To the Graduate Council:

I am submitting herewith a dissertation written by Ingrid Edgemon Hensley entitled "Effects of Diazoxide on NPY Content in Discrete Brain Nuclei of Lean and Obese Zucker Rats." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Psychology.

James E. Lawler, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor and
Dean of The Graduate School

EFFECTS OF DIAZOXIDE ON NPY CONTENT IN DISCRETE BRAIN NUCLEI OF LEAN AND OBESE ZUCKER RATS

A Dissertation
Presented for the
Doctor of Philosophy Degree
The University of Tennessee, Knoxville

Ingrid Edgemon Hensley
August 1999

DEDICATION

This doctoral dissertation research is dedicated to the memories of

Jimmy Catherine Snodgrass Kiser Brey

Her courage, strength, and unwavering faith

were a testament to her

independent and optimistic spirit which

will remain in the hearts and minds of those who knew her.

and

Valery (Konstantinovich) Kroutchinin

His love for both the principles and practice of

brain science as well as his kind, gentle nature

and his enthusiasm for all of his work were

an inspiration to all who knew him.

They fought the good fight and are sorely missed.

ACKNOWLEDGMENTS

The research presented in this doctoral dissertation would not have been possible without the assistance of many individuals. I should begin by extending my thanks to Baker-Norton Pharmaceuticals, Dr. Marvin Brown of the Alanex Corporation, and the Department of Pediatrics Research at the University of Tennessee Medical Center. They provided the drugs, antibodies, and supplies needed to carry out the laboratory aspects of this project. I also need to acknowledge the many individuals and the colleagues of their laboratories who provided both equipment and technical expertise in various aspects of this study: Dr. Jeffrey Becker, Dr. Roger Carroll, Dr. Jay Wimalasena, Dr. Robert Hall, and Dr. Michael Karlstad. Special appreciation is also extended to Dr. Sarah Leibowitz of Rockefellar University and her colleague Jordan Dourmashkin. They generously shared their research protocols and entertained my many questions on multiple occassions.

In addition to these individuals, I must recognize those people who, throughout my graduate career, taught me how to perform the technical aspects of research with knowledge and accuracy, Pam Stott, Susan Naylor, Melanie James, Meg Buchholz, Andrea Wood Youngstrom, Guanping Zheng, Ching Hsuang Wang, and Shenggang Li. Their talent in conducting research is a demonstration of how vital people "behind the scenes" are for accomplishing great tasks. I also need to thank beyond words Sidney J. Holshouser. He is without a doubt an exceptional technician and friend. His knowledge for conducting research and his dedication to gathering data that

are precise continue to be the model that I set for myself. He helped in every aspect of this current research. His steady humor made the many long hours of laboratory work involved in this project bearable.

I would like also to thank the members of my doctoral committee, Dr. Kathleen Lawler, Dr. Debora Baldwin, Dr. Ramin Alemzadeh, and Dr. James Lawler, for their patience and contributions to the process of conducting the current project. As a student, I was fortunate to participate in research in the labs of Dr. Kathleen Lawler and Dr. Debora Baldwin and to have both of them as instructors. The experiences that I gained in their labs and classrooms are invaluable. Dr. Ramin Alemzadeh took a chance on an unknown commodity, a student of psychology who had almost no background in obesity research, and provided me with the opportunity to conduct this project. I will never forget, or be able to show him, how much I appreciate the chance that he took in providing me with this opportunity. Finally, I must express my appreciation to Dr. James Lawler. He welcomed me into his lab, when I had no background to be there, and while I was there, he showed me how to conduct research with enjoyment and integrity, always seeking the truth and allowing it to be made known. He continues to be not only my mentor, but also my good friend.

I cannot conclude my statement of acknowledgments without also mentioning my family and friends. Without the multiple forms of support provided by my immediate family, extended family, and friends throughout my graduate education, I would never had been able to reach this moment in my

career as a student. I must extend special thanks to my parents for providing me with a pure love for gaining knowledge and understanding of this world. They have encouraged and provided every educational opportunity available throughout my life. For those things and so many others, I will never be able to thank them enough.

Finally, I must acknowledge the person who continually supported, encouraged, and coached me through each day of graduate school, my husband, Mark. He knew when to comfort and cajole me gently and when to use tough love. His amazing cooperation and patience are beyond my understanding and made the long days of studying and research possible.

ABSTRACT

Neuropeptide Y (NPY) is a powerful stimulant of feeding behavior and metabolic efficiency. Located throughout hypothalamic and extra-hypothalamic nuclei, NPY responds to circulating indices of nutritional status like insulin. Obese Zucker rats possess an autosomal recessive trait to become hyperphagic and hyperinsulinemic. Obese Zucker rats also demonstrate chronically elevated levels of NPY throughout the hypothalamus. Some researchers speculate that obese rats possess an insensitive insulin-NPY signaling mechanism resulting in the elevated NPY of these animals. Previous research with the insulin antagonist diazoxide (DZ) demonstrates the drug to be effective at lowering circulating insulin levels of obese rats as well as reducing their food intake and weight gain across the period of treatment. In addition, these peripheral indices of metabolic normalization are accompanied by enhanced levels of insulin in the brain as measured in cerebrospinal fluid. It is possible that the enhanced entry of insulin into the brains of the obese rats causes a reduction in NPY in hypothalamic and extra-hypothalamic nuclei resulting in normalized metabolism and feeding behavior. Thus, the purpose of the current research was to determine if DZ administration across the same time frame that led to enhanced entry of insulin into the brain leads to suppressed hypothalamic and extra-hypothalamic NPY levels. Female lean and obese Zucker rats, approximately six weeks of age, were placed on DZ (150 mg/kg/day) or placebo suspension for about four weeks. Following drug treatment, animals

remained free fed or were fasted overnight (12 to 16 hours) before rapid sacrifice by decapitation. Serum was obtained for measurement of insulin and glucose levels, and brains were removed, frozen, and later sliced and punched to obtain the nuclei of interest: ARC, ME, DMH, LH, VMH, PFH, CNA, PVNp, PVNm, AHA, SO, SCN, BNST, MPOA, and LPOA. Overnight fasting lowered insulin of control obese and lean rats, yet lowered glucose only in leans. Fasting also elevated ME and combined ARC-ME and lowered LH NPY levels of obese rats with no effects occurring in leans. DZ treatment reduced weight gain in obese rats only, lowered glucose of both lean and obese rats, but failed to significantly lower insulin of either genotype. Fasting led to more exaggerated effects on NPY than free feeding. DZ elevated ARC, combined ARC-ME, and VMH NPY levels of free fed obese rats, but only elevated LH levels of lean rats. For fasted animals, DZ elevated LH and PFH of obese rats whereas the drug elevated BNST and lowered CNA of lean rats. DZ treatment appears to affect both hypothalamic and extra-hypothalamic NPY concentrations of lean and obese Zucker rats. However, the etiology of those changes (e.g., synthesis, release, or transport) remains unknown.

TABLE OF CONTENTS

CHAPTER PAGE

I. INTRODUCTION 1

Rationale 1

Overview of Research Project and Statement of Hypotheses 1

II. REVIEW OF THE LITERATURE 6

Obesity and Abnormal Insulin Regulation 6
Human Studies 6
Insulin and Obesity 7
Hyperinsulinism and Food Intake 9
Animal Studies: Zucker rats 10
History 11
Hyperinsulinism 12
Feeding Behavior 13
Brain-Insulin Model 14

Central Controls of Feeding and Metabolics: NPY 17
Central Nervous System Features of NPY 17
Distribution 18
Receptors 21
Cellular Characteristics 22
Pathways 23
Characteristics of NPY as a Feeding Motivator 27
Primary Feeding Characteristics 33
Feeding-Related Behaviors, Motivation, and Nutrient Preferences 36
Metabolism, Independent of Feeding 42
Feeding Receptor Specificity 46
Food Deprivation 50
Relationship of NPY and Insulin 53
Relationship of NPY and Zucker rats 54

NPY, Eating Disorders,
and Obesity in Humans 56

Diazoxide as an Insulin Antagonist 63
History and Actions 63
Medical Treatments 64

Our Progress to Date 65

III. METHOD 69

Animals 69

Tissues 69

Serum Analyses 70

Neuropeptide Y and Protein Assays 70

Statistical Procedures 72

IV. RESULTS 76

Manipulation Check 76
Serum Glucose and Insulin 76
NPY Concentrations 76
Free fed and Fasted Obese Rats 78
Free fed and Fasted Lean Rats 78

Free Fed Rats 78
Body Weight Data 82
Serum Glucose and Insulin 84
NPY Concentrations 84
Controls 84
Obese Rats 84
Lean Rats 88

Fasted Rats 88
Body Weight Data 88
Serum Glucose and Insulin 91
NPY Concentrations 91
Controls 93
Obese Rats 93
Lean Rats 96

Combined ARC-ME Analysis 96
NPY Concentrations 96
Controls 99
Free Fed Rats 99
Fasted Rats 99

V. DISCUSSION AND CONCLUSIONS 100

REFERENCES 114

VITA 147

LIST OF TABLES

TABLE		PAGE
1.	Hypothesized Inter-Hypothalamic NPY Pathways	25
2.	Hypothesized Intra-Hypothalamic NPY Pathways	28
3.	Pioneering NPY-Feeding Studies	32
4.	NPY Radioimmunoassay Characteristics	74
5.	Manipulation Check for Fasting Effects of Control Lean and Obese Zucker Rats	77
6.	Diazoxide Effects on Body Weight, Glucose, and Insulin Levels of Free Fed Zucker Rats	83
7.	Diazoxide Effects on Body Weight, Glucose, and Insulin Levels of Fasted Zucker Rats	90

LIST OF FIGURES

FIGURE		PAGE
1.	Model for Placebo Control Obese Zucker Rats	4
2.	Model for Diazoxide-Treated Obese Zucker Rats	5
3.	Hypothesized Hypothalamic NPY Pathways in the Rat Brain	29
4.	NPY Radioimmunoassay Standard Curve	75
5.	Effects of Feeding Manipulation on NPY Levels of Control Lean and Obese Zucker Rats	79
6.	Manipulation of NPY Levels by Fasting in Control Obese Rats	80
7.	Manipulation of NPY Levels by Fasting in Control Lean Rats	81
8.	Summary Figure for Effects of Diazoxide Treatment on NPY Levels of Free Fed Lean and Obese Zucker Rats.	85
9.	NPY Content in Brain Nuclei of Free Fed Lean and Obese Zucker Controls	86
10.	NPY Content of Control and Diazoxide-Treated Free Fed Obese Zucker Rats	87
11.	NPY Content of Control and Diazoxide-Treated Free Fed Lean Zucker Rats	89
12.	Summary Figure for Effects of Diazoxide Treatment on NPY Levels of Fasted Lean and Obese Zucker Rats	92
13.	NPY Content in Brain Nuclei of Fasted Lean and Obese Zucker Controls	94

FIGURE PAGE

14. NPY Content of Control and Diazoxide-Treated Fasted Obese Zucker Rats 95

15. NPY Content of Control and Diazoxide-Treated Fasted Lean Zucker Rats 97

16. NPY Content of the Combined ARC-ME across Genotype, Diet, and Drug Groups 98

CHAPTER I.

INTRODUCTION

Rationale

Obesity is a major health problem in modern American with over 30% of the adult population considered overweight. Second to smoking, obesity has been labeled the most preventable cause of premature death. Millions of dollars are spent by consumers each year on diet plans, health club memberships, and elective surgery, yet the percentage of the population defined as clinically obese continues to rise. The inability to shed pounds and keep them off is only fostered by decreased physical activity and increased food intake. In spite of efforts to correct these problems, many people are unable to lose weight because they do not target the correct metabolic features that plague them as individuals. By learning more about the various physiological systems involved in obesity and its related syndromes, utilizing drug and animal models, scientists can better understand the complex events underlying weight gain and maintenance and create more successful behavioral and medical treatments for obesity.

Overview of Research Project and Statement of Hypotheses

Obesity is a common physiological state that influences both physical and psychological health. Hyperinsulinism and concomitant insulin resistance, along with altered dietary intake, are common characteristics of

the obese individual. Research using animal models of obesity suggests that suppressing plasma insulin may decrease body weight and feeding behaviors via a centrally-mediated signal. Obese rats placed on a drug that suppresses insulin release, diazoxide (DZ), show improved brain tissue binding of circulating insulin along with normalized metabolism and a decrease in food intake. It is possible that this improved insulin binding produces a decrease in hypothalamic and extra-hypothalamic levels of the peptide neuropeptide Y (NPY) which normally stimulates feeding and metabolic efficiency. The following study was designed to test the effects of DZ treatment on hypothalamic and extra-hypothalamic levels of NPY in hyperinsulinemic obese Zucker rats. The hypotheses of the study were:

I. The significance of DZ as an insulin and body weight regulator in lean and obese Zucker rats

A. Do lean and obese Zucker rats placed on DZ (150 mg/kg/day) for approximately four weeks show a significant decrease in serum insulin while maintaining glucose levels in the control range?

B. Do lean and obese Zucker rats, following four weeks of DZ treatment, have lower body weights than their respective placebo controls?

II. The significance of DZ as an insulin regulator of hypothalamic and extra-hypothalamic NPY content of lean and obese Zucker rats

A. Do obese Zucker rats, following DZ treatment, show a lower concentration of NPY across hypothalamic and extra-hypothalamic nuclei associated with feeding and metabolics compared to placebo controls? Are the greatest changes in NPY concentration located in the arcuate, median eminence, and paraventricular nuclei?

B. Do lean Zucker rats, following DZ treatment, maintain NPY concentrations in hypothalamic and extra-hypothalamic nuclei associated with feeding and metabolics at slightly lower but equivalent levels to those of their corresponding placebo controls?

C. Do DZ-treated lean and obese rats display different patterns of NPY concentration changes following DZ treatment based on being freely fed or fasted overnight?

Figures 1 and 2 demonstrate the hypotheses for the DZ-treated and placebo-treated obese rats for this study.

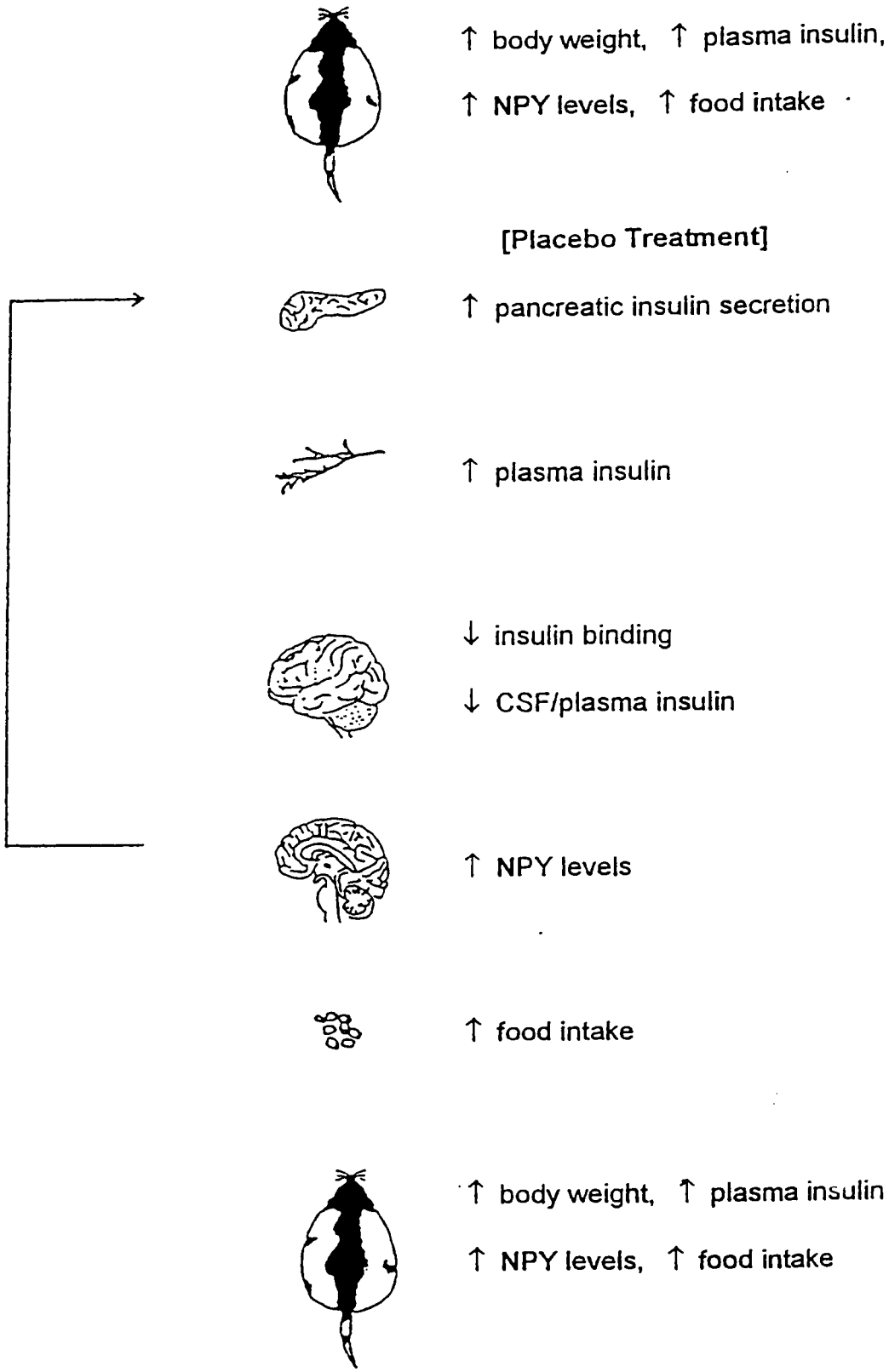


Figure 1. Model for Placebo Control Obese Zucker Rats

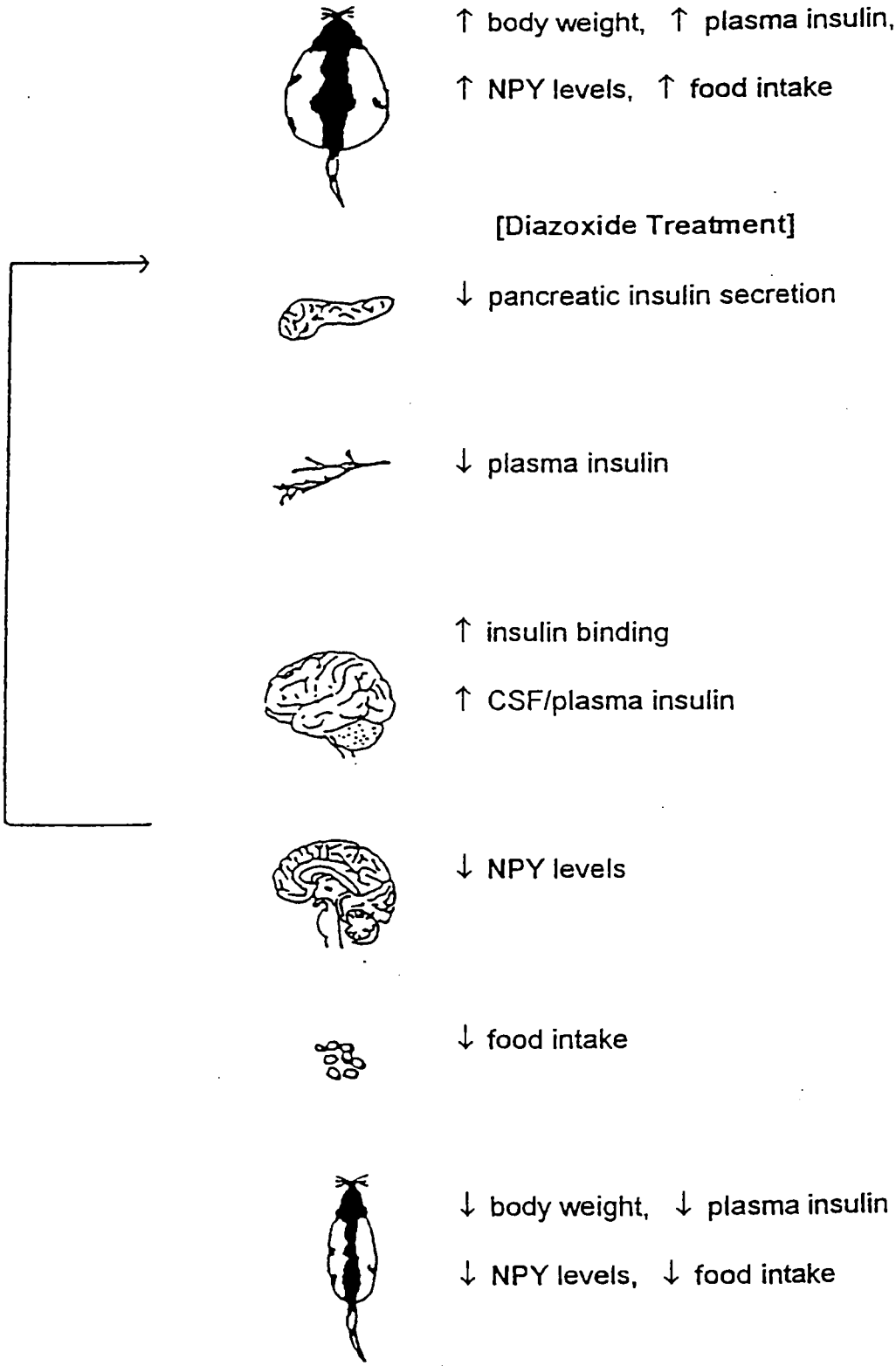


Figure 2. Model for Diazoxide-Treated Obese Zucker Rats

CHAPTER II.

REVIEW OF THE LITERATURE

Obesity and Abnormal Insulin Regulation

Human Studies

Between 1988 and 1994, the Centers for Disease Control estimated that almost 35% of American adults are overweight (CDC, 1997). Of the adults, 36% of women and 33% of men have body weights in excess of the desired weight for their height. Further, 14% of children and 12% of adolescents are considered overweight. These rates are reported to be higher than similar measurements taken only a few years earlier (CDC, 1997). This elevated body mass is associated with increased risk for certain forms of cancer, arthritis, heart disease, diabetes, and respiratory dysfunction such as sleep apnea (review, Pi-Sunyer, 1993). Obesity not only affects physiological health but also psychological well-being. Body weight has an inverse relationship with self-esteem in adolescent females (Martin et al., 1988). Beyond the mind-body health effects, obesity and its related conditions cast a strong economic burden on obese individuals and the medical community. Excess health care costs for overweight, 40-44-year-old females alone, compared to non-overweight cohorts, are projected to exceed $15 billion over the next 25 years (Gorsky, Pamuk, & Williamson, 1996). A reduction in body weight among Americans would relieve economic

pressures, improve psychological well-being, and improve physical health and longevity. Identification of common characteristics in the obese population may provide an avenue by which to target behavioral and medical treatments to accomplish these ends.

