

# TABLE OF CONTENTS

<b>FOREWORD</b> .....	iv
<b>TABLE OF CONTENTS</b> .....	v
<b>LIST OF FIGURES</b> .....	ix
<b>I. Overview</b> .....	1
A. Introduction.....	1
B. Literature Survey.....	5
1. The role of the sympathetic nervous system in thermoregulation .....	5
a. Sympathetic nervous system control of BAT thermogenesis.....	6
b. Sympathetic nervous system control of cutaneous vascular responses .....	9
2. Effects of fenfluramine on body temperature regulation.....	12
a. Effects of fenfluramine on BAT thermogenesis .....	13
b. Effects of fenfluramine on the cutaneous vasculature.....	14
c. Effects of fenfluramine on metabolic rate.....	17
3. The role of serotonin in thermoregulation.....	17
C. Specific Objectives of Research.....	20
<b>II. Methods</b> .....	23
A. Care and Housing of Animals .....	23
B. Measurement of Body Temperature by Temperature Telemetry and Computerized Data Acquisition System .....	23

C. Measurement of Tail Skin Temperature Using Thermocouples in a Controlled Temperature Environment.....	25
D. Measurement of Oxygen Consumption Using Open Circuit Calorimetry.....	27
E. Collection of Tissue Samples for Biochemical Analyses .....	27
F. Measurement of Catecholamines by High Performance Liquid Chromatography.....	30
G. Statistics .....	31
<b>III. Pharmacologic Assessment of the Contribution of the Sympathetic Nervous System to the Maintenance of Body Temperature in Conscious Rats.....</b>	<b>32</b>
A. Protocols.....	34
B. Results .....	35
1. Effects of saline, 1ml/kg, i.p., on body temperature, locomotor activity, adrenal and BAT catecholamine content.....	35
2. Effects of phentolamine, 2 mg/kg, i.p., on body temperature, locomotor activity, adrenal and BAT catecholamine content.....	36
3. Effects of propranolol, 3 mg/kg, i.p., on body temperature, locomotor activity, adrenal and BAT catecholamine content.....	36
4. Effects of phentolamine, 2 mg/kg, i.p., and propranolol, 3 mg/kg, i.p., on body temperature, locomotor activity, adrenal and BAT catecholamine content.....	41
5. Effects of chlorisondamine, 3 mg/kg, i.p., on body temperature, locomotor activity, adrenal and BAT catecholamine content.....	42
C. Discussion .....	47

<b>IV. Evaluation of the Effects of Fenfluramine on Sympathetic Nervous System Regulation of Body Temperature</b> .....	54
A. Protocols.....	57
B. Results .....	60
1. Effects of dl- fenfluramine, 10 mg/kg, i.p., on body temperature and BAT NE content.....	60
2. Effects of d-fenfluramine, 3 and10 mg/kg, i.p., on body temperature and catecholamine content of sympathetically innervated tissues.....	61
3. Effects of pentolinium, 10 mg/kg, i.p., and fluoxetine, 10 mg/kg, i.p., pretreatment on d-fenfluramine-induced BAT NE depletion.....	64
C. Discussion .....	68
<b>V. Evaluation of the Effects of d-Fenfluramine on the Cutaneous Vasculature and Total Metabolic Heat Production</b> .....	75
A. Protocols.....	78
B. Results .....	81
1. Effects of d-fenfluramine, 10 mg/kg, i.p., on tail skin and body temperature of rats kept at 16 <sup>0</sup> C.....	81
2. Effects of pentolinium pretreatment on d-fenfluramine-induced increases in tail skin temperature and hypothermia.....	82
3. Effects of d-fenfluramine, 10 mg/kg, i.p., on tail skin and body temperature of rats kept at 28 <sup>0</sup> C (thermoneutrality) .....	83
4. Effects of fluoxetine pretreatment on d-fenfluramine induced increases in tail skin temperature and hypothermia.....	83
5. Effects of d-fenfluramine and pentolinium on whole body oxygen consumption .....	87

C. Discussion .....	97
<b>VI. Conclusions</b> .....	104
<b>VII. Bibliography</b> .....	109

## LIST OF FIGURES

Figure 1.	Sympathetic pathways involved in thermoregulation .....	8
Figure 2.	The chemical structures of fenfluramine and amphetamine .....	11
Figure 3.	The sites of actions of fenfluramine .....	16
Figure 4.	Effect of chlorisondamine, 3mg/kg, i.p., on body temperature of rats 24-hours after surgery to implant temperature transmitter .....	26
Figure 5.	Effect of chlorisondamine, 3 mg/kg, i.p., on body temperature of rats after five days of recovery following surgery to implant temperature transmitter .....	26
Figure 6.	Measurement of body temperature using temperature telemetry and computerized data acquisition system and tail skin temperature using thermocouples .....	28
Figure 7.	Laboratory arrangement for performing experiments in a temperature-controlled environment .....	29
Figure 8.	Effects of saline, 1ml/kg, i.p., on body temperature and locomotor activity of rats kept at 22 <sup>0</sup> C (n=7) and 4 <sup>0</sup> C (n=7) .....	37
Figure 9.	Effects of saline, 1ml/kg, i.p., on adrenal epinephrine and norepinephrine (NE) content and brown adipose tissue (BAT) NE content in rats kept at 22 <sup>0</sup> C (n=7) and 4 <sup>0</sup> C (n=7) .....	38
Figure 10.	Effects of phentolamine, 2mg/kg, i.p., on body temperature and locomotor activity of rats kept at 22 <sup>0</sup> C (n=7) and 4 <sup>0</sup> C (n=7) .....	39
Figure 11.	Effects of phentolamine, 2mg/kg, i.p., on adrenal epinephrine and norepinephrine (NE) content and brown adipose tissue (BAT) NE content in rats kept at 22 <sup>0</sup> C (n=7) and 4 <sup>0</sup> C (n=7) .....	40
Figure 12.	Effects of propranolol, 3 mg/kg, i.p., on body temperature and locomotor activity of rats kept at 22 <sup>0</sup> C (n=7) and 4 <sup>0</sup> C (n=7) .....	43
Figure 13.	Effects of propranolol, 3 mg/kg, i.p., on adrenal epinephrine and norepinephrine (NE) content and brown	

