the excess heat, since d-fenfluramine produced a constriction of the cutaneous vasculature at 28^0C. Although, others have reported that d-fenfluramine increased body temperature at 28^0C, they attributed the hyperthermia to only the activation of BAT thermogenesis. Thus, an important finding of our study is that d-fenfluramine produces cutaneous vasoconstriction at 28^0C. The vasoconstrictive effect is independent of the ambient temperature at which the animal is maintained because d-fenfluramine produced vasoconstriction at 16^0C also. Similarly, the activation of BAT thermogenesis by d-

fenfluramine also occurs at all ambient temperatures. d-fenfluramine mediated BAT activation appears to depend upon fenfluramine-induced serotonin release since fluoxetine blocks this effect. Thus, d-fenfluramine stimulates thermogenesis in brown fat and caused cutaneous vasoconstriction, both actions are inappropriate at the environmental temperature, 28^0C, at which the animal is maintained and consequently produces hyperthermia.

As shown in Fig. 35, our results indicate that d-fenfluramine treatment resulted in a significant hypothermia at 22^0C. However, some reports in the literature have indicated that d-fenfluramine produced no change in body temperature at 22^0C. The fact that we observed a significant hypothermia is because we measured body temperature by telemetry, which eliminated temperature artifacts due to handling related stress involved in using rectal thermometers, which leads to an increase in body temperature. At 16^0C, d-fenfluramine produced a marked hypothermia. In fact, the magnitude of the hypothermia at 16^0C was no greater than the magnitude of hypothermia at 4^0C. Our results indicate that at 16^0C, d-fenfluramine treatment resulted in a dilation of the cutaneous vasculature. The effects of d-fenfluramine on the cutaneous vasculature, vasodilation and vasoconstriction are mediated via fenfluramine-induced release of serotonin, since fluoxetine blocked both these effects. However, the vasodilation and the consequent heat loss cannot completely account for the hypothermia to d-fenfluramine because pentolinium produced a greater cutaneous dilation, which lasted for a longer duration of time, yet produced a lesser hypothermia. Therefore, we speculated that d-fenfluramine might interfere with non-BAT heat generating mechanisms that include increased locomotor activity and shivering.

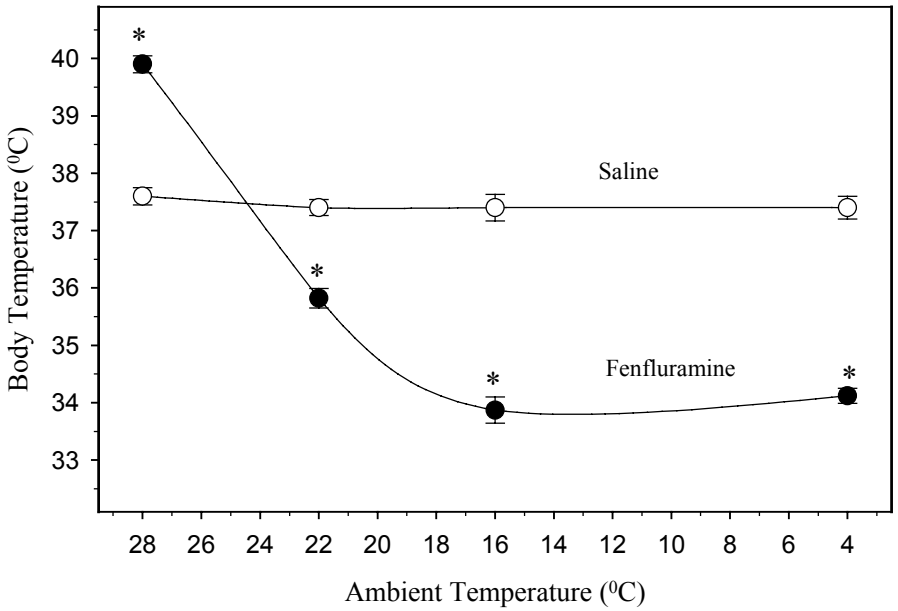

Fig. 35. Summary of the effects of d-fenfluramine, 10 mg/kg, i.p., or saline on body temperature, one hour post-treatment, of rats kept at different ambient temperatures. The data presented in this figure is consolidated from the results of chapters IV and V. At 28^0C, d-fenfluramine treatment resulted in hyperthermia. However, at 22, 16 and 4^0C, d-fenfluramine treatment resulted in varying degrees of hypothermia. The body temperature responses of d-fenfluramine treated animals are significantly different from the corresponding saline treated controls (as determined previously by Repeated measures ANOVA).

d-fenfluramine treatment did not affect locomotor activity. However, we visually determined that d-fenfluramine treated animals did not shiver. On the other hand, animals treated with pentolinium were observed to be markedly shivering and the heat generated through shivering appears to compensate for the heat loss that occurs following pentolinium treatment since the animals recovered from the hypothermia. Thus, we concluded that d-fenfluramine interfered with shivering thermogenesis and the heat generated through BAT activation was not sufficient to offset the heat loss and thus d-fenfluramine produces hypothermia.