Insulin and Obesity. One common physiological feature that has been observed and commented on regarding obese populations is elevated plasma insulin and a resistance to the effects of insulin at target cells (Karam, Grodsky, & Forsham, 1963; Rabinowitz & Zierler, 1962; Wigand & Blackard, 1979). Insulin is a hormone synthesized by the beta cells located in the islets of Langerhans of the pancreas. Insulin is secreted at basal levels, but in much greater quantities in response to glucose following a meal. Insulin serves to enhance glucose entry into cells and acts to promote the storage of nutrients as larger, more complex sugars known as glycogen, or as fats for later liberation during times of fasting and nutrient deficiency (Guyton & Hall, 1996).

Rabinowitz (1962) and Karam (1963), along with their respective colleagues, were among the earlier researchers to notice elevated insulin levels and insulin resistance in obese individuals. Obese individuals in the Rabinowitz study had nearly three times the level of plasma insulin compared to control subjects and were less responsive to the action of insulin. When insulin was infused into obese subjects, glucose clearance from the forearm was reduced compared to controls. Karam et al. (1963) also found higher

basal insulin and hyperinsulin secretion following glucose infusion in adult, nondiabetic obese subjects. These obese subjects also displayed sustained insulin levels longer than non-obese subjects.

Since these early observations, there has been considerable debate about whether insulin secretion is the primary disorder or whether insulin resistance arises first leading to hyperinsulinism. Wigand and Blackard (1979) described this controversy and pharmacologically suppressed insulin release in obese individuals for seven days. Insulin suppression led to a decrease in glucose tolerance, but an increase in insulin receptor numbers. They claim that if insulin resistance had been the primary pathology, they would not have achieved those results. Using a similar protocol, Ratzmann, Ruhnke, and Kohnert (1983) added support to those findings when they reduced hyperinsulin secretion to glucose and improved glucose clearance in obese individuals despite failing to reduce basal insulin levels. The failure to reduce basal insulin levels detectably in the study may have been due to the short drug treatment duration of four days. Very recently, Sigal and colleagues (1997) followed subjects for about 16 years and found that high acute insulin secretion to glucose challenge may predict weight gain, especially in those sensitive to insulin. While arguments continue about the order of events in the development of hyperinsulinism and insulin resistance, perhaps greater discussion revolves around the events underlying these insulin changes and how they may be linked to another common feature of obesity, altered dietary intake.

Hyperinsulinism and Food Intake. A discussion of statistics compiled by the Centers for Disease Control indicates that food intake has increased across the same recent time period as the rise in the rate of obesity (CDC, 1997). Nevertheless, earlier researchers argued that obese individuals fail to consume more food than non-obese people (Braitman, Adlin, & Stanton, 1985). The original events that trigger the excess release of insulin in obese individuals are not well understood. Some researchers claim that obese individuals possess a sensory system that is overresponsive to external, or environmental, cues causing them to eat in response to stimuli like advertising and time of day (Schachter, Goldman, & Gordon, 1968) whereas others cast doubt on this notion (Rodin, 1981). In any case, it is possible that high calorie, particularly carbohydrate, binges provoke equally enhanced bursts of insulin release, and as the pattern continues, it eventually leads to elevated basal insulin and glucose-stimulated insulin release. Grey and Kipnis (1971) noted a high percentage of the diet of their obese subjects was composed of carbohydrates. They also found that high carbohydrate intake contributes to increased insulin secretion in obese subjects. Low carbohydrate, isocaloric diets decreased basal insulin secretion in these subjects while a high carbohydrate diet reversed the basal insulin levels. Rodin, Wack, Ferrannini, and DeFronzo (1985) found evidence that hyperinsulinism may, in turn, drive increased food intake. Non-obese, pre-fasted subjects reported increased palatability of sucrose solutions and feelings of hunger when plasma insulin levels were artificially elevated. Given the opportunity to consume a liquid meal, these subjects drank more

than non-hyperinsulinemic groups. Furthermore, glucose levels did not differentiate the responses of the hyperinsulinemic subjects.

Elevated plasma insulin levels in obese subjects may result from altered dietary intake. Further, the increased insulin levels may provoke greater desirability of food and greater food consumption regardless of signals of peripheral nutrition status. It is difficult to determine which event (excessive feeding versus elevated insulin) precedes the other in obesity. In either case, a concomitant reduction in plasma insulin and food intake may aid in normalizing the metabolic state of some obese individuals.

Animal Studies: Zucker rats

The use of animal models in the study of feeding and metabolism provides more precise control over the behavioral and physiological factors that contribute to obesity than can be achieved by studying humans. For example, early studies involving select lesioning in rodents identified brain nuclei critical in feeding behavior (review, Keesey & Powley, 1975). Recent advances in genetic techniques have allowed researchers to explore the developmental aspects of obesity in rodents that are born unable to produce certain biochemicals (Erickson, Clegg, & Palmiter, 1996). Additionally, the breeding of animals having naturally occurring obesity has facilitated the study of factors underlying obesity. One of the most widely studied animal models of obesity, the Zucker "fatty" rat, was discovered when it appeared

"spontaneously" in a breeding colony in Stow, Massachusetts (Zucker & Zucker, 1961).

History. Theodore and Lois Zucker first noted the appearance of the obese genotype around 1961 when they found that about one-fourth of the rats in a segment of their breeding colony were very overweight compared to their leaner littermates (Zucker & Zucker, 1961). The Zuckers performed a series of breeding studies and determined that the obese body type is transmitted by an autosomal allele which was named *fa* for *fatty*. Because the trait is recessive, only animals *fa/fa* have the obese phenotype. The dominant allele, named *Fa*, is associated with the lean littermates that can be either heterozygous, *Fa/fa*, or homozygous, *Fa/Fa*, for the lean body type (Zucker & Zucker, 1961). The littermates are distinguishable based on body type by weaning, at three to four weeks of age, at which time the obese rats have nearly twice the amount of body fat of leans (Zucker & Antoniades, 1972). Obese rats develop higher body weights, shorter tibia lengths, and a less developed overall skeletal frame than leans (review, Martin, Harris, & Jones, 1986). In addition to these obvious physical differences, the blood of obese animals becomes lactescent as they age (Zucker & Zucker, 1961). This milky-white sheen is accompanied by elevated lipid and cholesterol levels (Zucker & Antoniades, 1972; Zucker & Zucker, 1962). A number of organ-system disruptions have also been observed in "fatties." Many obese rats develop granulosis and hydronephrosis of the kidneys, and nearly all are sterile (Zucker & Zucker, 1961; Zucker & Zucker, 1962). "Fatties" can only

be produced by breeding *Fa/fa* lean pairs. Thus, Zucker obese rats differ from their lean counterparts not only in physical appearance, but also in a host of systemic abnormalities.

Hyperinsulinism. In spite of these characteristics, obese Zucker rats may be most noted physiologically for their severe elevation in plasma insulin. This hyperinsulinism appears by three to four weeks of age even as the rats maintain plasma glucose levels within normal limits (Zucker & Antoniades, 1972). Along with the elevated insulin are increases in the size, number, and morphology of pancreatic islets housing the beta cells that produce insulin (York, Steinke, & Bray, 1972). Obese rats have a distinct developmental pattern in insulin levels showing a steady increase with age, which peaks at around 15 weeks of age and corresponds closely in time with insulin resistance, leading to an almost diabetic state of elevated plasma glucose in response to glucose challenge (Zucker & Antoniades, 1972). After 15 weeks of age, insulin levels decline to those that were reached at around eight weeks of age. Obese Zucker rats may have a propensity to hypersecrete basal insulin as early as one week of age (Tenenbaum et al., 1994). In addition to hypersecretion of basal insulin, obese rats over secrete insulin in response to glucose as early as 21 days of gestation (Tenenbaum et al., 1994). This hyperresponsiveness continues into adulthood (Schade & Eaton, 1975). Rohner-Jeanrenaud, Ionescu, & Jeanrenaud (1983) suggest that hypervagal activity may be involved in the hyperinsulinism. Resistance of muscle to incorporate glucose into glycogen stores may accompany the

elevated insulin levels, as noted in older obese animals compared to leans (Stern, Johnson, Greenwood, Zucker, & Hirsch, 1972). Hence, obese Zucker rats have a primary disorder of insulin regulation, maintaining excessive insulin at basal levels and when glucose-evoked.

Feeding Behavior. Studies also delineate that obese rats have different feeding patterns from lean animals. Overfeeding in "fatty" rats versus leans begins to emerge as early as 17 days of age (Stern & Johnson, 1977). "Fatty" rats continue to ingest more food than leans with the greatest trend in hyperphagia between five and 15 weeks of age, overlapping the period of basal insulin surge. "Fatty" rats consume more food, but differ in meal patterns. They eat larger meals rather than eating as frequently as leans, and they eat more during the light phase of the light-dark cycle than do leans (Becker & Grinker, 1977). "Fatties" may also be more behaviorally motivated to seek food. Vasselli, Cleary, Jen, and Greenwood (1980) observed a greater rate of bar pressing to receive food in obese rats than leans at 16 weeks of age. Diet choices may also differ between the genotypes with obese rats selecting diets with higher percentages of fat and lower percentages of protein when presented with nutrient choices (Castonguay, Hartman, Fitzpatrick, & Stern, 1982). However, this hyperphagia does not appear to be necessary for the early increase in weight found in pre-weanling "fatties" (Buchberger & Schmidt, 1994). Research supports this energy efficiency produced by feeding obese rats restricted diets (Zucker, 1975). Further, food restriction may not change glucose

responsiveness to resistance (York & Bray, 1973). Nevertheless, restricted diets lengthen the life span of "fatty" rats whereas high fat diets tend to shorten the life span (Zucker & Zucker, 1962). Although excessive feeding may not be the catalyst for the obesity in these animals, continued overfeeding only exacerbates the existing condition and may be a key feature during the climb in insulin levels and concomitant insulin resistance.

Brain-Insulin Model. While the disruption of peripheral metabolics in Zucker "fatty" rats is indisputable, the relationship between the peripheral and central nervous system is less well understood. Insulin has been postulated to be a circulating factor that signals to the brain peripheral nutrition status (Schwartz, Figlewicz, Baskin, Woods, & Porte, 1992a). Studies suggest that peripheral insulin is transported into the central nervous system via a saturable transport system (review, Schwartz et al., 1992a), even though insulin may be manufactured in small amounts in hypothalamic tissue (Young, 1986). According to the central-peripheral insulin model of Schwartz and colleagues (1992a), circulating insulin levels rise during feeding as glucose is absorbed in the digestive tract. This increased secretion of plasma insulin gains entry into the brain via the saturable blood brain barrier so that the insulin binds to receptors (Baskin, Porte, Guest, & Dorsa, 1983), most likely in the hypothalamus (Havrankova & Roth, 1978; Havrankova, Schmechel, Roth, & Brownstein, 1978; Van Houten, Posner, Kopriwa, & Brawer, 1980), signaling to the brain that feeding behavior has been successful in acquiring nutrients and that feeding should cease. This

model was originally designed to account for energy homeostasis and feeding behavior in normal animals. Based on observations of feeding patterns and metabolic status, obese rats appear to have a disruption in the communication of insulin levels from the peripheral to the central nervous system (review, Schwartz, Figlewicz, Woods, Porte, & Baskin, 1993). It is possible that circulating insulin fails to enter the brain in relation to release or that brain tissue does not respond to the insulin appropriately when it has gained entry.

Stein, West, Figlewicz, Porte, and Woods (1985) have found that obese rats maintain equivalent cerebrospinal fluid (CSF) insulin concentrations compared to leans in spite of maintaining elevated plasma insulin. Subsequently, Stein et al. (1987) infused insulin into the periphery of lean and obese rats and observed a smaller change in CSF insulin levels of obese rats relative to leans. Melnyk (1987) noted that six-week-old obese rats have lower insulin binding and fewer high affinity binding sites in hypothalamic tissue compared to leans, although binding in cortical tissue is similar between genotypes. These findings run parallel with those of Ikeda et al. (1986) who infused insulin into the third ventricle of lean and obese rats to find that obese rats did not respond to insulin infusion with a decrease in feeding and body weight as did leans. These researchers suggested that faulty postreceptor mechanisms may exist in the obese rats to explain the lack of an anorectic effect of insulin. This assumption may also be applicable to the results of Wilcox et al. (1989) who found greater insulin binding in

arcuate and dorsomedial hypothalamic nuclei of obese rats versus leans by three to four months of age. This is an age at which CSF insulin levels are elevated for obese rats compared to leans, with estimated CSF/plasma insulin ratios being lower in obese rats (Stein et al., 1983). Based on these findings, it is possible that a shift occurs in the brain-peripheral insulin signaling mechanism prior to the three to four month age period at which time insulin levels peak. The elevated central insulin levels during this time may indicate an attempt by the brain to buffer itself from rising levels which surge at around 15 weeks of age. This concept is supported by Schwartz et al. (1990) who examined brain capillary insulin binding just prior to the three to four month age window. They noted a decrease in the number of insulin binding sites in obese rats compared to leans, possibly indicating hyperinsulinism-induced down-regulation of insulin receptors. It appears that the elevated plasma insulin evident in these animals and peaking at three to four months of age may compromise brain responses to peripheral nutrition status, as mapped by the hyperphagic feeding patterns during this same time frame.

Disruptions in insulin regulation and feeding patterns occur early in the development of the obese Zucker rats. The communication of nutrient status to the brain may be compromised by an inability to relay or recognize circulating insulin levels. A particularly vulnerable period for the "fatties" appears to be five to 15 weeks of age. Thus, obese Zucker rats have a number of both central and peripheral abnormalities that are either primary or

secondary to their obesity. Intervention during this vulnerable period of development may alleviate further excessive feeding and metabolic disturbance.

Central Controls of Feeding and Metabolics: NPY

Central Nervous System Features of NPY

NPY is a peptide within the pancreatic polypeptide family which includes such members as pancreatic polypeptide (PP), avian pancreatic polypeptide (APP), and peptide tyrosine tyrosine (PYY; Allen & Balbi, 1993). Expression of encoding mechanisms for the peptide have been detected in the brains of rats as young as 16-days post-conception (Larhammar, Ericsson, & Persson, 1987), and mature peptide has been identified in hypothalamic and brainstem samples of rats as young as 14 days post-conception (Allen, McGregor, Woodhams, Polak, & Bloom, 1984a). Studies from humans indicate that the genes encoding NPY may be localized to chromosome 7 (Takeuchi et al., 1986).

Rat NPY is originally derived from a 98-amino-acid precursor known as prepro-NPY (Allen & Balbi, 1993; Higuchi, Yang, & Sabol, 1988). Prepro-NPY is made up of four segments: a signal peptide, 29 amino acids; the neuropeptide, 36 amino acids; a "post-translational processing site," 3 peptides; and the C-terminal flanking peptide of NPY, also known as CPON, 30 amino acids (Allen & Balbi, 1993, p. 49). After the prepro-molecule has

been cleaved several times, the active 36-amino-acid NPY molecule remains. Due to the tyrosine residues (abbreviated Y) flanking the peptide and its distribution in the central nervous system, the peptide was named neuropeptide tyrosine, abbreviated NPY (Allen & Balbi, 1993).

NPY is considered a highly "conserved" peptide in that it shares many chemical and structural similarities to other peptides within its family and across different species (reviews, Allen & Balbi, 1993; Allen, Novotny, Martin, & Heinrich, 1987; Larhammar, Soderberg, & Blomqvist, 1993, p. 30). For example, rat and human NPY have been mapped to be structurally identical with only one amino acid making the peptide different from porcine extracts (Allen & Balbi, 1993; Allen et al., 1987). Furthermore, Higuchi and colleagues (1988) estimated the similarity between amino acids of rat and human NPY encoding mRNA to be as high as 94%. In addition, NPY has been found to have chemical characteristics similar to several of the other peptides in the pancreatic family (Allen et al., 1987). Allen et al. (1984a), Allen and Balbi (1993), and Higuchi et al. (1988) propose that this continuity and the presence neonatally implies a strong adaptive and developmental relevance for the peptide.

Distribution. Shortly after its isolation from porcine brain extracts by Tatemoto and colleagues (1982), investigations began to map and characterize NPY brain systems. Allen et al. (1983) used immunoreactive techniques to detect NPY-like activity in the frontal, parietal, and occipital

regions of the neocortex in addition to the hippocampus and thalamus. NPY concentrations in the amygdala and selected regions of the hypothalamus were measured at three to five times the cortical levels. Activity in the midbrain periaqueductal gray rivaled the hypothalamic levels whereas activity in the cerebellum and pons was sparse. These findings led Allen and colleagues (1983) to describe NPY as distributed in rostral-caudal bands with concentrations diminishing in the caudal direction. NPY-containing cell bodies were identified in the cortex, basal ganglia, hypothalamus, amygdala, and brainstem with many fibers in the stria terminalis and specific hypothalamic regions. Chronwall et al. (1985) and DeQuidt and Emson (1986) extended Allen et al.'s (1983) report to detail not only the concentration of peptides and relative fiber and cell body distributions, but also to identify more clearly fiber and cell body locations. Although the absolute magnitude of concentrations of NPY for discrete brain regions was not consistent across those studies, the relative proportion of peptide among the regions remained similar, suggesting that methodological techniques were responsible for the variations.

The high concentration of NPY found in the hypothalamus prompted researchers to examine more closely the distribution of NPY within that structure. Located on the ventral surface of the brain, sandwiched beneath the thalamus, and bordered by the optic tracts, the hypothalamus is a collection of discrete cell bodies surrounding the third ventricle. Some of the bilateral cell clusters included in this region are the arcuate (ARC), median

eminence (ME), dorsomedial (DMH), ventromedial (VMH), anterior (AHA), posterior (PH), and paraventricular (PVN) nuclei, along with a collection of periventricular cells lining the third ventricle. These cell groups form the core of the hypothalamus. Flanking the core are the perifornical (PFH) and lateral (LH) hypothalamic nuclei. In addition, the medial (MPOA) and lateral (LPOA) preoptic bodies and supraoptic (SO) and suprachiasmatic (SCN) nuclei are located rostral to the core. Detailed, graphic depictions of these relationships are located in the rat brain atlas of Paxinos and Watson (1997).

NPY-immunoreactivity has been identified in diffuse distributions throughout the hypothalamic nuclei in association with different cell structures. A number of research teams (Allen et al., 1983; Chronwall et al., 1985; DeQuidt & Emson, 1986; Lantos, Gorcs, & Palkovits, 1995) have identified the largest proportion of cell bodies in the hypothalamic core at the ARC-ME with fewer in the VMH and DMH and more concentrated activity along the medial periventricular lining. Fewer cell bodies have been identified in the more rostral and lateral regions. In contrast, dense networks of fibers were mapped throughout the hypothalamus, especially in the SCN (Pelletier, 1990). Some of the densest bands of fibers have been found in the cells lining the third ventricle and dorsal region of the VMH, parvocellular portion of the PVN (PVNp), ME, and dorsal ARC (Chronwall et al., 1985; DeQuidt & Emson, 1986). Similarly, NPY activity has also revealed a wide distribution of receptor sites (review, Michel, 1991; Nakajima, Yashima, &

Nakamura, 1986; Unden, Tatemoto, & Bartfai, 1983; Unden, Tatemoto, Mutt, & Bartfai, 1984).

Receptors. To date, at least six different receptor subtypes binding NPY have been identified (reviews, Gehlert, 1994; Gehlert, 1998; Grundemar, Sheikh, & Wahlestedt, 1993; Wahlestedt & Reis, 1993). Some subtypes may be G-protein coupled, as indicated by effects on adenylate cyclase and intracellular calcium mobilization (Bard, Walker, Branchek, & Weinshank, 1995; Eva, Keinanen, Monyer, Seeburg, & Sprengel, 1990; Gehlert, 1994; Krause, Eva, Seeburg, & Sprengel, 1992; Rose et al., 1995). Studies involving radiolabeled NPY showed highest concentrations of NPY binding sites to be in the cerebral cortex, hypothalamus, and hippocampus, with lowest binding occurring in the cerebellum (Unden et al., 1983; Unden et al., 1984). Martel, St. Pierre, and Quirion (1986) found similar density distributions with moderate densities in the geniculate nuclei of the thalamus and olfactory nucleus. These results mirror those of the concentration and distribution of NPY throughout the brain as described earlier. In a later study, Martel and coworkers (1988) placed greater focus on binding within specific regions of the rat hypothalamus in addition to extra-hypothalamic areas where they found low to moderate densities of binding in the LH, AHA, and bed nucleus of the stria terminalis (BNST). Unexpectedly, they found fewer binding sites in the SCN and central nuclei of the hypothalamus where NPY content is relatively elevated.

Studies of receptor distribution for the specific subtypes of receptors indicate that few Y5 receptors are located in the hypothalamic brain region while they are more numerous in the brain stem (Dumont, Fournier, & Quirion, 1998) unlike Y1 receptors which have been mapped specifically in the ARC (Zhang et al., 1994). Broberger, Landry, Wong, Walsh, and Hokfelt (1997) and Mikkelsen and Larsen (1992) have found evidence that Y2 and Y1 receptors, respectively, are expressed in the ARC and suggest that there is greater expression in the ARC than PVN, especially for Y1 receptors. At the same time, other researchers claim that Y2 receptors are primarily pre-synaptic and Y1 receptors post-synaptic (Doods et al., 1996; review, Gehlert, 1994; Wahlestedt, Yanaihara, & Hakanson, 1986). The close localization of NPY mRNA and Y2-mRNA suggests that Y2 receptors may act as a negative feedback system controlling the synthesis and/or release of NPY in the medial hypothalamus. Additionally, Y4 encoding mRNA has been detected in the hypothalamus and much of the brain (Bard et al., 1995).

Cellular Characteristics. Pelletier (1990) has characterized the microstructure of NPY cells using electron-microscopy and staining techniques. He found the peptide to be stored in dense core vesicles within the cell bodies, axons, and cytoplasm of neurons and most noticeably in unmyelinated tissues. Using the immunogold technique, Pelletier (1990) analyzed the ultrastructure of NPY synaptic connections in several hypothalamic nuclei. For an estimated 20% of the connections, terminal

endings formed synaptic connections with dendritic tissue, leading Pelletier (1990) to conclude that NPY's primary function is likely as a "classical" neurotransmitter or neuromodulator. Sawchenko and Pfeiffer (1988) used light and electron-microscopy to confirm Pelletier's (1990) results showing specific fiber staining in the medial (parvocellular) PVN along with fibers in the lateral (magnocellular) region, PVNm. In contrast to the other findings, they observed as high as 70% of the PVN terminal contacts to be with dendritic tissue.