	adipose tissue (BAT) NE content in rats kept at 22 <sup>0</sup> C (n=7) and 4 <sup>0</sup> C (n=7) .....	44
Figure 14.	Effects of phentolamine, 2 mg/kg, i.p. and propranolol, 3 mg/kg, i.p., on body temperature and locomotor activity of rats kept at 22 <sup>0</sup> C (n=7) and 4 <sup>0</sup> C (n=7).....	45
Figure 15.	Effects of phentolamine, 2 mg/kg, i.p. and propranolol, 3 mg/kg, i.p., on adrenal epinephrine and norepinephrine (NE) content and brown adipose tissue (BAT) NE content in rats kept at 22 <sup>0</sup> C (n=7) and 4 <sup>0</sup> C (n=7).....	46
Figure 16.	Effects of chlorisondamine, 3 mg/kg, i.p., on body temperature and locomotor activity of rats kept at 22 <sup>0</sup> C (n=7) and 4 <sup>0</sup> C (n=7) .....	48
Figure 17.	Effects of chlorisondamine, 3mg/kg, i.p., on adrenal epinephrine and norepinephrine content (NE) and brown adipose tissue (BAT) NE content in rats kept at 22 <sup>0</sup> C (n=7) and 4 <sup>0</sup> C (n=7) .....	49
Figure 18.	Effects of saline or dl-fenfluramine , 10 mg/kg, i.p., on body temperature of rats kept at 22 and 4 <sup>0</sup> C (n=7/group).....	62
Figure 19.	Effects of saline or dl-fenfluramine, 10 mg/kg, i.p., on brown adipose tissue (BAT) norepinephrine (NE) content of rats kept at 22 and 4 <sup>0</sup> C (n=7/group).....	63
Figure 20.	Effects of saline or d-fenfluramine, 3, 10 mg/kg, i.p., on body temperature of rats kept at 22 and 4 <sup>0</sup> C (n=7/group).....	65
Figure 21.	Effects of saline or d-fenfluramine, 3 and 10 mg/kg, i.p., on brown adipose tissue (BAT) and white adipose tissue (WAT) norepinephrine (NE) content of rats kept at 22 <sup>0</sup> C and 4 <sup>0</sup> C (n=7/group).....	66
Figure 22.	Effects of saline (Sal) or pentolinium (Pent), 10mg/kg, i.p. pretreatment on d-fenfluramine (FEN), 10 mg/kg, i.p. induced changes in brown adipose tissue (BAT) norepinephrine (NE) content (n=7/group) .....	69
Figure 23.	Effects of saline (Sal) or fluoxetine (Fluo), 10mg/kg, i.p. pretreatment on d-fenfluramine (FEN), 10 mg/kg, i.p. induced changes in brown adipose tissue (BAT) norepinephrine (NE) content (n=7/group) .....	70

Figure 24.	Effects of saline or d-fenfluramine, 10 mg/kg, i.p., on tail skin and body temperature of rats kept at 16 <sup>0</sup> C (n=7/group) .....	84
Figure 25.	Effects of saline or pentolinium, 10 mg/kg, i.p., on tail skin and body temperature of rats kept at 16 <sup>0</sup> C (n=7/group).....	85
Figure 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) .....	86
Figure 27.	Effects of increasing ambient temperature on body and tail skin temperature of rats (n=7) .....	88
Figure 28.	Effects of saline or d-fenfluramine, 10 mg/kg, i.p., on tail skin and body temperature of rats kept at 28 <sup>0</sup> C (n=7/group) .....	89
Figure 29.	Effects of saline or fluoxetine, 10 mg/kg, i.p., on tail skin and body temperature of rats kept at 16 <sup>0</sup> C (n=7/group).....	90
Figure 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) .....	91
Figure 31.	Effects of saline or d-fenfluramine, 10 mg/kg, i.p., on whole body oxygen consumption (VO <sub>2</sub> ) and body temperature of rats kept at 16 <sup>0</sup> C (n=7/group) .....	93
Figure 32.	Effects of saline or pentolinium, 10 mg/kg, i.p., on whole body oxygen consumption (VO <sub>2</sub> ) and body temperature of rats kept at 16 <sup>0</sup> C (n=7/group).....	94
Figure 33.	Effects of d-fenfluramine (Fen), 10 mg/kg, i.p., pretreatment on pentolinium-induced increases in whole body oxygen consumption (VO <sub>2</sub> ), measured 30 minutes after treatment, in rats kept at 16 <sup>0</sup> C (n=7/group) .....	95
Figure 34.	Effects of pentolinium (Pent), 10 mg/kg, i.p., pretreatment on d-fenfluramine-induced increases in whole body oxygen consumption (VO <sub>2</sub> ), measured one hour after treatment, in rats kept at 16 <sup>0</sup> C (n=7/group).....	96
Figure 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 .....	106

Figure 36. Summary of the mechanisms by which d-fenfluramine  
produces hypothermia ..... 108