In support of our conclusion, we found that the pentolinium treatment increased total metabolic heat production to a much greater magnitude compared to d-fenfluramine treatment. And pentolinium, being a ganglionic blocker, cannot activate sympathetically mediated BAT thermogenesis. Even though, d-fenfluramine increased total metabolic heat production through BAT activation, the heat generated could not compensate for the heat lost and thus produces hypothermia.

In summary, the experiments in this thesis have provided insights into the multiple actions of d-fenfluramine that compromise the maintenance of body temperature as indicated in Fig. 36.

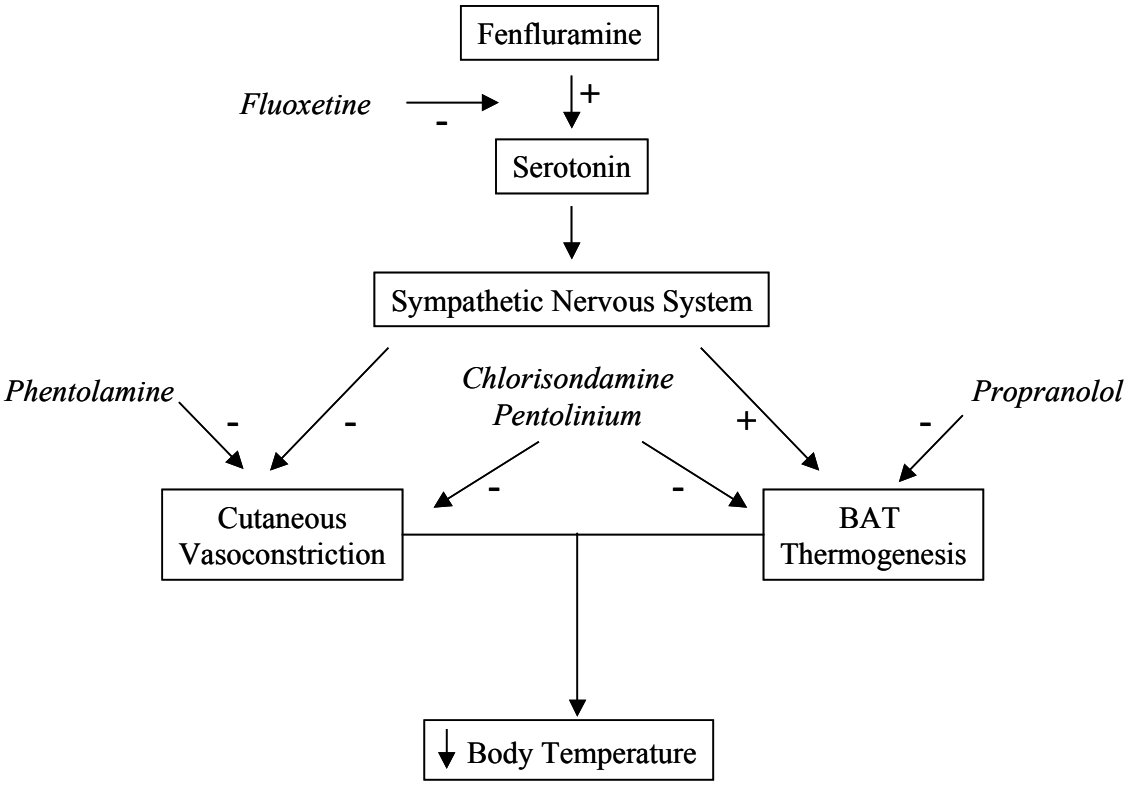

Figure 36. Summary of the mechanisms by which d-fenfluramine produces hypothermia. The results of the experiments in this dissertation indicate that even though d-fenfluramine activates brown adipose tissue (BAT) thermogenesis, the heat generated could not compensate for the heat loss produced due to cutaneous vasodilation and thus produces hypothermia. The effects of d-fenfluramine on BAT and the cutaneous vasculature were produced via release of central serotonin and through subsequent modulation of the sympathetic nervous system.