Pathways. Given the distribution of NPY cell bodies, fibers, and receptors, it was of interest to determine the principal pathways of NPY-containing neurons within the hypothalamus and between the hypothalamus and extra-hypothalamic structures. Most of these pathways have been mapped using retrograde tracing techniques combined with immunoreactions for NPY (double labelling) or using physical pathway disruption (via chemical lesioning or knife cuts severing neurons). The pathways can be grouped based on inter-hypothalamic and intra-hypothalamic connections.

Employing retrograde tracing techniques, Magnuson, O'Donohue, and Gray (1984) have identified some inter-hypothalamic pathways by infusing Fast Blue dye into the dorsomedial region of the vagal nuclei of the medulla of male rats. Fibers and terminals contacting the labeled regions were highlighted in ARC, LH, PVN, BNST, central nucleus of the amygdala (CNA), and periventricular nuclei. Similar techniques in the same lab established

projections of NPY fibers from the DMH to similar medullary regions (Gray, O'Donohue, & Magnuson, 1986; Gray, O'Donohue, Watson, & Magnuson, 1984). Severing tissues in the stria terminalis using knife cuts, Allen, Roberts, Bloom, Crow, and Polak (1984b) identified projections from the stria terminalis to the BNST and SCN. Sawchenko et al. (1985) used True Blue retrograde techniques to trace fibers containing NPY that project from the rostroventral lateral medulla (RVLM) and rostral medulla to the SO and PVN, finding dense patches in the PVNp and more diffuse activity in the PVNm. These studies suggest that pathways of NPY fibers project from the hypothalamic core to the autonomic brain stem and vice versa.

Using a combined fluorogold and labeled-latex bead procedure, Card and Moore (1989) traced pathways between the SCN and geniculate nuclei of the thalamus, a visual relay center. They found projections of intergeniculate leaflet (IGL) cells onto the SCN. Specifically, NPY precursor CPON fragments were identified in IGL and SCN pointing to the presence of mature NPY. The authors suggest the role of this geniculohypothalamic tract in circadian entrainment. Similarly, Vanhatalo and Soinila (1996) used fluorogold retrograde tracing to find an NPY pathway from rostral (i.e., supraoptic) and medial (PVN) regions of the hypothalamus to the posterior pituitary, perhaps indicating a role for hypothalamic NPY as a neurosecretory agent. Readers are referred to Table 1 for a listing of procedures and results for these inter-hypothalamic pathway studies.

Table 1. Hypothesized Inter-Hypothalamic NPY Pathways

Reference	Tracing Technique	Origin	Termination Site
Magnuson et al. (1984)	Fast Blue retrograde tracing	ARC, LH, PVN, periventricular nuclei regions, CNA, BNST	vagal nuclei of medulla
Gray et al. (1984)	Fast Blue retrograde tracing	DMH	vagal nuclei of medulla
Gray et al. (1986)	Fast Blue retrograde tracing	ARC, PVN, LH, DMH, CNA, BNST	dorsal vagal complex (NTS & dorsal vagal nucleus)
Allen et al. (1984)	knife cuts	stria terminalis	BNST, SCN
Sawchenko et al. (1985)	true blue fluorescent tracing	RVLM, rostral NTS	PVN, SO
Card & Moore (1989)	labeled-latex beads & fluorogold retrograde tracing	IGL of thalamus	SCN
Vanhatalo & Soinila (1996)	fluorogold	rostral and medial hypothalamus	posterior pituitary

To gain a better understanding of NPY connections within the hypothalamus, Bai and colleagues (1985) lesioned the ARC in rats and examined NPY immunoreactivity in other hypothalamic nuclei. A marked decrease in ipsilateral activity in the PVN and DMH following surgery led the authors to conclude the fibers of NPY project from the ARC to the PVN and DMH within the hypothalamus. Using a stainless steel knife, Sahu, Kalra, Crowley, & Kalra (1988) bilaterally transected the tegmentum of the midbrain. After a two-week waiting period, they found evidence that some SCN, ARC, and VMH NPY-containing neurons receive their projections from within the hypothalamus or thalamus, unlike the MPOA, PVN, ME, and DMH which had markedly reduced NPY levels following surgical intervention. Another intra-hypothalamic connection was uncovered by Kawano and Masuko (1993) as fibers were found to project from the MPO to the PVN. Other studies by Kerkerian and Pelletier (1986) show that destruction of the ARC by monosodium L-glutamate (MSG) during the neonatal period (c. two to 10 days of age) reduces NPY fibers in the PVN as well as ARC and ME, but not the SCN and SO cell bodies, in three-month-old rats. Further, this procedure leads to increased appearance of cell bodies in PVN, particularly the PVNm. The authors also offer the possibility of genome plasticity involvement. However, Abe et al. (1990) treated neonatal rats with MSG subcutaneously during the first five days of life. At 14 weeks of age, brains were sliced and regions were extracted to reveal NPY concentrations. MSG led to stunted growth in the rats and increased serum lipids and insulin. It also decreased PVN levels of NPY. These results may indicate sparing of NPY fibers

passing through the ARC. Together, these results suggest a dual origin of NPY fibers from within the hypothalamus or thalamus (rostral origin) or the brainstem (caudal origin). A summary of the intra-hypothalamic pathway studies is located in Table 2, and a graphic depiction of the hypothesized pathways can be found in Figure 3.

Characteristics of NPY as a Feeding Motivator

Functionally, the role of NPY has been implicated in learning and memory (Heilig, 1993), vascular tissue regulation (Walker, Grouzmann, Burnier, & Waeber, 1991), and mental disturbances like Alzheimer's disease, anxiety, and depression (Heilig, 1993). Perhaps the most reliable area of NPY effects has been found in feeding behavior. Research in this field was pioneered by three separate research groups nearly simultaneously. One group claims to have set about examining NPY as a potential feeding peptide based on their own previous brain research involving human pancreatic polypeptide, hPP, which is structurally related to NPY and shown centrally to evoke feeding bouts (Clark, Kalra, Crowley, & Kalra, 1984). The second group of authors undertook the task of testing NPY's feeding effects in a specific hypothalamic region, the PVN, which is a potent orexigenic site for the neurotransmitter norepinephrine, which is colocalized with NPY in neurons projecting to that region (Stanley & Leibowitz, 1984). The third group conducted a multistage study to replicate Clark et al.'s (1984) findings and test for interactions of drugs (Levine & Morley, 1984).

Table 2. Hypothesized Intra-Hypothalamic NPY Pathways

Reference	Tracing Technique	Origin	Termination Site
Bai et al. (1985)	electrolytic lesion of the ARC	ARC	PVN, DMH
Sahu et al. (1988)	knife transection at tegmentum	hypothalamus/ thalamus	SCN, ARC, VMH
		brainstem	MPOA, PVN, ME, DMH
Kerkerian & Pelletier (1986)	L-MSG in ARC	ARC	PVN
Kawano & Masuko (1993)	colloidal gold-horseradish-peroxidase	MPO	PVN

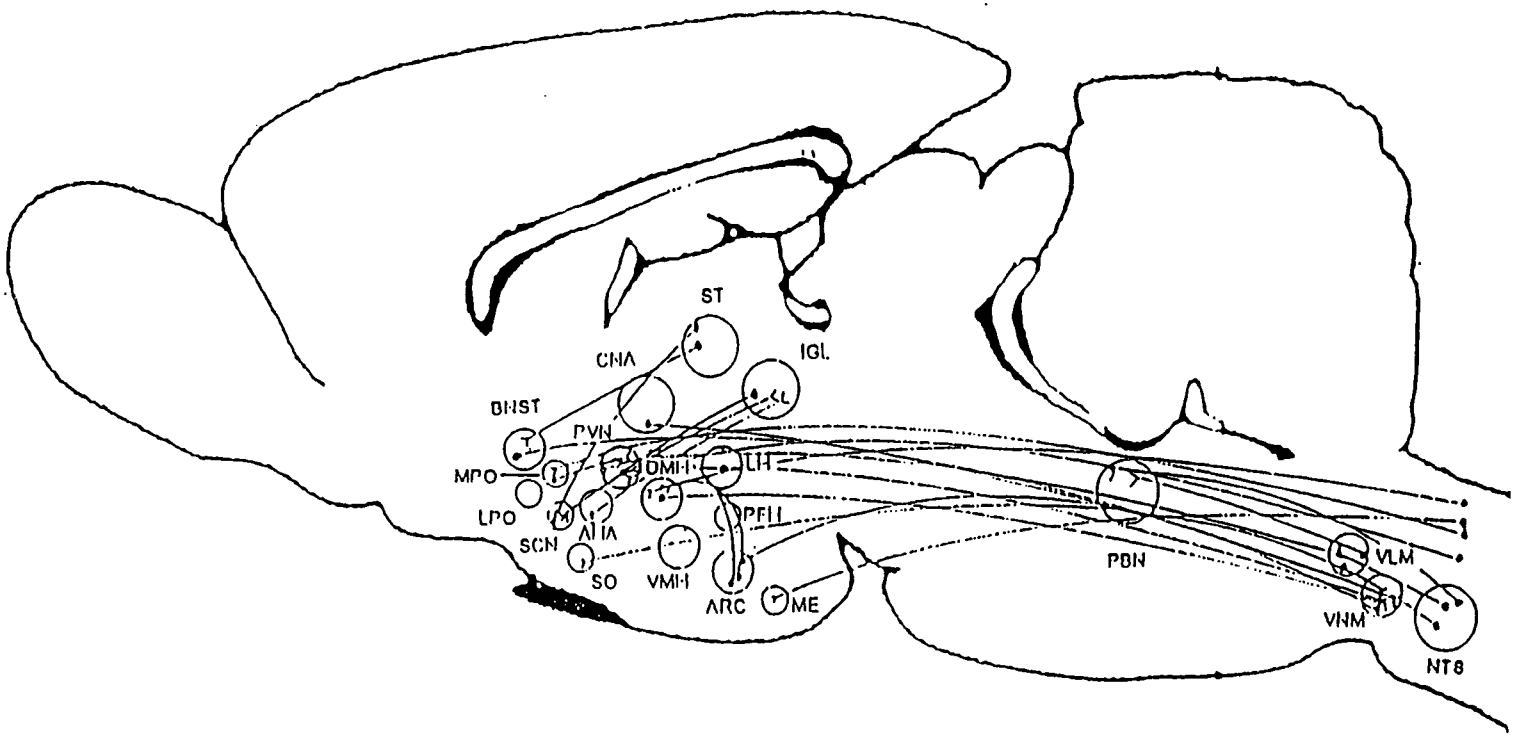


Figure 3. Hypothesized Hypothalamic NPY Pathways in the Rat Brain. Origin and termination sites are indicated by cell bodies (solid circles, o) and terminal buttons (carrots, >). Abbreviations: AHA, anterior hypothalamic area; ARC, arcuate nucleus; BNST, bed nucleus of stria terminalis; CNA, central amygdaloid nucleus; DMH, dorsomedial nucleus of hypothalamus; IGL, intergeniculate leaflet of thalamus; LH, lateral hypothalamus; LPO, lateral preoptic area; ME, median eminence; MPO, medial preoptic area; NTS, nucleus of the solitary tract; PBN, pontine parabrachial nucleus; PFH, perifornical hypothalamus; PVN, paraventricular hypothalamus; SCN, suprachiasmatic nucleus; SO, supraoptic nucleus; ST, stria terminalis; VLM, ventrolateral medulla; VMH, ventromedial hypothalamus; VNM, vagal nuclei of medulla.

Clark et al. (1984) implanted cannulae into the third ventricle (3V) of ovarectomized rats. Following two hours of food deprivation, rats were given two doses of saline (3µl), hPP, or NPY (2 or 10 µg), approximately one hour apart. Feeding behavior was monitored for one hour following infusion. Both peptides elicited a greater feeding response, at either dosage, than the vehicle. The pattern of responses following the second dose was similar to the first. NPY produced about 2.25 grams greater food consumption than hPP, about 0.75 grams, compared to the vehicle. In addition, at both doses, NPY reduced the latency (c. 15 minutes) and increased the duration of feeding (c. 15 minutes) whereas only the high dose led to the same effect for hPP (c. 35 minute latency and six minute duration) although the effects were less dramatic. Thus, NPY proved to be an effective feeding peptide not only in terms of increasing consumption, but also in terms of reducing latency and increasing the duration of feeding.

In their similar study, Stanley and Leibowitz (1984) used adult rats to examine the orexigenic effects of NPY administered into the PVN. Cannulae were implanted to target the PVN, and rats were satiated before testing with a milk-mash diet consisting of chow, sucrose, and evaporated milk. Saline vehicle (0.3 µl) or 0.1 or 0.33 µg of NPY was infused in a counterbalanced order and food intake was monitored for up to four hours following infusion. The 0.5-hour consumption (about 4.75 grams) was equivalent to food consumed in four hours by control animals. By the four-hour mark, consumption had nearly doubled the 0.5-hour reading. The 0.1 µg dose

elicited a transient response with only the one-hour reading being significant and the consumption equivalent to the 0.33 µg dose at 0.5 hours. Furthermore, the latency of the 0.33 µg dose was approximately 10 minutes, spanning up to 0.5 hours to begin consumption.

The third study was actually a series of studies that Levine and Morley (1984) conducted in an attempt to replicate Clark et al.'s (1984) findings in animals not subject to surgical intervention and to test the interaction of NPY with other neurotransmitters in producing the feeding effects. Saline (5 µl) or NPY was infused into the right lateral ventricle (rLV) of male rats at doses 10, 5, 1, or 0.2 µg as well as 5 µg/0.5 µl intraperitoneally. Food intake was measured after one, two, four, and 24 hours. The 5 and 10 µg doses increased feeding at one, two, and four hours. There was a rapid increase in feeding with both doses from injection until two hours postinjection reaching a plateau at 10 grams. Neither the 1.0, 0.1 µg, nor peripheral doses affected feeding at any time point. No effects were significant at 24 hours post-injection. See Table 3 for a diagram of these pioneering studies.

The results of these initial studies are significant in establishing NPY's role in feeding for a number of reasons. Because the doses and routes of drug administration varied, the similarity of findings across studies suggests a robustness of NPY as an orexigenic agent, rather than a highly specific site and dose effect. In addition, both male and female rodents were tested, with females ovarectomized. Again, the similarity of results points to NPY as a

Table 3. Pioneering NPY-Feeding Studies

Reference:	Clark et al. (1984)	Stanley & Leibowitz (1984)	Levine & Morley (1984)
Route:	3rd ventricle	PVN	lateral ventricle
Dose:	2 or 10 µg NPY	0.1 or 0.33 µg NPY	0.1, 1, 5, 10 µg NPY
Time Evaluated:	1 hour post-injection	4 hours post-injection	4 & 24 hours post-injection
Effects:	2 and 10 µg NPY ↑ food intake ↓ feeding onset latency ↑ feeding duration	0.1 µg transient ↑ feeding 0.33 µg ↑ intake over 0.5, 1, 2, and 4 hours post-injection 0.33 µg ↓ feeding onset latency	5 and 10 µg ↑ feeding from 1 to 4 hours post-injection
Test subject:	ovarectimized rats	male rats	male rats
Feeding state:	fasted 2 hours	satiated	**not indicated

nonselective stimulator of food intake. Finally, animals were tested in both satiated and mildly fasted states. Administration of NPY produced increased feeding regardless of feeding status indicating that NPY's effects may supersede feeding status.

Over the years following these early studies, a number of other characteristics were uncovered regarding NPY-induced feeding. These characteristics include the duration of NPY effects across doses, nuclei within the hypothalamus susceptible to NPY's orexigenic effects, permanency of continuously administered NPY on feeding and body weight, and effects of fourth ventricle infusion on feeding behavior.

Primary Feeding Characteristics. Since the pioneering studies established NPY as a feeding peptide, other studies have demonstrated that NPY-induced feeding can have effects lasting longer than the four hours tested in those original studies (Levine & Morley, 1984; Stanley & Leibowitz, 1984). Stanley and Leibowitz (1985) extended their earlier study to increase the dose of NPY given into the PVN of rats and follow the duration of effects beyond four hours. Food intake following NPY doses ranging from 0.033 to 10 µg in saline (0.3 µl) administered into the PVN was followed up to 22 hours postinjection. The lowest dose administered (0.033 µg) failed to elicit an increase in feeding compared to saline controls. However, a dose of 0.33 µg increased feeding up to four hours following injection, with a plateau occurring for intake between one and two hours. The lowest dose to produce

a continued increase in feeding up to 22 hours postinjection was 1.0 µg with both the 3.3 and 10 µg doses producing a similar pattern of effects. More interesting was the finding that a 10 µg dose of NPY elicited an increase in feeding such that four hours following administration the intake rivaled a day's consumption in a control animal. Thus, NPY may produce sustained increases in food intake with a single sufficient dose.

Seeking to determine if continuous administration of NPY would lead to a permanent increase in feeding and/or body weight, Stanley, Kyrkouli, Lampert, & Leibowitz (1986) administered 1 µg dose of NPY three times daily for 10 days in the PVN of rats maintained on a high fat (33% vegetable oil-chow) diet. Following the 10 days of drug exposure, rats were monitored for 21 additional days. Maturation and saline controls were used for comparison. Across the 10 days of treatment, NPY rats showed a continuous pattern of increased body weight and increased feeding with intake plateauing around day four, whereas both control groups maintained similar feeding patterns throughout this period and the 21-day follow-up period. Body weight continued to rise gradually beyond day four in the maturation controls. Between 4 and 10 days, food intake of NPY rats was about twice that of controls, and body weight nearly 100 grams in excess of control levels. Immediately following cessation of the drug, food intake of NPY rats dropped below the level of maturation controls, but gradually increased to equivalent levels by the end of the follow-up period. Body weight also decreased but in a more gradual pattern to levels similar to maturation

controls at the end of the follow-up period. Carcass decomposition of a subgroup revealed that NPY rats had about 218% higher fat weight/carcass composition than saline controls.

Further research has demonstrated that NPY not only affects the PVN of the hypothalamus, but other nuclei composing the region. Stanley, Chin, and Leibowitz (1985a) explored NPY's orexigenic effects in other brain sites by infusing NPY into the PVN, LH, VMH, MPOA, amygdala, periaqueductal area of the midbrain (PAG), and the thalamus of adult rats. Rats were satiated with a milk-mash diet prior to testing. NPY doses ranging from 0.033 to 1.0 µg were administered. A dose of 0.033 µg dose failed to evoke feeding. However, 0.33 µg evoked feeding when administered in the PVN, VMH, and LH. The 1.0 µg dose evoked feeding in the MPOA. For these "effective" doses, food intake increased between two and four times over saline values during the hour following infusion. Neither the amygdala, PAG, nor the thalamus responded with increased feeding at any dose. The hypothalamus, particularly the medial portion, appears to be sensitive to the orexigenic effects of NPY whereas extra-hypothalamic sites are not as sensitive.

Steinman, Gunion, and Morley (1987) added to these findings by infusing NPY into the 3V and fourth ventricles (4V) of rats at 5 and 10 µg and following feeding for two hours postinjection. Both doses increased feeding responses by two fold over saline (3 µl) controls levels when administered to

either location. The 3V appears more sensitive since there was an increase from 4.1 to 5.8 grams in intake with the 3V treatment compared to the 4V (4.7 to 4.5 grams) with the 5 and 10 µg doses, respectively. However, the authors failed to find a difference in the latency to feed.

Thus, NPY appears to have a non-tolerant effect on feeding that can last up to nearly a day following administration of a minimum dose of 1.0 µg into the PVN. The effects of NPY are not site specific to the PVN only. Other nuclei such as the LH, VMH, and MPOA are also susceptible to NPY's orexigenic effects. In addition, administration of the peptide into any of the cerebral ventricles may lead to an increase in feeding behavior. NPY appears to be a strong motivator to increase food intake. Subsequent studies have demonstrated that NPY may affect feeding through motivational mechanisms of feeding-related behaviors.

Feeding-Related Behaviors, Motivation, and Nutrient Preferences. In addition to feeding itself, NPY appears to orient animals to produce behaviors to seek and acquire food. In their early NPY study, Clark et al. (1984) reported that in addition to increasing feeding behavior, NPY reduced grooming compared to saline injected animals. Similarly, Levine and Morley (1984) infused 5 µg/5µl of NPY into the rLV of rats and monitored behaviors (eating, drinking, moving, rearing, and grooming) for two hours following drug administration. During the first hour postinjection, NPY animals spent about one-third of the time inactive and one-third eating, substantially less and

more than saline controls, respectively. Thus, NPY rats spent more time moving in addition to feeding. During the second hour, these differences seemed to disappear. These findings seemed to indicate that NPY enhances not only feeding behavior, but also greater time in activity although these behavioral effects may have a short duration. However, these studies were conducted in the presence of food making it difficult to ascertain what kinds of behaviors would be evoked by NPY in a situation void of food.

To test how the absence of food affects NPY-induced behaviors, Stanley and Leibowitz (1984) also administered 0.33 μg of NPY into the PVN of rats and monitored behaviors including locomotor activity, grooming, rearing, resting, eating, sleeping, and drinking in the presence or absence of food for one hour postinjection. When food was not present, animals spent more time drinking, less time grooming, less time sleeping, and more time (about three times more time) in upper-body activity than saline controls. On the other hand, when food was present, these animals spent about four times more time eating and twice as much time drinking as controls. However, no other behaviors were different from controls. These data suggest that NPY seems to shunt behaviors from eating to low activity and away from grooming and sleep when food is absent, possibly indicating increased exploratory behavior in search of food.

To further examine this hypothesis, Morley, Levine, Gosnell, Kneip, and Grace (1987) again looked at behavior in rats following 5 μg/5 μl of NPY

into the rLV. Trained observers, blind to treatments, observed behaviors for two hours following injection. Animals were infused with saline or NPY and placed in their home cage with food and water removed or in a novel cage containing empty food cups. NPY animals placed in home cages sans food and water moved more, explored the empty food shutes, and decreased resting and grooming compared to controls. Similar to Stanley and Leibowitz's (1984) findings for rats when food is absent, animals demonstrated greater movement and exploratory behavior when in a familiar environment, possibly indicating a search of food. In contrast, NPY animals placed in the novel environment did not increase moving or exploration of the food cups, but they decreased grooming. During the first hour, NPY rats displayed an unusual head-turning behavior, while resting on their bellies, and spent more time proportionally "chattering" their teeth than saline controls. The significance of these behaviors is unclear. However, the teeth chattering may be a stereotypical response to the consummatory conditioning with feeding behavior. These data suggest that NPY will drive animals to consume food when it is present or engage in exploratory behaviors to seek food in a familiar environment when food is not present. In a novel environment, data are not as clear, possibly due to an unforeseen stress response. However, these studies employed designs to examine food seeking behaviors, not simply behaviors related to food consumption. It is possible that NPY modulates behaviors associated with food consumption.

In their paper on NPY-induced feeding behaviors, Seeley, Payne, and Woods (1995) describe feeding as involving two types of behavioral responses. Appetitive responses include behaviors like orienting and movements to locate and acquire food, as have been mentioned in previous studies. Consummatory behaviors, on the other hand, are more stereotyped behaviors related to ingestion, like chewing and licking. To evaluate the consummatory components of feeding, Seeley et al. cannulated the 3V of rats and bilaterally implanted intraoral catheters. These instruments consisted of a catheter anchored to the skull and passing into the mouth through which solutions could be delivered at the researcher's discretion. Following varying doses of NPY (3 to 30 µg in 5 µl), no dose affected intraoral intake. That is, animals consumed as much sucrose solution through the catheter following any NPY dose as they did following saline. However, 24-hour food deprivation increased the intraoral intake. In addition, when rats were given access to a sucrose solution in a standard sipper, a 9.5 µg dose of NPY increased drinking compared to saline. These data support NPY as having primary effects on the appetitive aspects of feeding rather than the consummatory behaviors.

In addition to driving animals to seek food, NPY may motivate animals to work to overcome obstacles or to withstand noxious stimuli to obtain food. Flood and Morley (1991) have assessed the motivation to seek food in food-deprived mice receiving 3V infusions of NPY. In this three part study, following a delay, mice infused with NPY were tested with NPY (0.5 to 5 µg in

2 µl) to determine if they would press a lever to receive a milk solution more than saline controls over 30 minutes following infusion. These animals had previously been trained to press the lever for milk using continuous reinforcement. Mice were also tested to assess the degree of hunger in the animals by having them receive a tongue shock (0.3 mA DC) each time they attempted to drink from the sipper. In this second study, a 0.2 µg NPY dose was added. Animals were monitored until they refrained from sipping for 90 seconds following infusion. In part three, pre-fed mice were again administered NPY, and following a 30-minute delay, mice were given a milk-quinine solution, and a variety of doses of NPY, ranging from 0.25 to 1.5 µg, were administered. For part one, the authors found that only a 1.0 µg dose increased lever pressing beyond saline levels. On the other hand, in part two, all doses of NPY increased the mean number of shocks tolerated to receive the solution in an inverted U relationship according to dose. Interestingly, the 1.0 µg dose again produced results at least two times that of the other doses or saline. For the final study group, mice receiving the 0.75 and 1.5 µg doses consumed more quinine-adulterated milk than the saline controls. The response evoked by the 1.5 µg dose in a mouse given the quinine solution was equivalent to that evoked by 0.25 µg when a mouse was given regular, unadulterated milk.

NPY appears to orient animals to seek and consume food when it is available. When food is absent, NPY administration leads animals to increase exploratory behaviors, perhaps in search of food. If food is directly

administered into the mouth, NPY-infused animals will not consume more food than saline controls. However, when given the chance to acquire the solution, they will consume more than controls. Further, animals will tolerate more tongue shocks, work to receive food by bar pressing, and tolerate greater quantities of quinine-adulterated milk following NPY infusion. Nonetheless, NPY does not seem to drive animals to select non-specific nutrients. Food seeking behaviors seem to show preferences for nutrients high in carbohydrates.

One example of how NPY affects macronutrient preference can be found in the study by Stanley, Daniel, Chin, and Leibowitz (1985b). These researchers administered NPY (330 ng) or saline (0.3 µl) into the PVN of satiated rats and measured food intake one hour postinjection. Animals were initially given simultaneous access to three freshly prepared diets of high carbohydrate (HC, sucrose, corn starch, and dextrin), protein (HP, casein), and fat (HF, lard) and were pre-exposed to the diets for two weeks prior to the experiment. Diets were placed in glass dishes in the cages so that animals could ingest any of the diets, and diets were supplemented with vitamins and minerals. Later, animals were given access to all diets or pairs, HP-HF, HC-HF, or HP-HC, and intake was measured over one hour postinjection. NPY increased total food intake compared to saline infusion when animals were given access to all three diets or any pair. NPY produced greater consumption of the HC diet relative to saline control or the other diets. NPY increased carbohydrate consumption nearly seven-fold

compared to saline whereas NPY did not affect protein or fat consumption under the three diet conditions. When animals were given access to diet pairs, carbohydrates were again the diet of choice in the HC-HF and HC-HP pairs. However, protein consumption increased in the HC-HP pair given NPY although to a lesser extent (about 40%) compared to carbohydrates. When given access to fats and proteins only, protein consumption and fat consumption increased, although fat consumption increased to a slightly greater extent than protein consumption. Thus, PVN doses of NPY appear to elicit nutrient-specific ingestion favoring carbohydrates over proteins and fats for up to one hour following infusion. Subsequent studies modified the designs to test macronutrient preference across longer time periods (Morley et al., 1987), following chronic infusion of NPY (Beck, Stricker-Krongrad, Nicolas, & Burlet, 1992b; Stanley, Anderson, Grayson, & Leibowitz, 1989), and when presented with novel flavors or with saline solutions (Lynch, Grace, Billington, & Levine, 1993). In all cases, there again appeared to be a distinct preference for carbohydrate diets.

Metabolism, Independent of Feeding. Independent of the multiple behavioral changes associated with NPY-induced feeding is the ability of NPY to affect metabolism. As an orexigenic agent, NPY is responsible for enticing an organism to take in greater quantities of nutrients. As a metabolic agent, NPY continues this function to conserve energy. Moltz and McDonald (1985) conducted a two-part study to examine how NPY may act as a metabolically efficient agent. Cannulae were implanted to target the 3V of

ovarectimized rats. NPY was injected and following a 30-minute waiting period, animals were sacrificed. In another group of animals, NPY was given at differing doses and blood was sampled from the jugular vein over two hours following infusion. In the first group, NPY led to an increase of plasma insulin levels twice the level of controls. In the second group, the higher dose (5 µg/2µl) of NPY infused led to a rise in insulin within 30 minutes. The levels were four times higher than the other groups. The insulin levels of this NPY-treated group remained elevated over the next 1.5 hours. The lower dose of NPY (0.5 µg/2µl) had no effect on insulin.

In a similar study, Sainsbury, Cusin, Doyle, Rohner-Jeanrenaud, and Jeanrenaud (1996) found that NPY injected intracerebroventricularly (ICV) into lean Zuckers over six days tended to increase glucose-stimulated insulin as well as basal insulin beyond levels of controls, even when rats were fed the same diet consumed by saline-treated controls. In addition, expression of *ob* protein producing leptin in white adipose tissue increased with NPY when animals were pair fed and returned to control levels with the cessation of drug over two weeks. Leptin expression is an index of white fat mass and may serve as a reference for obesity via adiposity (review, Caro, Sinha, Kolaczynski, Zhang, & Considine, 1996). For these animals, insulin secretion normalized shortly after NPY infusion was terminated. Accompanying the enhanced storage of nutrients is a resistance to insulin effects at muscle and reduced fat mobilization. Utilizing lean Zucker rats, Zarjevski (1993, 1994) and Vettor (1994) and their colleagues chronically

infused NPY ICV over a one-week period. Following treatment, lipoprotein lipase (LPL, an index of storage of nutrients as fat) activity in white adipose tissue and liver was assessed as well as serum glucose and insulin. Chronic NPY increased body weights and fat pad weights. NPY also elevated insulin and increased LPL activity. Further, most of these effects occurred in rats fed ad lib or pair fed to 85% of control levels (Zarjevski et al., 1993). In a later study, Zarjevski et al. (1994) found that similar treatment increased GLUT4 activity in white adipose tissue without changing muscle GLUT4. Because GLUT4 is a glucose transporter, these results suggest shunting of nutrients from muscle to fat by NPY. To explore the duration of chronic NPY on metabolism, Vettor et al. (1994) followed animals for seven days after cessation of NPY infusion. Over the recovery period, elevated feeding levels returned to control levels along with body weight, plasma insulin, and GLUT4 in white adipose tissue.

Additionally, Billington, Briggs, Harker, Grace, and Levine (1994) injected multiple doses of NPY into the PVN of male rats over 24 hours and examined changes in uncoupling protein (UCP) of brown adipose tissue and LPL. UCP is an index of fat breakdown. NPY was shown to decrease UCP mRNA and increase LPL mRNA compared to controls regardless of whether animals were fed ad libitum or pair-fed to control intake.

Complementing the insulin and fat metabolism results are tests of NPY's effects on energy efficiency, as gauged by energy expenditure and

respiratory quotient. Menendez et al. (1990) found an increase in respiratory quotient in the absence of a change in activity or energy expenditure when they infused NPY into the PVN of rats. They also noted that depending on the dose, respiratory quotients exceeded or fell below saline control levels in a time-dependent fashion. The authors interpreted this outcome to mean that NPY enhances carbohydrate metabolism along with fat synthesis.

Thus, NPY acts as an energy promoter by increasing energy storage and reducing energy breakdown. When nutrient stores are liberated, fat stores are preserved in favor of carbohydrate expenditure. Based on this information, blocking the transmission of NPY's signal from the brain to the peripheral tissues may normalize metabolic status. Rohner-Jeanrenaud and colleagues (1995; 1996) have summarized NPY's over-activation of feeding behavior and the hypothalamic-pituitary-adrenal axis, but, more specifically, they have described its part in the vagal-pancreatic pathway, by which the vagus nerve mediates pancreatic insulin secretion. A link between NPY and metabolic controls independent of feeding may be found in NPY-containing nuclei that lie along this parasympathetic vagal pathway. Neurons projecting to the vagal control centers have been mapped to contain NPY, as discussed previously. Nuclei along these paths include the PVN, ARC, DMH, LH, and extra-hypothalamic nuclei such as the lateral bed nucleus of the stria terminalis (l-BNST), and the medial portion of the central nucleus of the amygdala (m-CNA) which connect to the dorsal motor nucleus of the vagus (DMX; Gray et al., 1986). Li, Xu, Rowland, and Kalra (1994) have noted

cellular activity in the PVN, SO, l-BNST, DMH, m-CNA, and nucleus of the solitary tract (NTS), and to a lesser extent in other nuclei, following ICV infusion of NPY. The distribution of activity, as measured by expression of the immediate early gene c-fos, differs whether or not animals are exposed to food. These findings suggest that diverse NPY activity along vagal pathways may contribute to the hyperinsulinism found in the presence of elevated NPY. Sainsbury et al. (1997) have supported this proposal by finding that vagal activity mediates NPY-induced elevations in basal and glucose-stimulated insulin release since vagotomy abolishes those effects. Lending support to this notion, Rohner-Jeanrenaud and colleagues (1983) found evidence for the role of the vagus in the hyperinsulinemic state of the obese Zucker rat.

Nonetheless, attempts to block NPY's effects centrally and reduce these peripheral effects have not been very successful. Beck and colleagues (1994) failed to change either feeding or metabolic activity, as reflected in a lack of weight loss, following administration of the NPY antagonist PYX-2. More research is needed to understand the pathways and receptor couplings responsible for NPY's feeding-metabolic effects before such pharmacological intervention will be successful.

Feeding Receptor Specificity. Although at least six different NPY-binding receptors have been identified, there are differences in feeding responses based on stimulation of individual receptors. In rats, the Y1, Y2, and Y5 receptors appear to be most involved in mediating NPY's orexigenic

effects (review, Gehlert, 1998). Widdowson et al. (1997) demonstrated that Y2 and Y5 receptors respond to chronic food restriction and high-caloric intake. Animals restricted to 60% of regular food intake or placed on a mash diet of chow, condensed milk, and sucrose had a decrease in binding at receptors insensitive to the Y1-sensitive compound BIBP3226 when compared to controls. Autoradiographic maps showed the decreased binding to hypothalamic and thalamic as well as amygdaloid sites. The authors suggest that increased NPY synthesis and/or secretion due to the fasting and high-calorie states led to a down-regulation of receptors in the regions sensitive to feeding. Thus, in terms of feeding restriction and caloric modification, Y2 and Y5 receptors may be more responsive than Y1 receptors to NPY-mediated effects.

The potential role of the Y5 receptor in feeding was first proposed based on *in situ* hybridization identification of Y5 mRNA within feeding areas of the hypothalamus, specifically in the LH, PVN, SO, and ARC. Y5 mRNA was also identified in the central amygdala (Gerald et al., 1996). Therefore, Gerald and colleagues conducted pharmacological analyses to determine the sensitivity of Y5 to evoke feeding. Administering doses of different receptor stimulators into the rLV, the team measured food intake over a four-hour follow-up period. They found that NPY fragments binding to Y5 receptors *in vitro* tended to produce greater food intake than fragments which bind more specifically to other receptors such as Y2 and Y4. Taken together with the Widdowson (1997) research, it appears that the Y5 receptor may be directly

related to NPY-induced feeding and respond to changes of endogenous NPY based on nutritional status. Subsequent research by Haynes, Arch, Wilson, McClue, and Buckingham (1998) using similar techniques lends support to the Y5-receptor effects on feeding, but those researchers hesitate to label Y5 as an exclusive receptor for feeding behavior. They favor combined actions of the Y1 and Y5 receptors.

Evidence points to a distinction in function between Y1 and Y2 receptors when it comes to macronutrient consumption. Leibowitz and Alexander (1991) allowed rats to self-select diets of carbohydrates, fats, or proteins during an overnight period immediately following infusion of Y1 and Y2 receptor selective compounds into the PVN. The Y1 agonist [Leu^{31},Pro^{34}]NPY preferentially increased carbohydrate consumption and reduced protein consumption. On the other hand, Y2 stimulation by the fragment NPY[13-36] produced an overall decrease in feeding with specific reductions in carbohydrates and proteins. Kalra, Dube, Fournier, and Kalra (1991a) also examined Y1 and Y2 receptor stimulation and feeding; however, they did not examine macronutrient preferences. They administered the same receptor agonists into the PVN or 3V of rats and measured feeding over a two-hour period. Interestingly, they noted an increase in feeding when the Y2 agonist was given compared to saline. Nonetheless, when the Y1 agonist was infused and food intake was compared to that of rats receiving the Y2 agonist, the Y1 receptor was clearly associated with greater food

intake than the Y2 receptor whether fragments were infused into the PVN or 3V.

Stanley, Magdalin, Seirafi, Nguyen, and Leibowitz (1992) examined Y1 and Y2 mediated feeding using different agonists targeted in a different brain region, the PFH. Other research has established the PFH as the hypothalamic site most sensitive to NPY-induced feeding (Stanley, Magdalin, Seirafi, Thomas, & Leibowitz, 1993). Food intake over four hours following infusion of the Y1 agonist [Pro^{34}] NPY and Y2 agonist C2-NPY was measured. The Y1 agonist resulted in a larger, dose dependent response of feeding than the Y2 agonist. However, the Y1 receptor agonist still did not result in as robust of a feeding response as NPY infused into the PFH.

Kanatani et al. (1996) extended these Y1-Y2 receptor studies to test effects of Y1 antagonism on feeding behavior. Following an overnight fast, rats were given the Y1 antagonist 1229U91 or saline in the rLV. When given access to food, 1229U91-treated rats ate less up to four hours following administration. 1229U91 also reduced the feeding response when it was paired with infused NPY. Thus, over two hours following infusion of both compounds, animals ate nearly 50% less than rats given NPY alone.

Looking back on evidence presented earlier that Y2 receptors may be located presynaptically and Y1 receptors postsynaptically, it is of interest that Y2 agonists suppressed food intake. The exact mechanisms underlying this

effect are not well understood. Leibowitz and her colleague (1991) suggest an interaction of Y2 stimulation with release of monoamines. However, it is also possible that Y2 receptors act as autoreceptors to self-regulate synthesis and release of presynaptic NPY. Others suggest that there may be receptor subtypes sharing the characteristics of both Y1 and Y5 receptors that have yet to be identified (Haynes et al., 1998). Nevertheless, Y1 and Y5 receptors appear to be most directly associated with orexigenic behavior while Y2 receptors may act to buffer those feeding effects.

Food Deprivation. Thus far, NPY has been shown to increase feeding, motivate feeding behavior, and selectively alter macronutrient consumption when infused from exogenous sources. Many of these studies, however, were performed in animals that had previously been satiated attesting to the independence of NPY as an orexigenic agent. However, the food orienting characteristics of the peptide suggest that endogenous stores of NPY should change dramatically as nutrient availability decreases. In addition, many studies have examined the mechanism for the change (i.e., increased synthesis or changes in transport and release).

A number of studies point to the change in concentration of NPY across the hypothalamus as duration of food deprivation is enhanced (e.g., Beck et al., 1990b). However, Calza et al. (1989) employed immunocytochemistry techniques to examine PVN changes within 72 hours of fasting in adult male rats. NPY immunoreactivity was significantly greater

in 72 hour deprived than ad lib control rats. Thus, food deprivation seems to spur an increase in NPY perhaps to motivate animals to seek nutrients. Other studies have addressed more directly the mechanisms that produce the increase in NPY.

Increased synthesis may be responsible for the elevation in NPY found in fasted animals. For example, White and Kershaw (1990) dissected tissue from the hypothalamus of 72-hour fasted, overnight fasted, or ad lib fed adult rats. NPY precursor preproNPY mRNA was localized to cell regions using *in situ* hybridization. Rats fasted for 72 hours had higher preproNPY mRNA levels than overnight fasted rats which had levels higher but not significantly different from ad lib controls. There was an increase in the expression in ARC of 72-hour food deprived rats versus controls, but no differences in reticular nuclei of the thalamus or cortex based on feeding.

In addition to synthesis, the possibility that food restriction alters the release and transport of NPY has been evaluated both *in vitro* and *in vivo*. Dube, Sahu, Kalra, and Kalra (1992) explored basal and evoked *in vitro* release of NPY in bilateral PVN and VMH of adult male rats given ad lib food or food deprived for four days. Food deprivation decreased body weight and increased basal PVN-NPY release compared to ad lib controls. KCl-evoked release of NPY further increased release in food deprived rats over controls and appeared to be dose dependent based on KCl concentration. No real changes in basal release between ad lib and deprived rats were evident, but

release increased from both ad lib and deprived rats with KCl in a dose-dependent fashion in VMH. Thus, it appears that the primary release site of increased ARC synthesis may be the PVN, more so than the VMH. It also seems that deprivation may not only increase basal release in the PVN, but also enhance sensitivity to evoked release.

Kalra, Dube, Sahu, Phelps, and Kalra (1991b) took this research further by testing release *in vivo* using push-pull cannulae (PPC). Male rats were tested during the light cycle and were ad lib fed or allowed access to food for four hours each day. During the refeeding period, PPC measurement of extracellular NPY was performed in PVN of controls, food restricted or a food restricted group without food present. Food restricted animals had greater NPY release measured compared to ad lib controls and refed-food restricted rats. However, early during the measurement period, no differences between refed groups were found. Refed animals dropped to control levels shortly after perfusion began. Sacrifice concentrations of NPY across the MPO, ME, ARC, and VMH did not change during the period, although DMH levels rose modestly in restricted rats near the end of perfusion, suggesting that the primary fasting-refeeding circuit involves mechanisms that are mediated in the ARC-PVN pathway. Thus, NPY release appears primarily to follow the PVN pathway and occurs based on degree of deprivation, although individual patterns may differ with a similar average release.

Relationship of NPY and Insulin. Neuropeptide Y activity in the hypothalamus responds to fasting and feeding status. Therefore, NPY probably responds primarily or secondarily to a peripherally-generated index of nutrient availability. As discussed previously, insulin is a hormone released at basal levels and in greater levels following feeding. Insulin also is capable of entering the central nervous system. An antagonistic relationship between circulating insulin and central NPY has been established (review, Schwartz et al., 1993). Support for this relationship comes from drug manipulation of insulin levels. In 1988, Pierson et al. demonstrated that nearly complete elimination of peripheral insulin secretion elevates NPY levels in the hypothalamus by increasing NPY synthesis, as measured by NPY m-RNA. Insulin production was destroyed in this study by streptozotocin (STZ) administration. STZ is an antibiotic that selectively destroys the pancreatic beta cells following as little as one dose. STZ-treatment also elevates NPY in the PVN, ARC, and other regions of the hypothalamus and is reversed by insulin therapy (Sahu et al., 1992, 1990). Marks, Li, Schwartz, Porte, and Baskin (1992) have noted increases in NPY mRNA in the ARC of fasted animals that corresponded to decreases in serum insulin levels as the animals were fasted. Insulin is released at basal levels and in response to the presence of food. As plasma insulin levels increase, NPY levels in the hypothalamus decrease whereas insulin disruption increases NPY. NPY levels correspond positively with food intake and adipose conservation. Thus, NPY levels respond to circulating nutrient status to modulate nutrient availability.

Relationship of NPY and Zucker rats. Previously discussed literature describes obese Zucker rats as being hyperphagic with lower CSF insulin levels relative to hyperinsulinemia in the periphery. These factors point to obese Zucker rats as a possible model of elevated hypothalamic NPY (review, White & Martin, 1997). Research supports this model across various ages of the animals.

Beck, Burlet, Bazin, Nicolas, and Burlet (1993) have identified differences in obese and lean NPY levels as early as 30 days of age. Obese rats were found to have higher levels of NPY in the ARC-ME at weaning age. There was a marked increase in NPY across a number of hypothalamic nuclei, including ARC, ME, PVN, DMH, LH, LPOA, MPOA, and the nucleus accumbens, from 16 to 30 days of age in both lean and obese rats. Ten-week-old obese rats also have higher NPY levels in the ARC-ME whereas leans have higher levels in the VMH (Beck, Burlet, Nicolas, & Burlet, 1992a). On the other hand, 18-week-old freely fed obese rats have higher NPY content in the PVN, AHA, VMH, DMH, and ARC-ME than leans (McKibbin et al., 1991). At six months of age, the differences between lean and obese NPY concentrations are more divergent with obese rats having higher NPY levels than heterozygous lean rats at ARC-ME, PVN, and SCN. Heterozygous leans, in turn, have higher levels than obese rats in the VMH, similar to previously mentioned findings in 10-week-old rats. At six months of age, differences are also present in lean genotypes with heterozygotes having higher content at the ARC-ME than homozygotes (Beck et al., 1990a).

The elevated NPY levels found across obese rats are likely due both to increased synthesis of NPY, as measured by mRNA expression in ARC tissue, compared to lean rats (Sanacora, Kershaw, Finkelstein, & White, 1990) and differences in the release of NPY. Using the PPC technique, Dryden, Pickavance, Frankish, and Williams (1995) measured greater release of NPY from PVN of "fatty" rats compared to leans.

In summary, obese rats appear to maintain higher levels of NPY across a number of hypothalamic nuclei across a range of ages than leans. Exaggerated synthesis and release of NPY may explain some differences between lean and obese rats at specific nuclei. In spite of these well replicated findings, there remains speculation about how NPY and hypothalamic tissue interact to influence feeding and metabolism in the different genotypes.

The brains of obese rats may not accurately detect and respond to peripheral nutritional state. Some researchers have failed to find a change in hypothalamic NPY expression following periods of fasting as long as 72 hours (Sanacora et al., 1990), leading them to propose that the brains of obese rats are insensitive to the food deprivation because they inaccurately sense the body as being in a chronic state of nutritional need. Thus, the brains of these animals fail to increase NPY following food deprivation. Schwartz et al. (1991) have supported this claim using 16-week-old rats by finding that ICV infusion of insulin does not produce a decrease in

preproNPY mRNA at ARC in fasted obese rats as it does in fasted leans. Along these same lines, not all studies find that obese rats respond to ICV infusion of NPY with increased food intake (Brief, Sipols, & Woods, 1992) while others find mild increases in feeding time without changes in meal size (Stricker-Krongrad et al., 1994). At the same time, others have found NPY responses in obese rats following food restriction. At six months of age, increased NPY occurs for food restricted obese rats compared to similar leans and free fed leans in blocks of medial hypothalamic tissue (Williams et al., 1991). Further, chronic diet restriction increases preproNPY mRNA in both lean and obese rats (Pesonen, Huupponen, Rouru, & Koulu, 1992).

It is possible that the hyperphagic window of insulin surge from approximately five to 15 weeks of age results in an insensitivity of NPY to the normally inhibitory effects of insulin on NPY synthesis and/or release. Perhaps intervention during this period leading to greater hypothalamic insulin sensitivity will modulate NPY expression and blunt some of the hyperinsulinemic pathology observed in these animals.

NPY, Eating Disorders, and Obesity in Humans. Given the relationship of NPY to food intake and response of endogenous levels to indices of nutrient availability like insulin and food deprivation, it is possible that NPY may be implicated in eating disorders in humans. However, before these notions can be examined, it must be recognized that NPY is distributed throughout the human brain in patterns similar to those of rats, as previously

described. Adrian et al. (1983) mapped the distribution of NPY-like activity in human brains using postmortem tissue samples from males and females having no history of psychological or neurological disorders. Radioimmunoassays and immunocytochemistry revealed higher concentrations of NPY activity in rostral than caudal brain areas. Specifically, high concentrations were found in caudate and putamen of the basal ganglia. Other areas containing concentrated activity were the limbic system, including the hippocampus and amygdala, hypothalamus, and motor cortex. However, many of these areas had concentrations at half the level of the basal ganglia.

More specific analyses of the hypothalamus, removed six hours after death, from male and female patients aged 40 to 70 years, uncovered rostrocaudal paths from the SO to the mammillary bodies with high amounts of NPY activity near the 3V and relatively less activity in the more ventral areas like the ME and the more lateral hypothalamic regions. The most concentrated region of NPY was in the VMH. Some bands of activity running rostrocaudal from the PVN were also observed (Pelletier, Desy, Kerkerian, & Cote, 1984). Although it should be noted that only four subject samples were used in each of these studies, the time from death to fixation of tissue probably did not affect the viability of NPY. Tests for stability in rat brain samples show that NPY levels appear to be stable up to 48 hours before brain tissue is preserved. The researchers conducting those studies examined human brain samples and found results relatively similar to those

of Adrian and colleagues (1983) in whole brain NPY distributions (Dawbarn, Hunt, & Emson, 1984).

With regard to eating disorders in humans, Kaye, Berrettini, Gwirtsman, and George (1990) have examined CSF levels of NPY in female patients, average age about 25 years, fitting the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (III-R) criteria for anorexia and bulimia. Anorectic patients had maintained body weights less than 75% of average weight for at least six months to be included in the study whereas bulimics maintained weights in the normal range. Nearly all anorectics experienced amenorrhea with only one-third of bulimics having that condition. CSF was obtained by lumbar puncture shortly after admittance (onset) to treatment, when patients were considered to be at the peak of the disorders. Anorectics were sampled again at short-term (three to four weeks) and later at long-term points (average 31 months) following treatment to restore body weight. Bulimics were sampled at onset and again 30 days after admittance. Control subjects not having eating disorders were sampled for reference.

At the onset of treatment, anorectics had higher CSF-NPY levels than controls, bulimics at both measurement times, and anorectics at long-term sampling. In addition, short-term measurements of anorectics were higher than controls and bulimics at either measurement time. Thus, anorectics at the peak and during short-term weight gain maintain NPY levels higher than

controls, long-term weight-recovered anorectics or bulimics. Other findings by Kaye et al. (1989) suggest that as anorectics regain weight and progress from six weeks of weight recovery to at least six months of recovery, weight restoration is associated with decreases in CSF-NPY. It appears that NPY is more likely involved in anorectic conditions than bulimic conditions. Interestingly, it is the anorectics who continue to have amenorrhea following weight restoration who also continue to maintain elevated NPY. The authors of these projects suggest that NPY's relationship to gonadal-stimulating hormones like luteinizing hormone releasing hormone (LHRH) in the hypothalamus may be responsible for the disturbed menstrual cycles. Thus, it is possible that NPY may act in either a primary or secondary way with respect to anorectic behavior. Endogenous NPY may respond to circulating indices of nutrient deficiency like decreased insulin to initiate feeding behavior in an attempt to reverse the negative energy balance. On the other hand, a primary disruption in NPY, such as receptor insensitivity, may lead to elevated NPY levels explaining a lack of feeding when CSF levels of NPY are elevated. Although both hypotheses may be plausible, they are difficult to test in human subjects due to the inability to collect pre-anorectic CSF samples and follow CSF-NPY changes comparatively across the course of the disorder. Nonetheless, a new animal model, the anorectic mouse, may be useful in answering some of these questions.

The *anx/anx* mouse is an autosomal recessive animal model for the anorectic phenotype. These animals can be phenotyped from non-anorectic

littermates by five days of age based on their emaciated appearance. By 20 days of age, their body weights are about 50% of non-anorectic littermates. The *anx/anx* mice demonstrate a restriction of food intake, about 50% of non-anorectic intake, rather than complete starvation, across the first 20 days of postnatal life. They have a host of physiological indices suggesting altered metabolism such as increased alkaline phosphatase, increased uric acid, decreased glucose, and increased blood urea nitrogen as well as neurological abnormalities like body tremors, lack of coordinated movement, and hyperactivity. Most *anx/anx* mice die by the age of 22 days (Maltais, Lane, & Beamer, 1984).

Fluorescence and *in situ* hybridization studies have revealed that at 20 days of age, the NPY content across many hypothalamic nuclei (MPOA, LH, PVN, and DMH) is decreased in *anx/anx* mice compared to controls although ARC levels show non-uniform distribution with increases in some regions of the nucleus. Prepro-NPY mRNA levels also show increases in activity in the ARC, cortex, and hippocampus of *anx/anx* compared to controls. Thus, the *anx* mutation may be associated with an increase in NPY synthesis, but a decrease in transport, possibly as a primary genetic deficiency in these mice, or as a secondary response to the emaciated physiology in an attempt to restore weight (Broberger, Johansen, Schalling, & Hokfelt, 1997). However, it should be noted that subsequent research challenges these findings by demonstrating a failure of ARC NPY mRNA to respond to the deficient state

in *anx/anx* mice, unlike 24-hour food-deprived non-anorectic mice that increase synthesis indices (Jahng, Houpt, Kim, Joh, & Son, 1998).

In contrast to states of energy deficiency in humans, other researchers have targeted obese populations for possible faulty regulation of CSF-NPY. It would appear possible for elevated NPY levels to result in increased storage of nutrients and/or increased feeding resulting in a state of overweight, positive energy balance. Brunani and colleagues (1995) examined NPY levels in obese and ideal weight women between the ages of 22 and 41 years. Obese subjects had an average body mass index in the morbid range (approximately 50) whereas control subjects were in the ideal range (approximately 23). Following an overnight fast, lumbar punctures were performed to obtain CSF from these women not having diabetes or other related endocrine disturbances. Plasma NPY levels were also measured. Neither CSF nor plasma NPY levels of obese participants differed significantly from controls. On the other hand, Strombom et al. (1996) examined females, average age 38 years, classified as obese (body mass index 35 to 36) with high and low waist-to-hip ratios. CSF obtained by lumbar puncture during nonmenstrual phases showed a decrease in the CSF-NPY of obese patients compared to a non-obese reference group.

The incompatible findings of the Brunani (1995) and Strombom (1996) studies should be considered with respect to the body mass of the participants. Subjects in Brunani's study had a body mass in the morbidly

obese range whereas Strombom's subjects had a less severe obese body mass. It is possible that NPY is more involved in weight changes during the phasic period in which weight gain is occurring or in which weight is maintained below the dangerously morbid levels. People having excessive body mass likely have a host of chronic, systemic metabolic features that operate differently from people with a lower body mass. These questions are not easy to address because they require CSF and plasma samples from an individual subject across a period of weight gain from an ideal body mass to a morbid range. Therefore, the difference in body mass of the subjects between the studies may mask the role that NPY plays in human obesity.

CSF NPY levels in obese human populations are inconsistent for determining whether the peptide plays a role in the origins or maintenance of the obesity syndrome. Moreover, the application of Y1 and Y5 "feeding" receptor findings to humans is questionable. A study of 93 Caucasian French families having a high rate of obesity showed little role for unusual activity of those receptors (Roche et al., 1997). In anorectic populations, however, NPY disruptions follow a more consistent pattern of elevation, yet the onset of NPY disruptions is more difficult to determine. The *anx/anx* mouse model points to a disruption in hypothalamic NPY levels seeming to indicate that the animals possess an inability to regulate NPY synthesis and transport as non-anorectic animals do. For both the negative and positive energy balance groups, it is difficult to determine to which indices NPY is responding to cause altered levels. It is possible that one circulating index

involves insulin. If this is the case, a number of drugs may be able to influence feeding and weight gain by modifying NPY-insulin communication. One drug that may have potential therapeutic use in this model is diazoxide.

Diazoxide as as Insulin Antagonist

History and Actions

Diazoxide, 7-chloro-3-methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide ($C_8H_7ClN_2O_2S$, molecular weight 230.7) is a benzothiadiazine drug that was first synthesized in the early 1960's (Baker-Norton, 1995; Black, 1968; Rubin et al., 1961). Diazoxide (DZ) was originally prescribed as an antihypertensive due to its action as a potent vasodilator of small vessels (Rubin et al., 1961; Rubin, Zitowitz, & Hausler, 1963). It did not take long for its actions as a metabolic regulator also to be recognized (Black, 1968). When orally administered, DZ suppresses insulin secretion from pancreatic beta cells by increasing cellular membrane permeability to potassium ions which leads to a decrease in calcium-stimulated exocytosis of insulin (review, Henquin, 1987; review, Lebrun, Antoine, & Herchuelz, 1992; Stanley, 1997). Thus, DZ appears more to prevent the release than the synthesis of insulin (Greenwood, Mahler, & Hales, 1976). Because of its ion regulating activities, DZ has become a popular agent for studying cellular activation and excitation in brain regions like the hippocampus (for example, Ben-Ari, Krnjevic, & Crepel, 1990). At the organ-system level in the periphery, DZ has been used in both human and animal research to explore the varying functions of

insulin. For example, Pronicka et al. (1995) administered DZ in pediatric cases of hyperinsulinism and studied insulin's control of its pancreatic counterpart glucagon. Leahy, Bumbalo, and Chen (1994) used DZ to blunt insulin secretion in 90%-pancreatectomized Wistar rats. This process produced improved glucose tolerance and supported the potential role of hyperinsulin secretion in noninsulin-dependent diabetes mellitus. These researchers concluded that the increased insulin response to glucose in hyperglycemia is due to excessive insulin secretion. In addition, Ratzmann et al. (1983) administered DZ to obese adults and found that it reduced glucose-stimulated insulin release after four to five days of treatment in spite of unchanged basal insulin levels. While it is a useful tool for exploring both cellular actions and insulin's various physiological roles, DZ is usually recognized for its medical properties.

Medical Treatments

Since DZ suppresses insulin secretion, it is not surprising that much of its therapeutic use has been in providing relief to individuals having hyperinsulinism of varying etiologies. As a metabolic drug, DZ was first used in treating hypoglycemia arising from primary pancreatic dysfunction (e.g., carcinoma or adenoma) as well as liver and pituitary disease (review, Black, 1968). Particularly, DZ has been successful in reducing hyperinsulinism-induced hypoglycemia in infants and children (Stanley, 1997). However, DZ is not effective as a treatment in individuals having mutations of the sulphonylurea receptor on the beta cell since this is the receptor which DZ

contacts to alter cellular permeability to potassium (review, Stanley, 1997). More recently, DZ has been used to improve insulin secretion in adult onset autoimmune diabetes (Bjork et al., 1996). Some researchers have proposed that the hypoinsulinism of the individuals is due to the overproduction of insulin by the pancreatic beta cells which leads to beta cell exhaustion. DZ treatment has modestly restored insulin secretion in some of these individuals by preventing the exhaustion process (Bjork et al., 1996).

Diazoxide has actions at the cellular level which make it a popular tool for researching cellular and peripheral physiology. Because DZ reduces insulin levels associated with hyperinsulinism, it is possible that DZ would be an appropriate treatment for reducing insulin levels which would, in turn, reduce insulin resistance and lead to normalization of metabolic processes and feeding behavior and subsequent weight loss in hyperinsulinemic obese individuals. Research by colleagues of this lab has begun the exploration of this possibility using the hyperinsulinemic obese Zucker animal model.

Our Progress to Date

In recent years, Alemzadeh and colleagues have explored the role of DZ in weight loss and improved metabolic functioning in female obese Zucker rats. In 1993, they published data on the effects of two different DZ doses, 50 mg/kg/day and 100 mg/kg/day, in lean and obese rats placed on the doses for 8 weeks. Results showed that the 50 mg dose was sufficient to lower insulin in comparison to placebo controls of both genotypes, but

otherwise, the dose had little effect on body weight, food intake, glucose tolerance or adipocyte insulin binding. The 100 unit dose produced more dramatic effects in both genotypes. DZ-obese rats failed to gain as much weight as controls, consumed more calories, had smaller diameter fat cells, maintained lower plasma glucose, and lower insulin levels. Further, DZ-obese rats had a more normal response to glucose challenge at lower insulin levels, indicating improved insulin sensitivity and responsiveness. On the other hand, DZ-lean rats became glucose intolerant, showing a diabetic response to glucose challenge, likely due to exaggerated DZ-induced insulin suppression. The higher DZ dose also increased the insulin binding capacity of adipocytes in lean and obese rats. Thus, Alemzadeh et al. (1993) concluded that a 100 mg/kg/day DZ dose improved insulin responsiveness in obese rats along with a decrease in body weight while producing a diabetic-like response in lean rats.

To investigate the dose-dependent effects of DZ on peripheral metabolics and weight loss further, Alemzadeh and colleagues (1996) extended their study to include more biochemical data to assess fat metabolism. They placed seven-week-old Zucker female rats on a 150 mg/kg/day dose of DZ for six weeks. Again, they found that obese and lean rats had lower insulin with DZ treatment. Obese rats had decreased weight gain and adipocyte diameter and lower glucose and triglyceride levels. Leans, for the most part, remained unaffected. Food intake was not altered for either genotype on DZ, nor were free fatty acid or cholesterol levels

changed. Glucose tolerance effects mirrored those of the 100 mg dose with improved tolerance in obese rats and a diabetic response in leans. DZ enhanced insulin receptor binding and receptor number in adipose tissue of obese rats and to a greater extent in leans as well as decreased insulin-stimulated lipogenesis.

Based on these studies, Alemzadeh and colleagues have concluded that DZ at a dose of 100-150 mg/kg/day given from 6-8 weeks will reduce weight gain, improve insulin sensitivity, and reduce the rate of fat production, yet will not produce a major effect on feeding behavior in obese rats. However, in the latter studies, food intake was measured during the final week of drug exposure when the shift in metabolic and behavioral controls may already have occurred. Furthermore, these earlier studies focused exclusively on peripheral controls and effects of DZ and utilized animals that had been fasted overnight and were in the postabsorptive phase of metabolics.

To extend these previous studies and answer some of these questions, research involving central insulin levels was performed (Alemzadeh & Holshouser, in press). Again, lean and obese seven-week-old female Zucker rats were placed on a 150 mg/kg/day dose of DZ. However, the drug duration was four weeks. In addition, at the end of the study animals were divided into free fed and fasted (12-18 hours, overnight) groups to assess biochemical data when recently consumed glucose is available in

the periphery. Plasma and CSF levels of insulin and brain capillary insulin binding as well as plasma leptin levels were measured. DZ obese rats were found to eat less and gain less weight than controls and to have lower plasma glucose levels. DZ lean rats, however, consumed food equivalent to controls and gained a similar amount of weight as controls. Plasma leptin levels were higher in control obese than lean rats. DZ treatment reduced obese leptin levels but was less consistent in lean rats, only lowering leptin in free fed animals. Free fed and fasted rats of both genotypes had lower plasma insulin when placed on DZ. Free fed and fasted DZ obese rats had higher CSF insulin and CSF/plasma insulin ratios when compared to controls. Free fed leans placed on DZ had higher CSF/plasma insulin ratios compared to controls. All groups placed on DZ had greater insulin binding to brain capillaries compared to controls. Compared to controls, both lean and obese DZ treated rats showed greater binding capacity for high and low affinity sites. The feeding changes noted in this study parallel those of Maggio and Vasselli (1990) who administered DZ to Zucker rats for 12 weeks to reduce feeding and body weight in obese rats. This most recent study points to the role of the central nervous system in the intermediate changes occurring up to 11 weeks of age when the adipocyte and glucose tolerance data were collected in the earlier studies. The improved insulin levels in the CSF and improved binding produced by DZ in the obese rats along with the lower food intake point to a shift in a central insulin-mediated control of feeding behavior and metabolics.

CHAPTER III.

METHOD

Animals

Forty-nine female Zucker obese (*fa/fa*) and 43 female Zucker lean (*Fa/?*) rats, approximately five weeks of age, were obtained from Charles River Laboratories (Wilmington, MA). These animals were housed in pairs in plastic cages on a 12-hour light-dark cycle with standard chow (Teklad 22/5, Madison, WI) and water available ad libitum, unless otherwise noted. Following one week of acclimation to laboratory conditions, approximately half of the animals of each genotype began a drug regimen with a dose (150 mg/kg body weight) of DZ, in oral suspension (Schering Corp., Kenilworth, NJ), given daily via gavage. The remaining animals of each genotype were given a chocolate-mint flavored placebo suspension (prepared by the University of Tennessee Medical Center Pharmacy) of the same dosage on a daily basis. The drug conditions were continued for approximately four weeks with animals receiving the drug seven days per week. Body weights were measured daily to titrate drug dosage.

Tissues

Following approximately four weeks of the drug condition, half of the animals in each condition maintained access to food or were fasted overnight, approximately 12 to 16 hours prior to sacrifice. Animals were

sacrificed by rapid decapitation between 0800 and 1200 hours. Brains were removed and immediately frozen on dry ice to maintain peptides and brain shape for consistency during later slicing. Trunk blood was collected and immediately centrifuged and then frozen so that serum samples could later be assayed for glucose and insulin.

Serum Analyses

Glucose and insulin samples from each animal were assayed in duplicate using commercially available kits. Glucose concentrations in sera were determined using an enzymatic method (Sigma Diagnostics, St. Louis, MO, No. 510). Insulin concentrations were assessed using a radioimmunoassay (RIA) kit available for rat serum determinations (Linco Research Inc., St. Charles, MO).

Neuropeptide Y and Protein Assays

Brains were sliced in 300 µm-thick slices. The rat brain atlas of Paxinos & Watson (1997) was used to locate the following nuclei: PVNp and PVNm, parvocellular and magnocellular regions of paraventricular nucleus; ARC, arcuate nucleus; ME, median eminence; VMH, ventromedial hypothalamus; LH, lateral hypothalamus; DMH, dorsomedial hypothalamus; PFH, perifornical hypothalamus; SCN, suprachiasmatic nucleus; MPOA, medial preoptic area; LPOA, lateral preoptic area; SO, supraoptic nucleus; AHA, anterior hypothalamus; lateral BNST, bed nucleus of the stria terminalis; medial CNA, central nucleus of the amygdala; and SIM, cerebellar

cortex. The cerebellar tissue was collected to serve as a control brain region since little NPY is located in that region (data not shown). Bilateral punches were made to collect the nuclei using a blunt needle (0.5 mm internal diameter). Following extraction, punches were placed on ice in microcentrifuge tubes, and 200 µl of 0.5 N acetic acid solution was added. The nucleus-punch solution was then boiled for 25 minutes and sonicated continuously for approximately one minute to disrupt the membranes and release the peptides. Samples were centrifuged, aliquoted, and then lyophilized via speed vac.

Neuropeptide Y concentrations were determined for each punch using a modified radioimmunoassay procedure (Allen et al., 1991) provided by Leibowitz and colleagues of Rockefeller University (Akabayashi et al., 1993). The brain tissue content per punch was measured using a modified Lowry protein assay (Lowry et al., 1951), also provided by Leibowitz and colleagues.

Briefly, following rehydration of samples with 500 µl RIA buffer (0.05 M phosphate buffer [pH~7.4] containing 0.1% sodium azide and 0.25% BSA), samples and porcine NPY standard were pipetted. Samples and standards were incubated with 100 µl of NPY antisera (1/75,000 final dilution), as provided by Dr. Marvin Brown and associates (Alanex Corp., San Diego, CA). Following 24 hours of incubation at 4° C, 100 µl of ^{125}-I NPY (Bolton-Hunter labeled, NEX 222, New England Nuclear, Boston, MA), dissolved in RIA

buffer (8,000-9,000 cpm/100 µl) containing 0.1% Triton X-100 was added to samples and standards to serve as tracer. Standards and samples were incubated again for 18 to 24 hours at 4° C. The final solution was precipitated using a 0.05% dextran (mol. wt. ~70,000) and 0.5% activated charcoal solution over one hour. The final supernatant, containing bound NPY, was read for one minute using a gamma counter. All samples were measured in duplicate. Following protein assay, final concentrations of peptide for each nucleus were reported as picograms of NPY per microgram of protein (pg NPY/µg protein).

Statistical Procedures

Two-way ANOVAs (genotype, drug, drug x genotype) for independent samples of free fed and fasted animals were conducted separately on body weight measurements, glucose and insulin levels, and NPY concentration for each nucleus of interest. Because the chief interest of this project involved the drug and genotype effects, only a manipulation check of the feeding status was performed. This process involved conducting two-way ANOVAs (diet, genotype, diet x genotype) on insulin, glucose, and NPY concentrations of nuclei to determine if fasting resulted in a change in physiological status compared to the free fed state. Exploratory t-tests and nonparametric Mann-Whitney U tests were conducted to follow up significant results from the ANOVAs and test for hypothesized effects of DZ on each genotype and differences between controls of each genotype. Results were considered statistically significant at $p<0.05$ with marginally significant results reported at

$p<0.10$. Statistics were computed using the JMP (SAS Institute, Trumbull, CT) and CRUNCH (Crisp Software, San Francisco, CA) software programs. Figures were produced using the SigmaPlot (Jandel Scientific, Sausalito, CA) program. Table 4 summarizes the characteristics of the NPY radioimmunoassay procedure used in this study, and Figure 4 summarizes the standard curve for the NPY radioimmunoassay.

It should be noted that animal numbers outlined in the beginning of the method section and the following data tables do not include three rats (two obese and one lean) that died during the course of drug treatment and approximately six other rats that were removed from data analysis due to incomplete serum data. Additionally, the number of brain nuclei present for each cell of NPY data explored ranges from four to 12 punches based on successful tissue extraction and assay sensitivity.

Table 4. NPY Radioimmunoassay Characteristics

Minimum sensitivity:	10 pg/tube
Maximum binding:	29% ± 1%
Nonspecific binding:	4% ± 1%
Interassay displacement:	
ID20	204 ± 9 pg
ID50	84 ± 4 pg
ID80	34 ± 2 pg
Interassay coefficient of variation on 100 pg standard point:	5%

Mean ± SEM

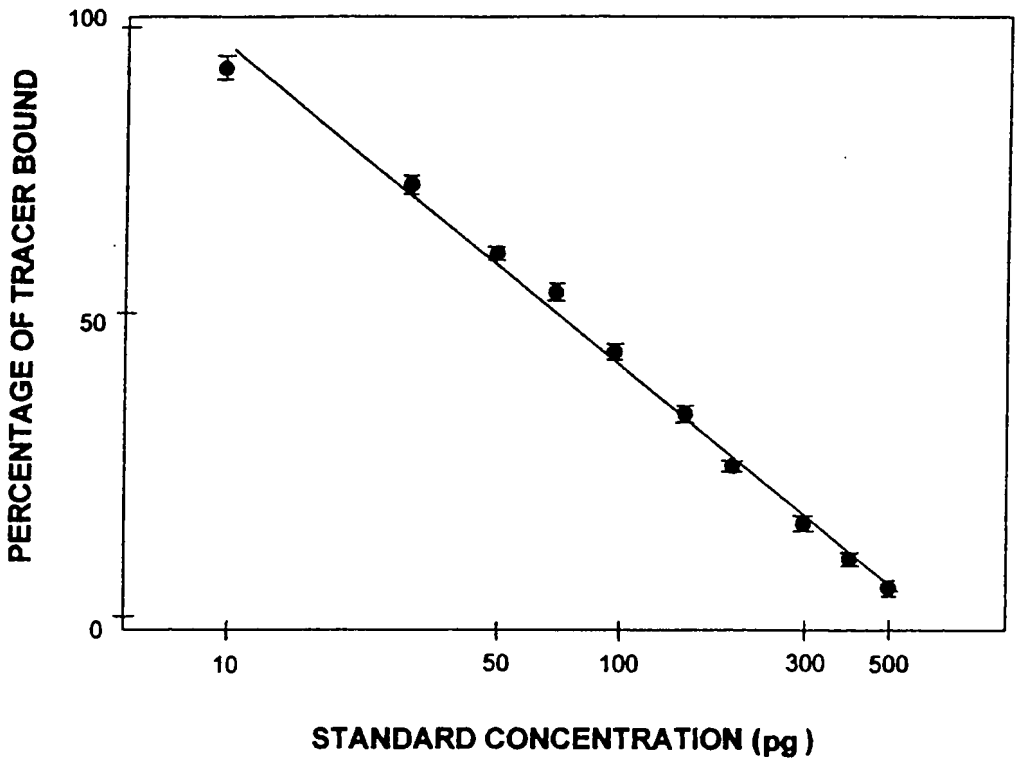


Figure 4. NPY Radioimmunoassay Standard Curve. Points indicate mean and standard error for each concentration (picograms; pg) included in the standard curves used across the series of assays for NPY.

CHAPTER IV:

RESULTS

Manipulation Check

To determine if overnight fasting produced a moderate state of negative energy balance, two-way ANOVAs testing for differences of serum glucose and insulin levels as well as central NPY concentrations in fasted and free fed controls were conducted.

Serum Glucose and Insulin

Table 5 shows that overall, control obese rats had higher glucose [obese, 7.9 $\pm$ 0.2; lean, 6.6 $\pm$ 0.2 nmol/L; $F(1,46)=35.11$, $p<0.01$] and insulin [obese, 0.61 $\pm$ 0.1; lean 0.10 + 0.01 pmol/ml; $F(1,46)=19.11$, $p<0.01$] levels than lean rats regardless of diet. Fasting led to a main effect of diet by suppressing insulin [$F(1,46)=5.8$, $p<0.05$] . Effects of dietary status according to genotype were more complex. Fasting reduced the glucose levels of lean rats [$t(21)=4.45$, $p<0.01$], but did not change glucose levels of obese rats. However, fasting reduced insulin levels of both obese [$U(14,13)=48$, $p<0.05$] and lean rats [$t(21)=5.6$, $p<0.01$] by over 50%.

NPY Concentrations

Two-way ANOVAs revealed a significant main effect of genotype in the ME [$F(1,30)=11.22$, $p<0.01$] with a marginal trend in the DMH

Table 5: Manipulation Check for Fasting Effects of Control Lean and Obese Zucker Rats

Subgroup	n	Serum Glucose (mmol/L)	Serum Insulin (pmol/ml)
Free Fed Obese	14	7.63 + 0.22	0.82 + 0.18**
Fasted Obese	13	8.11 ±0.24	0.38 ±0.11
Free Fed Leans	11	7.2 + 0.18**	0.15 + 0.01**
Fasted Leans	12	6.08 ±0.17	0.06 ±0.01

T-tests and Mann-Whitney U tests. Mean ± SEM.

Significant differences between same genotype (free fed v. fasted), ** $p<0.05$

[F(1,36)=3.23, p<0.10] as obese rats had higher levels than leans. An elevation of NPY in the ME of fasted rats compared to free fed rats was also found [F(1,30)=5.09, p<0.05]. An interaction of genotype x diet occurred in the LH [F(1,35)=4.48, p<0.05] as NPY levels of fasted obese rats were reduced to the level of free fed and fasted lean rats. Results of dietary manipulation on NPY are summarized in Figure 5.

Free fed and Fasted Obese Rats. As shown in Figure 6, fasting produced inconsistent changes in NPY concentration within the hypothalamus. Fasting reduced the LH levels of obese rats by about 40% [U(11,11)=24, p<0.05]. However, fasting led to a trend for a corresponding increase in NPY levels within the ME of obese rats [t(18)=2.09, p<0.10].

Free fed and Fasted Lean Rats. Figure 7 clearly demonstrates that the change in nutrient availability due to fasting produced no alterations in NPY levels of lean rats as levels remained consistent regardless of feeding state.

Free Fed Rats

Obese rats generally demonstrated higher body weights at the onset and end of the study with elevated insulin levels compared to lean rats. DZ-treatment appeared to reduce body weight gain in obese rats while not affecting lean weights. DZ-treatment also reduced glucose levels of both lean and obese rats without changing insulin levels of either genotype. NPY

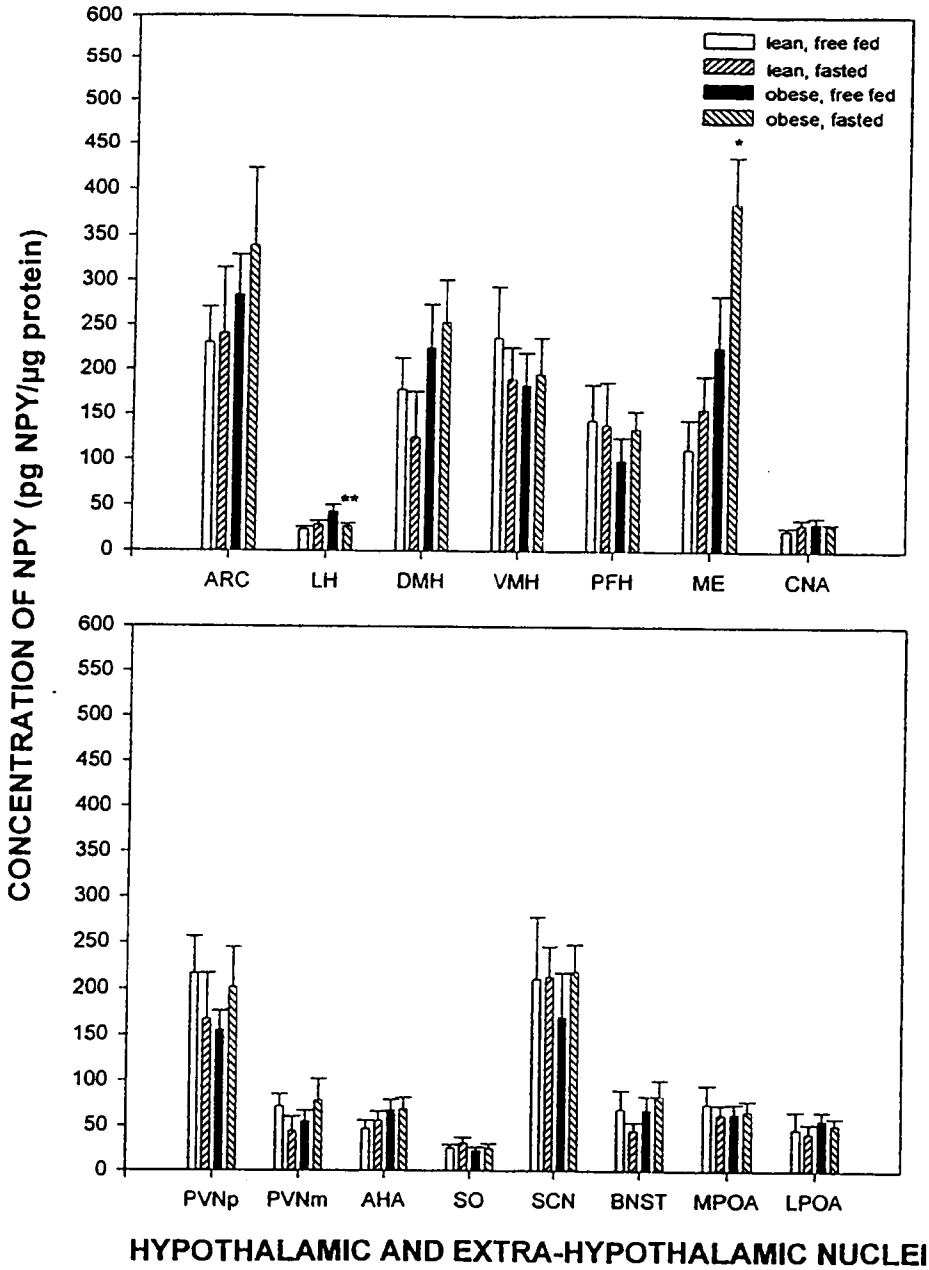


Figure 5. Effects of Feeding Manipulation on NPY Levels of Control Lean and Obese Zucker Rats. Bars indicate NPY levels (mean ± SEM) across nuclei for free fed and overnight fasted placebo-treated control animals, both lean and obese, to demonstrate the effects of fasting on NPY levels for animals of the same genotype (significant differences within genotype based on feeding status, ** $p<0.05$, * $p<0.10$).

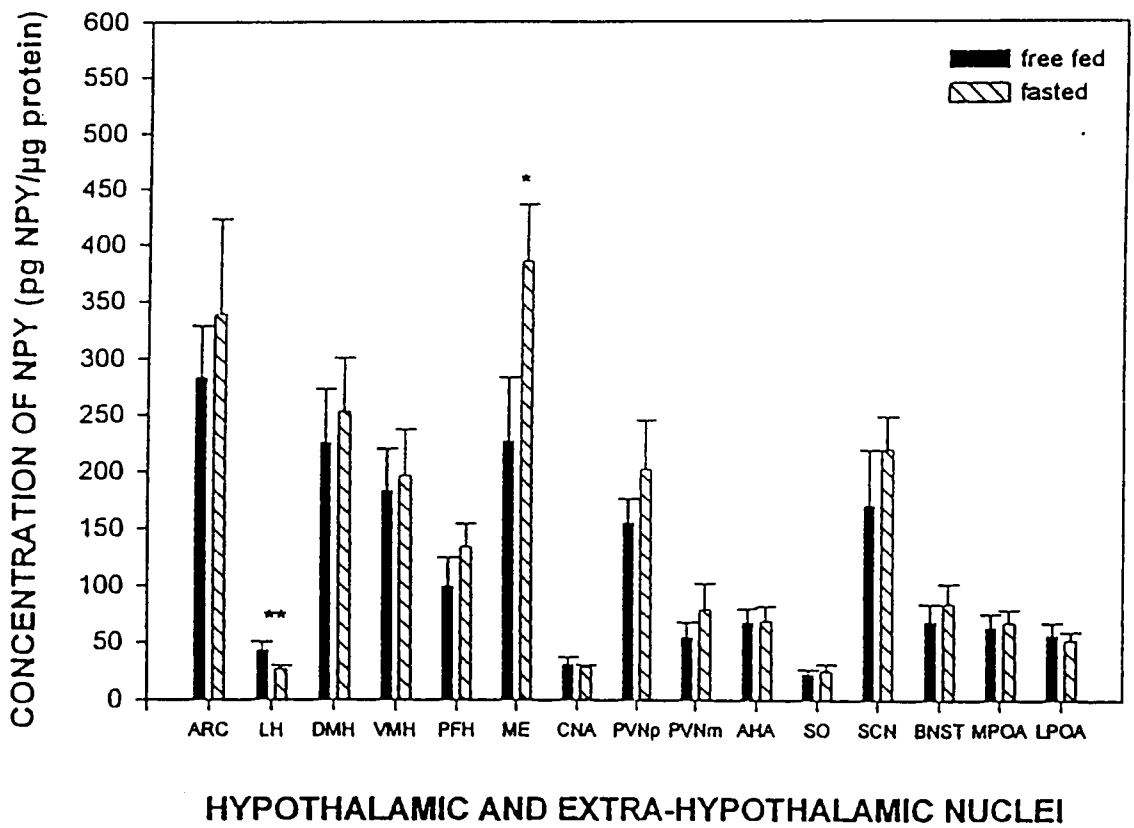


Figure 6. Manipulation of NPY Levels by Fasting in Control Obese Rats. Bars indicate NPY levels (mean ± SEM) for free fed versus overnight fasted placebo-treated obese Zucker Rats across several hypothalamic and extra-hypothalamic nuclei (significant differences based on feeding status, ** $p<0.05$, * $p<0.10$).

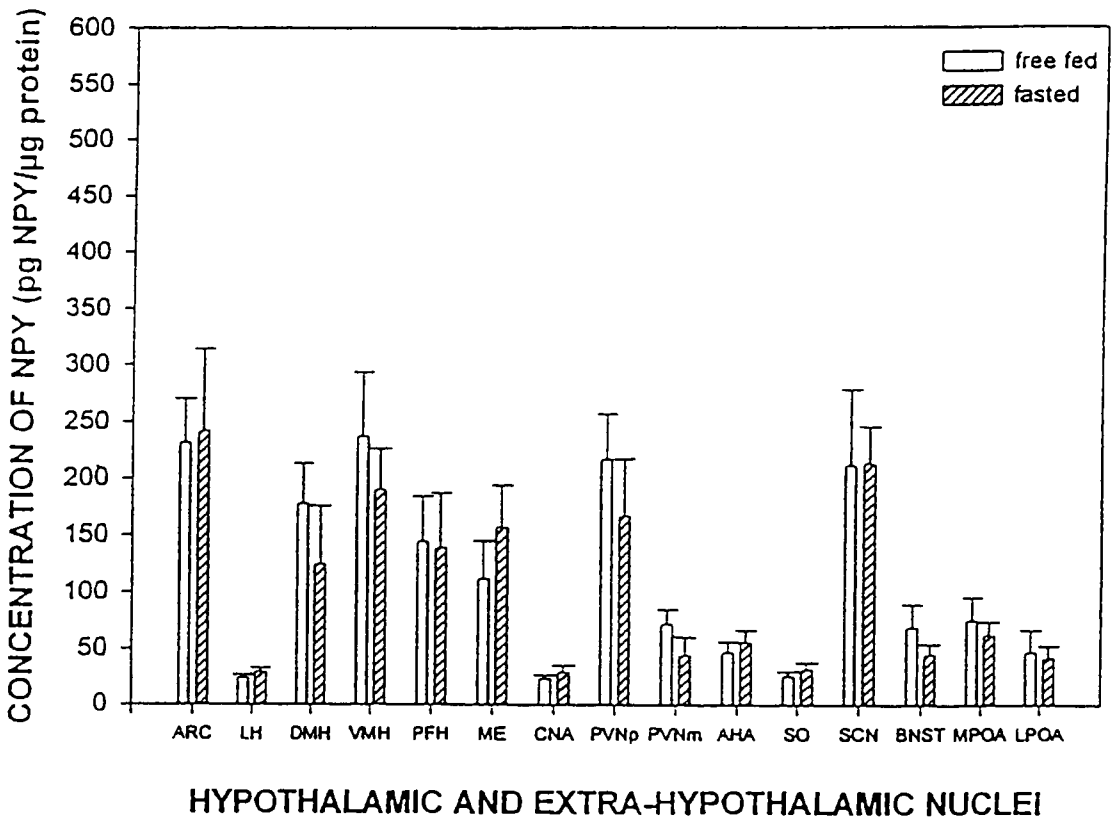


Figure 7. Manipulation of NPY Levels by Fasting in Control Lean Rats. Bars indicate NPY levels (mean ± SEM) for free fed versus overnight fasted placebo-treated lean Zucker Rats across several hypothalamic and extra-hypothalamic nuclei. No significant differences were found based on feeding status.

concentrations were affected more specifically based on genotype and drug treatment.

Body Weight Data

Body weight and serum data for free fed rats are outlined in Table 6. Obese rats had higher body weights at the onset of the study [$F(1,43)=55.59$, $p<0.01$] than leans rats (obese, 195 $\pm$ 7 g; lean, 145 $\pm$ 3 g). There was also an interaction of genotype and drug group for baseline weights [$F(1,43)=4.43$, $p<0.05$] as obese rats placed on DZ had lower weights than those given placebo at the onset of the study [$t(22)=2.54$, $p<0.02$] whereas lean rats had similar weights despite drug group. Across the course of drug treatment, obese rats gained approximately 35% more weight than lean rats [$F(1,43)=27.93$, $p<0.01$] regardless of drug group. Although no genotype x drug treatment interaction was significant, exploratory analysis revealed a trend for DZ to suppress body weight gain of obese rats compared to obese controls [$t(22)=2.22$, $p<0.05$; DZ, 31 $\pm$ 3 g; placebo, 37 $\pm$ 2 g]. Significant main effects showed that end-study body weights of the animals were higher in obese than lean rats [$F(1,43)=217.13$, $p<0.01$] and placebo than DZ-treated animals [$F(1,43)=24.26$, $p<0.01$]. An interaction of genotype x drug treatment [$F(1,43)=22.14$, $p<0.01$] revealed that drug treatment had no effect on end-study weights of lean rats whereas DZ-treated obese rats had lower body weights than placebo-treated obese rats [DZ, 257 $\pm$ 12 g; placebo, 330 + 9 g]. Thus, DZ may have led to lower body weights in obese rats without affecting lean rats.

Table 6: Diazoxide Effects on Body Weight, Glucose, and Insulin Levels of Free Fed Zucker Rats

Subgroup	n	Initial Wt (g)	Final Wt (g)	Wt Gain (per 100 g BW)	Serum Glucose (mmol/L)	Serum Insulin (pmol/ml)
DZ obese	10	178 ± 9**	257 ± 12**	31 ± 3**	6.85 ± 0.35*	0.72 ± 0.15
C obese	14	209 ± 8++	330 ± 9++	37 ± 2++	7.63 ± 0.22	0.82 ± 0.18++
DZ lean	12	144 ± 5	187 ± 2	23 ± 3	6.51 ± 0.13**	0.18 ± 0.03
C lean	11	146 ± 3	189 ± 3	23 ± 2	7.2 ± 0.18	0.15 ± 0.01

T-tests and Mann-Whitney U tests. Mean ± SEM.

Significant differences from genotype control (DZ v. C) ** $p<0.05$, * $p<0.10$

Significant differences from C lean (C obese v. C lean) ++ $p<0.05$, + $p<0.10$

Serum Glucose and Insulin

Insulin and glucose levels differed for animals of each genotype with obese rats having insulin levels nearly five-fold higher than lean animals [$F(1,43)=24.46$, $p<0.01$] and glucose levels more modestly elevated in obese rats [obese, 7.31 ± 0.2; lean, 6.83 ± 0.1 mmol/L; $F(1,43)=4.27$, $p<0.05$] regardless of drug treatment. DZ treatment had little effect on insulin levels of either genotype [$F(1,43)=0.26$, $p<.65$], but did reduce glucose levels of both lean and obese rats [$F(1,43)=10.49$, $p<0.01$].

NPY Concentrations

Two-way ANOVAs revealed main effects for genotype in the LH [$F(1,32)=6.12$, $p<0.02$] and ME [$F(1,31)=6.36$, $p<0.02$] with marginal effects in the AHA [$F(1,30)=3.47$, $p<0.10$] as obese rats had higher NPY levels than lean rats. Trends for main effects for drug treatment were found in the ARC [$F(1,30)=3.69$, $p<0.10$] and ME [$F(1,31)=3.06$, $p<0.10$]. No interactions of genotype x drug treatment were significant. Overall results of NPY levels of free fed rats are summarized in Figure 8.

Controls. Figure 9 shows that follow-up tests revealed effects only in NPY concentrations between obese and lean rats at the LH [$U(11,8)=14$, $p<0.05$] as obese levels nearly doubled lean levels.

Obese rats. As shown in Figure 10, hypothalamic nuclei were affected by drug treatment in obese rats. NPY levels at the VMH [$U(7,11)=9$, $p<0.01$]

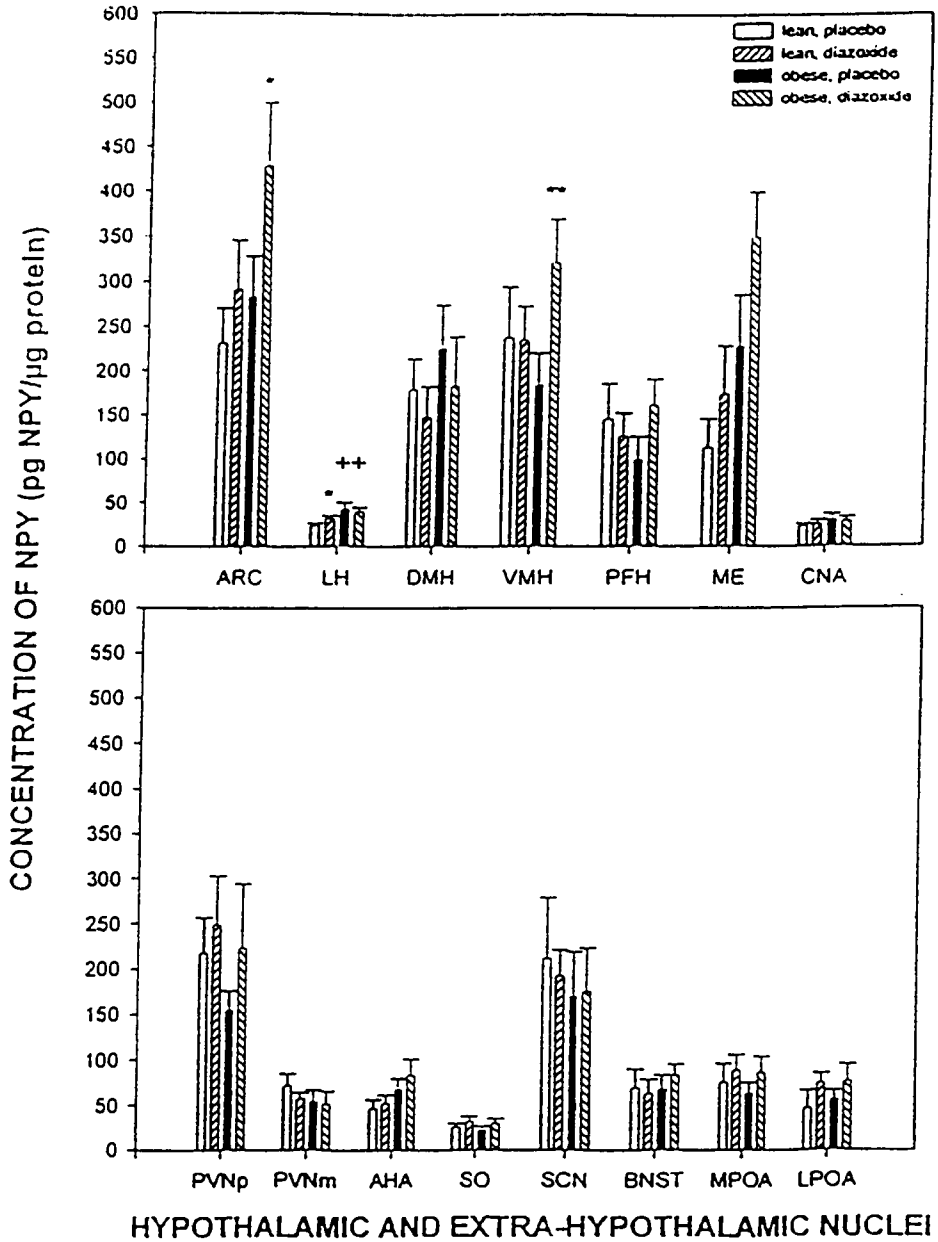


Figure 8. Summary Figure for Effects of Diazoxide Treatment on NPY Levels of Free Fed Lean and Obese Zucker Rats. Bars indicate NPY levels (mean ± SEM) for freely fed lean and obese rats given either placebo or diazoxide treatment. Significant comparison between genotype controls (lean v. obese), ++ $p<0.05$. Significant comparison between diazoxide and placebo treatments within genotype, ** $p<0.05$, * $p<0.10$.

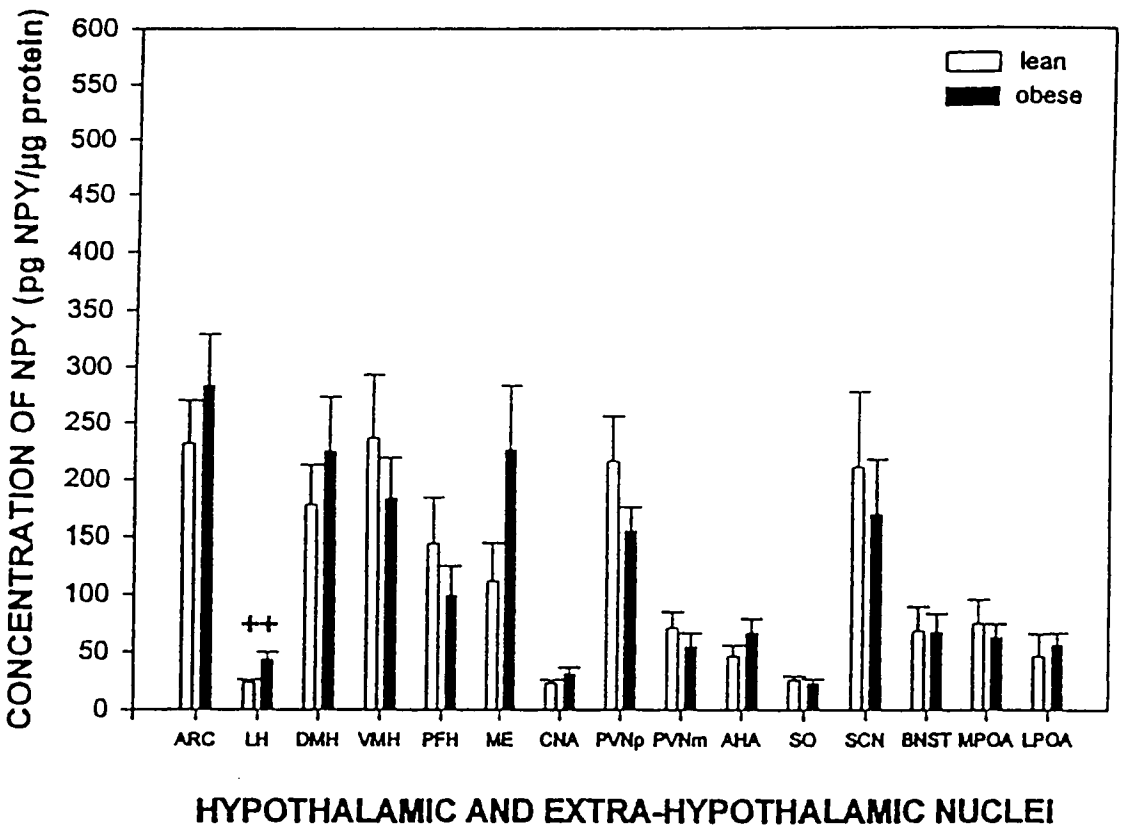


Figure 9. NPY Content in Brain Nuclei of Free Fed Lean and Obese Zucker Controls. NPY levels (mean ± SEM) for free fed placebo-treated lean and obese fats are shown. Significant differences between lean and obese animals for the same nucleus are indicated (++ $p<0.05$).

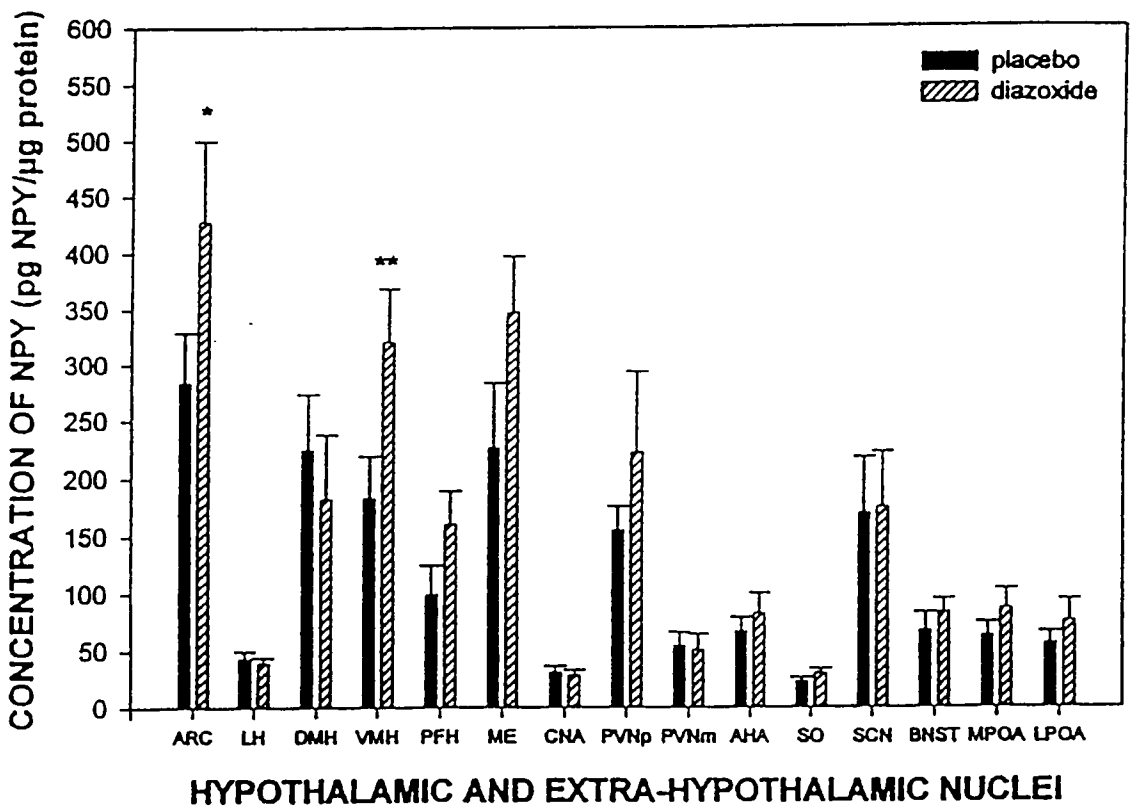


Figure 10. NPY Content of Control and Diazoxide-Treated Free Fed Obese Zucker Rats. NPY levels (mean ± SEM) are shown for freely fed obese rats treated with either a placebo or diazoxide. Significant differences between drug treatments within the same nucleus are noted (** $p<0.05$, * $p<0.10$).

nearly doubled with DZ-treatment, with a similar trend in the ARC [$t(13)=1.81$, $p<0.10$] compared to placebo treatment.

Lean rats. Figure 11 outlines effects of drug treatment on NPY content of free fed lean nuclei. Similar to control conditions, only LH levels of lean rats were affected by DZ-treatment, and again those levels showed a trend to be elevated compared to placebo-treated rats [$t(16)=1.81$, $p<0.10$].

Fasted Rats

Similar to the data from free fed rats, the data outlined below demonstrate that obese and lean Zucker rats had different body weights at the onset of the study (approximately 6.75 weeks and 7.25 weeks of age, for obese and lean rats, respectively). Drug treatment led to changes in body weight gains and serum glucose as well as central NPY levels which showed different patterns for rats of the obese versus lean genotypes.

Body Weight Data

Body weight and serum data for fasted rats are presented in Table 7. Lean and obese rats showed a distinct difference in body weights at the onset of this study [leans, 151 ± 4 g; obese, 188 ± 6 g; $F(1,41)=25.63$, $p<0.01$]. Weight gains across the study, however, varied depending on whether the animals were drug treated [$F(1,41)=8.51$, $p<0.01$]. Obese rats treated with DZ gained about 20% less weight than placebo-treated obese

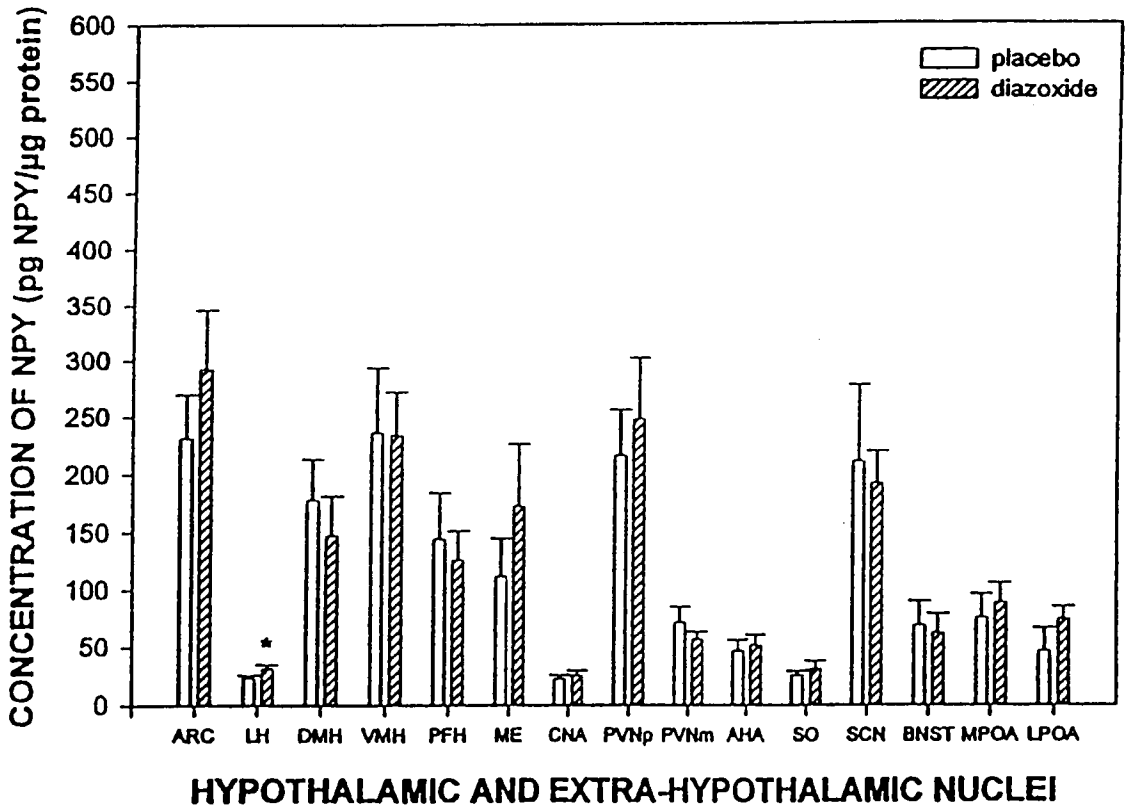


Figure 11. NPY Content of Control and Diazoxide-Treated Free Fed Lean Zucker Rats. NPY levels (mean ± SEM) are shown for freely fed lean rats treated with either a placebo or diazoxide. Significant differences between drug treatments within the same nucleus are noted (* $p<0.10$).

Table 7. Diazoxide Effects on Body Weight, Glucose, and Insulin Levels of Fasted Zucker Rats

Subgroup	n	Initial Wt (g)	Final Wt (g)	Wt Gain (per 100 g BW)	Serum Glucose (mmol/L)	Serum Insulin (pmol/ml)
DZ obese	12	178 ± 3	247 ± 6**	28 ± 1**	6.62 ± 0.30**	0.19 ± 0.05
C obese	13	197 ± 11^{++}	302 ± 9^{++}	35 ± 2^{++}	8.11 ± 0.24^{++}	0.38 ± 0.11^{++}
DZ lean	8	152 ± 3	190 ± 5	20 ± 2	5.08 ± 0.11**	0.05 ± 0.01
C lean	12	150 ± 5	179 ± 6	16 ± 2	6.08 ± 0.17	0.06 ± 0.01

T-tests and Mann-Whitney U tests. Mean ± SEM.

Significant differences from genotype control (DZ v. C) ** $p<0.05$, * $p<0.10$

Significant differences from C lean (C obese v. C lean) $^{++}$ $p<0.05$, $^{+}$ $p<0.10$

rats [U(12,13)=34, p<0.02] whereas DZ treatment had no effect on the body weight of lean rats. Consequently, the final body weights were lower in DZ-treated obese rats than obese controls [DZ, 247 ± 6g; placebo, 302 ± 9g; U(12,13)=7, p<0.01] unlike lean rats that had similar weights whether placebo- or DZ-treated (DZ, 190 ± 5g; placebo, 179 ± 6g). Therefore, DZ treatment appears to have influenced the metabolism of obese rats compared to leans.

Serum Glucose and Insulin

Overall, obese rats had higher glucose [F(1,41)=53.09, p<0.01] and insulin [F(1,41)=11.53, p<0.01] levels than leans at the end of the study. Specifically, insulin levels of lean rats were 20% of obese levels. Glucose levels were lower for rats following DZ treatment [F(1,41)=29.33, p<0.01]. DZ treatment reduced the glucose levels of obese and lean rats by about 20%. Although obese insulin levels appear to have been reduced by as much as 50% following DZ treatment, the results failed to reach significance [U(12,13)=64, p<0.47]. Therefore, DZ treatment appears to have reduced glucose levels of both lean and obese rats without suppressing serum insulin levels in either genotype.

NPY Concentrations

NPY concentrations throughout hypothalamic and extra-hypothalamic nuclei ranged from 400 pg/μg to less than 50 pg/μg concentration. Figure 12 shows the NPY concentration across all nuclei examined and all groups

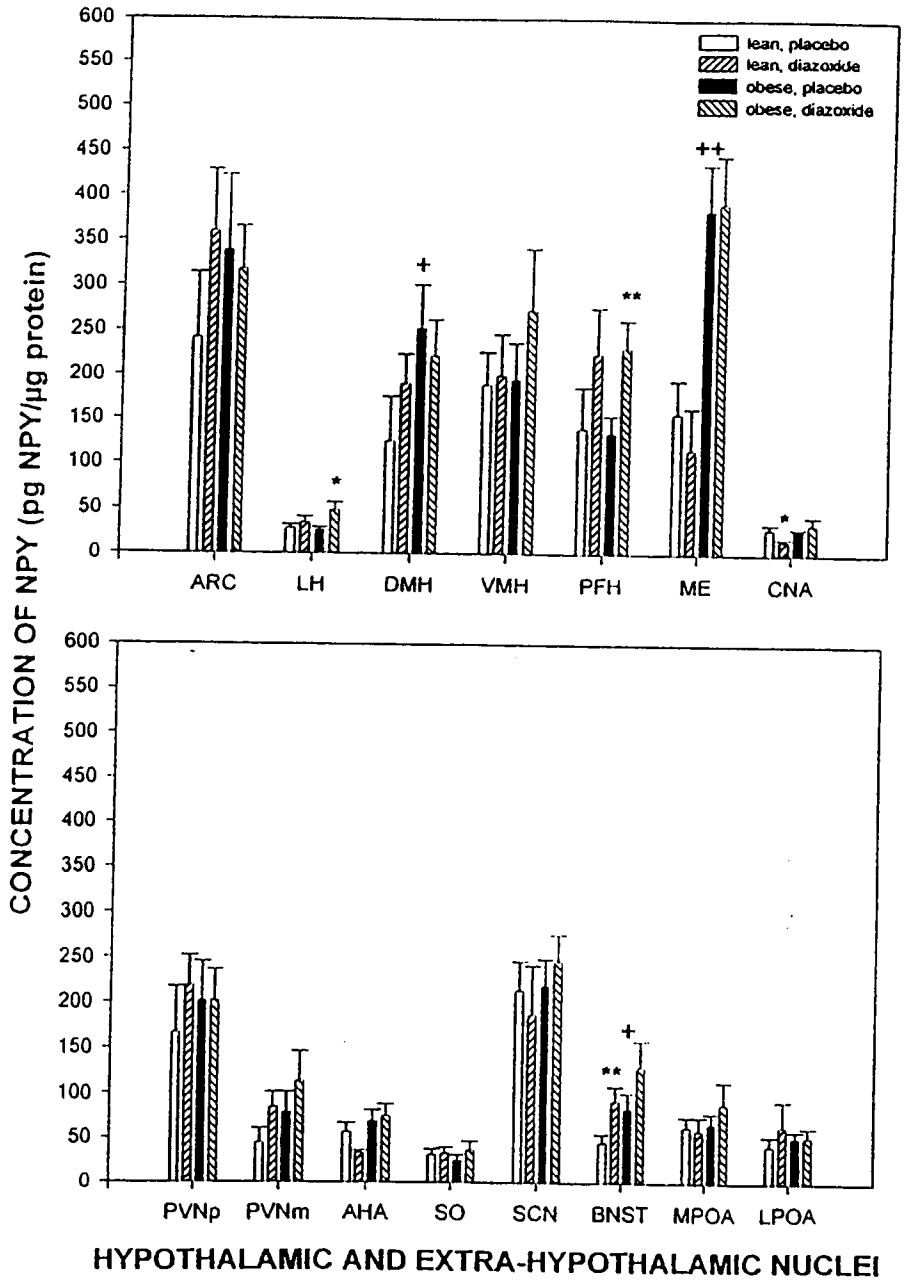


Figure 12. Summary Figure for Effects of Diazoxide Treatment on NPY Levels of Fasted Lean and Obese Zucker Rats. Bars indicate NPY levels (mean ± SEM) for fasted lean and obese rats given either placebo or diazoxide treatment. Significant comparisons between genotype controls (lean v. obese) are shown, ++ $p<0.05$, + $p<0.10$. Significant comparisons between diazoxide and placebo treatments within genotype, ** $p<0.05$, * $p<0.10$, are also displayed.

tested. Two-way ANOVAs revealed main effects of drug condition in the LH [$F(1,31)=6.83$, $p<0.05$], PFH [$F(1,28)=5.84$, $p<0.05$], and BNST [$F(1,25)=5.24$, $p<0.05$] as DZ treatment led to NPY elevations. Significant main effects of genotype were found in the ME [$F(1,31)= 27.0$, $p<0.01$] and AHA [$F(1,23)=5.52$, $p<0.05$] with marginal effects in the DMH [$F(1,32)=3.07$, $p<0.10$] and BNST [$F(1,25)=3.83$, $p<0.10$]. Obese rats generally had higher NPY levels than leans. No genotype x drug interactions were significant.

Controls. Figure 13 shows that control lean and obese rats had a trend for elevations of NPY across some hypothalamic nuclei. NPY concentration in the ME was higher by nearly 2.5 fold in obese compared to lean controls [$t(16)=3.48$, $p<0.01$]. In the DMH and BNST, marginal trends were found. However, the magnitude of the effects was less extensive with about a two fold increase in DMH [$t(17)=1.75$, $p<0.10$] and BNST [$t(13)=1.91$, $p<0.10$] of obese rats compared to leans.

Obese rats. Figure 14 demonstrates that drug treatment led to sparse increases in NPY in the hypothalamic nuclei of obese rats with no changes occurring in extra-hypothalamic nuclei. DZ treatment nearly doubled PFH levels of NPY in obese rats compared to controls [$t(16)=2.66$, $p<0.05$] whereas it led to a marginal effect of elevating LH levels [$U(8,11)=22$, $p<0.10$] in obese rats.

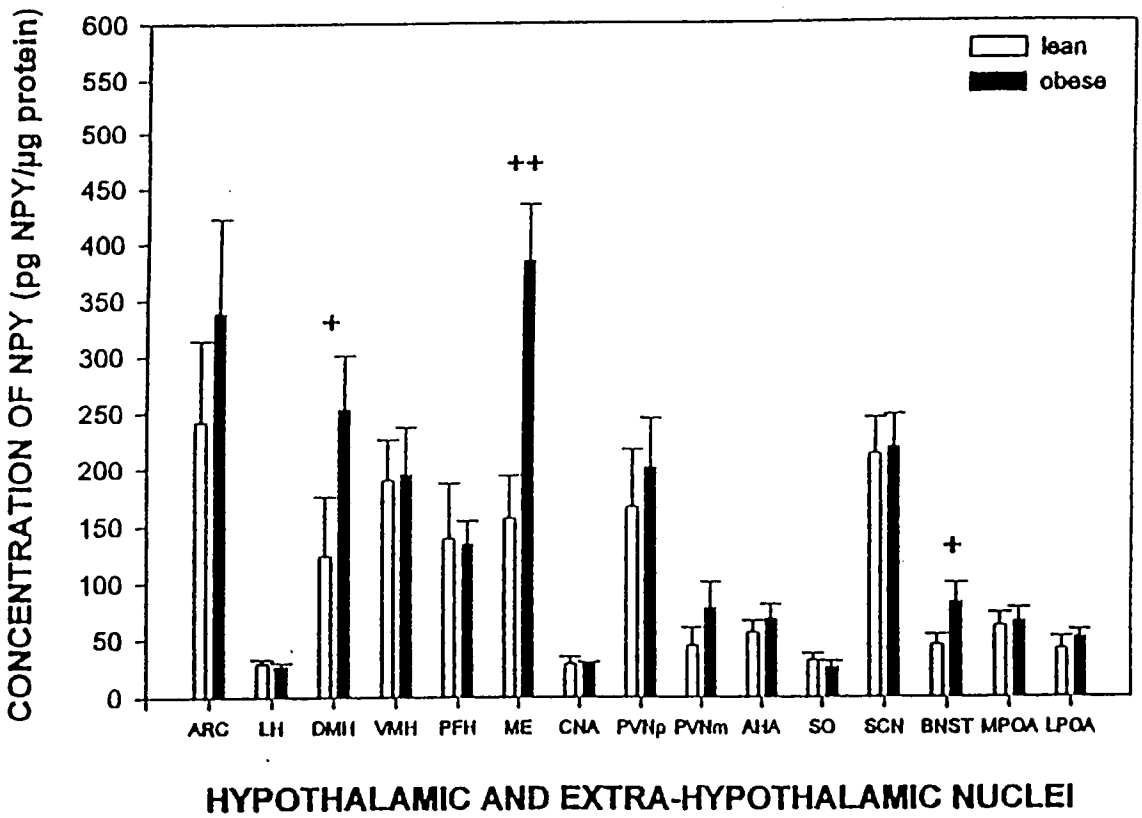


Figure 13. NPY Content in Brain Nuclei of Fasted Lean and Obese Zucker Controls. NPY levels (mean ± SEM) for fasted placebo-treated lean and obese fats are shown. Significant differences between lean and obese animals for the same nucleus are indicated (++ $p<0.05$, + $p<0.10$).

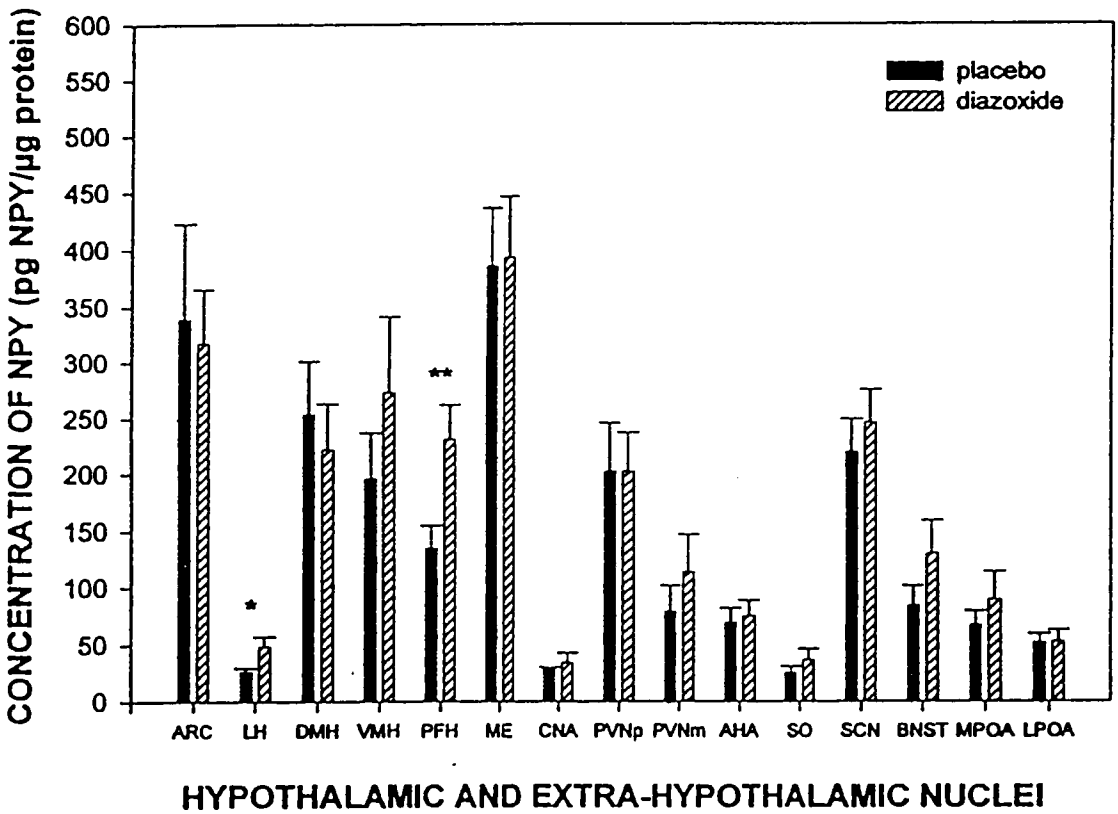


Figure 14. NPY Content of Control and Diazoxide-Treated Fasted Obese Zucker Rats. NPY levels (mean ± SEM) are shown for fasted obese rats treated with either a placebo or diazoxide. Significant differences between drug treatments within the same nucleus are noted (** $p<0.05$, * $p<0.10$).

Lean rats. Similar to obese rats, DZ treatment had few effects on NPY levels of lean rats. However, the effects were more diverse as the effects lay outside of the hypothalamus and failed to consistently increase NPY levels. NPY levels in the BNST of DZ-treated rats were nearly twice the level of control leans [$t(11)=2.7$, $p<0.05$]. Although no two-way effects were found for the CNA, a reverse effect was revealed for lean rats as NPY levels showed a trend for a decrease of nearly 50% following DZ treatment [$t(9)=1.84$, $p<0.10$]. Figure 15 outlines these results.

Combined ARC-ME Analysis

NPY Concentrations

Many researchers assess the concentration of NPY in the ARC and ME regions as a single unit by excising the wedge of tissue at the base of the 3V containing both nuclei (e.g., McKibbin et al., 1991). To facilitate comparison of the current results with such studies, analyses were performed on the combined NPY content of the ARC and ME nuclei of animals from which both nuclei were successfully extracted. Main effects of genotype [$F(1,20)=3.64$, $p<0.10$] and diet [$F(1,20)=5.79$, $p<0.05$] and an interaction of genotype x diet [$F(1,20)=2.99$, $p<0.10$] were found for controls. Free fed animals had main effects for genotype [$F(1,22)=4.53$, $p<0.05$] and drug [$F(1,22)=8.99$, $p<0.05$]. Fasted animals had a main effect of genotype [$F(1,19)=7.88$, $p<0.05$]. More specific analyses for the groups are described below. Figure 16 summarizes those findings and points to the most dramatic effects occurring in obese animals.

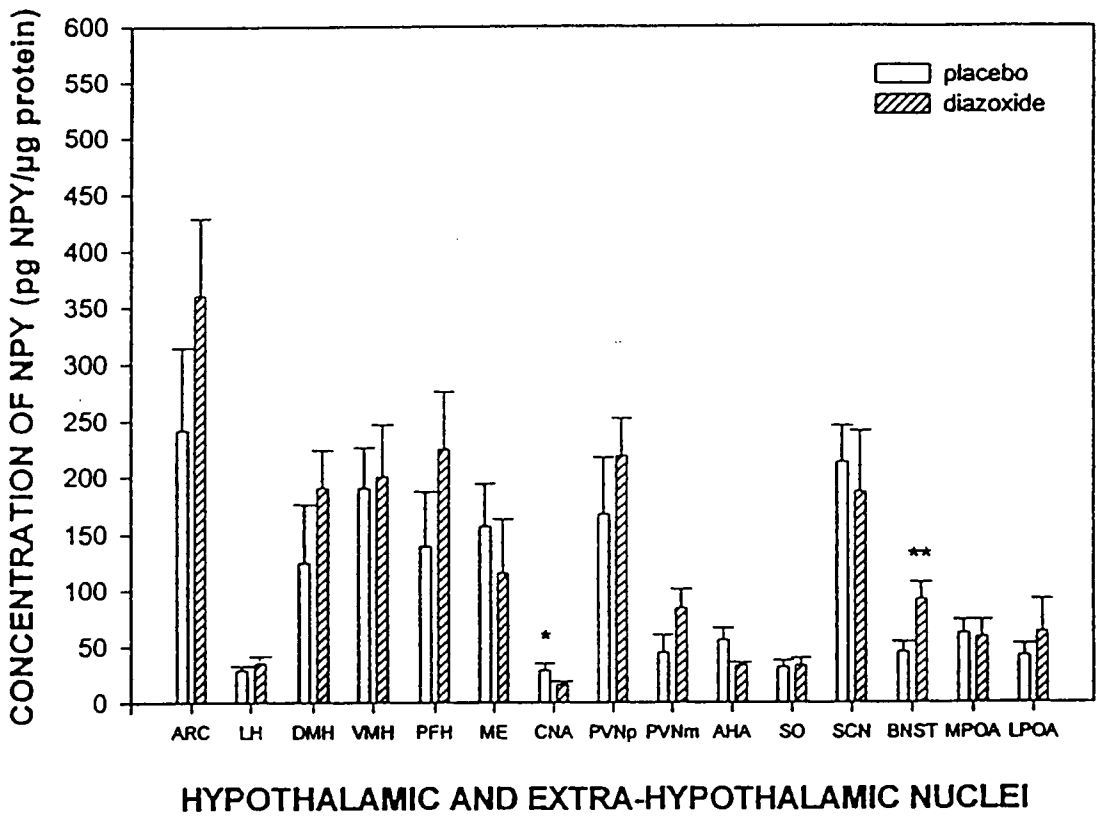


Figure 15. NPY Content of Control and Diazoxide-Treated Fasted Lean Zucker Rats. NPY levels (mean ± SEM) are shown for fasted lean rats treated with either a placebo or diazoxide. Significant differences between drug treatments within the same nucleus are noted (** $p<0.05$, * $p<0.10$).

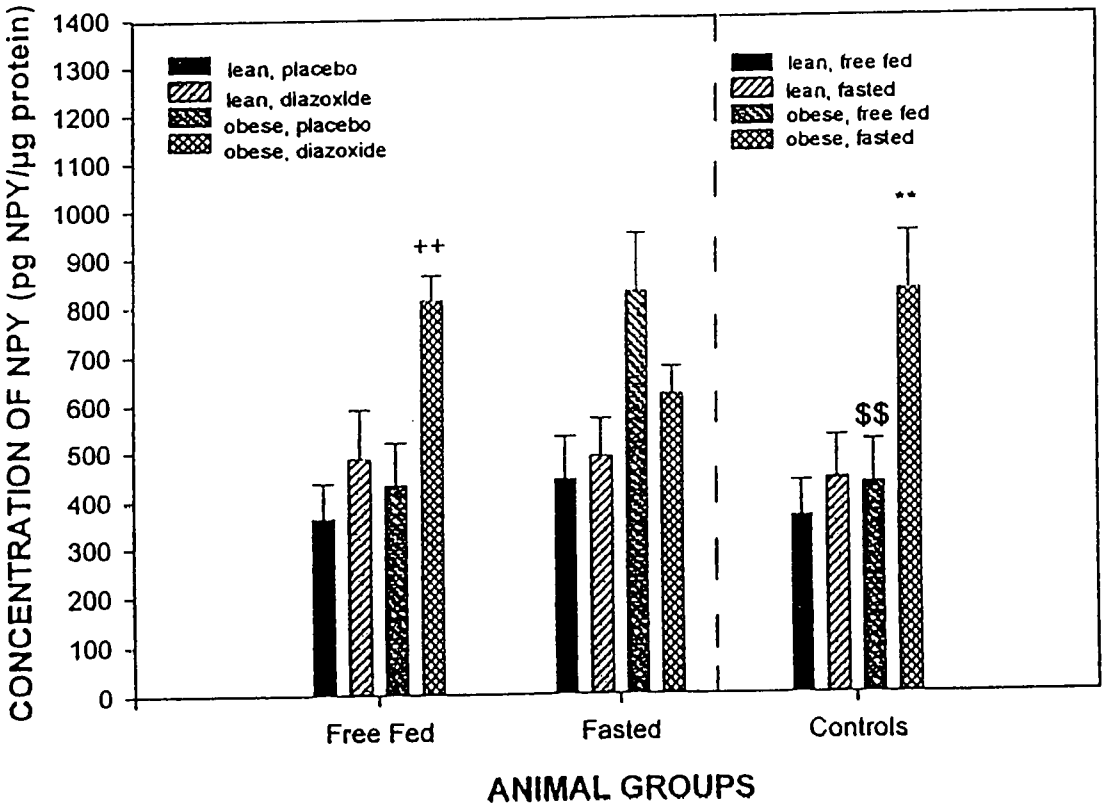


Figure 16. NPY Content of the Combined ARC-ME across Genotype, Diet, and Drug Groups. NPY levels (mean ± SEM) are shown for the addition of the ARC and ME content of subjects from which both nuclei were obtained. Statistically significant differences between animals of the same genotype and diet, but different drug treatments, are shown (++ $p<0.05$). Significant differences between control animals of the same diet, but different genotype (** $p<0.05$) , or same genotype, but different diet ($$ $p<0.05$), are also shown.

Controls. Control obese animals that were fasted had markedly higher NPY levels than fasted leans [$t(9)=2.66$, $p<0.05$] and free fed obese controls [$t(9)=2.72$, $p<0.05$].

Free Fed rats. NPY levels of DZ-treated obese rats freely fed diverged from other groups with higher NPY levels than control obese rats [$t(11)=3.62$, $p<0.05$]. Free fed lean levels did not appear to be influenced by drug treatment.

Fasted rats. Although a trend appears to have occurred for increased NPY in control obese rats compared to DZ-treated obese rats, those results failed to reach significance [$t(7)=1.72$, $p<0.15$; see Figure 16].

CHAPTER V:

DISCUSSION AND CONCLUSIONS

NPY is a powerful stimulant of feeding behavior and metabolic efficiency. Many circulating indices of nutrient availability, including insulin, have been proposed to regulate NPY. Previous research with the hyperinsulinemic, hyperphagic obese Zucker rat shows that the insulin antagonist DZ acts to reduce body weight in these animals along with reducing food intake and enhancing insulin levels in the brain (Alemzadeh & Holshouser, in press). The current study was designed to determine if the reduced food intake and weight gain along with enhanced insulin entry into the brain observed in DZ-treated obese rats is associated with a decrease in hypothalamic and extra-hypothalamic NPY concentrations.

Consistent with the previous research, obese rats entered the study with higher body weights and insulin levels than lean rats and gained less weight across the study when given DZ. Lean rat weights were unaffected by the drug treatment. Lower body weights with DZ have been shown not only to be associated with decreased feeding (Alemazadeh & Holshouser, in press), but also to correspond to smaller adipocyte diameters and increased insulin receptor binding, implying reduced insulin resistance in animals treated with a 100 mg/kg dose over an eight week treatment period (Alemzadeh et al., 1993).

Overnight fasting produced a mild state of negative energy balance as demonstrated by reductions in serum glucose and insulin in control leans. Control obese rats, however, showed reductions in insulin without a change in glucose. These results may point indirectly to the poor sensitivity of the glucose-insulin system of the obese rats or more directly to enhanced gluconeogenesis in the animals. Nevertheless, DZ treatment reduced serum glucose of both lean and obese rats, yet failed to significantly lower basal insulin levels. These findings are inconsistent with recent data obtained using the same dose and duration of DZ treatment (Alemzadeh & Holshouser, in press). Variability between animals due to age and drug duration based on staggering of groups in order to randomize sequencing may have contributed to these differences, but the similarity of the current animals compared to previous groups across all other parameters casts doubt on this source of incompatibility. The discrepancy between the insulin results of these studies is more than likely due to the types of tissues sampled. The previous research utilized plasma for insulin determinations while the current research utilized serum. The nature of serum samples to clot more readily than plasma may have led to trapping of insulin in clots suspended in sera, resulting in an inability to distinguish trace differences between insulin levels of drug and control groups. Nevertheless, the effectiveness of the drug as a metabolic agent was demonstrated by its reduction in obese body weights.

NPY levels of overnight fasted animals followed patterns previously observed with obese rats, showing responses mostly in the medial hypothalamus. Overnight fasting elevated control obese levels in ME and combined ARC-ME, yet lowered LH levels compared to free fed rats. Further, the combined ARC-ME of fasted obese rats exceeded lean fasted levels. Beck et al. (1992a) fasted obese rats for 48 hours and observed suppressed VMH levels compared to leans that had a decrease in PVN levels. Williams et al. (1991) restricted the diet of six-month-old obese rats to reduce their weights to those of age-matched leans over the course of about two weeks or one month. Restriction across both time periods led to increases in NPY of medial hypothalamic tissue blocks compared to free fed leans. On the other hand, McKibbin et al. (1991) observed little change in NPY of hypothalamic nuclei of obese rats undergoing food restriction to lose 25% of their body weight or during refeeding to original weight. Others have shown that, in non-obese animal models, the increases in NPY content associated with fasting may reflect an increase in NPY synthesis following 72 hours of food deprivation (White & Kershaw, 1990) and *in vivo* and *in vitro* release in the medial hypothalamus (Dube et al., 1992; Kalra et al., 1991b). Further, Schwartz et al. (1992b) fasted nonobese rats for 48 or 72 hours to increase NPY mRNA expression in the ARC, but attenuated those results with ICV insulin infusion. However, some researchers have evidence to suggest that deprivation does not increase preproNPY mRNA expression in obese rats (Sanacora et al., 1990). The findings may explain how enhanced entry of insulin into CSF of DZ-treated obese rats would fail to affect NPY of

obese rats based on central insulin sensitivity. Ikeda et al. (1986) infused insulin into the 3V of 16-week-old lean and obese rats for two weeks and found a decrease in weight and feeding in leans, but no change in obese rats. Stein et al. (1987) elevated plasma insulin in four-month-old rats to produce more elevated CSF insulin in lean, than obese rats. Further, Melnyk (1987) found a decrease in insulin binding in hypothalamic not cortical tissues of six-week-old obese rats compared to leans, whereas Schwartz et al. (1990) found a decrease in cortical binding of insulin in 10-week-old rats resulting from a decrease in sites corresponding to a time period of rapid increase in NPY mRNA in the hypothalamus of obese rats (Van Huijsduijnen, Rohner-Jeanrenaud, & Jeanrenaud, 1993). These findings also parallel those of Schwartz et al. (1991) that ICV insulin infusion following 48-hours of food deprivation reduces prepro-NPY mRNA elevations in lean but not obese rats.

Nevertheless, the severity of the deprivation protocol must be considered in NPY changes. Sanacora and colleagues fasted animals for 72 hours and the window for changes in expression may have already passed by the time they assessed expression. Pesonen and colleagues (1992) restricted feeding (50% of regular intake) for two weeks to stimulate NPY synthesis in both lean and obese rats. The less abrupt period of the latter study may indicate adaptation to the chronic restriction that is not revealed with the acute, severe restriction. Evidence of increased NPY mRNA in 24-hour deprived animals the same age as those of the current

study suggests increased NPY production in the hypothalamus of lean and obese rats following short periods of food deprivation (Jhanwar-Uniyal & Chua, 1993). Thus, elevations in NPY of control obese rats following fasting in the current study probably reflect an increase in NPY synthesis.

In addition to the central hypothalamic changes noted with fasting, the decrease in LH levels found in this study probably reflects changes in transport since lateral hypothalamic areas are densely innervated with neuron fibers (DeQuidt & Emson, 1986). The decrease in lateral and increase in medial levels of NPY following overnight fasting may indicate a shift in secretion to the primary ARC-PVN feeding circuit, although no changes in PVN levels were found. The failure of any treatment to affect PVN levels of NPY is unusual given the potent site-specific effects of this nucleus (e.g., Stanley & Leibowitz, 1984). The low concentration of NPY in the PVNp and PVNm relative to other nuclei in this study may be due to methodology. Because 15 nuclei were sampled for this study, brains were sliced to obtain several punches from each slice. PVN slices may have included rostral and caudal interstitial tissue of enough quantity to buffer the concentration of NPY obtained from each punch.

Free feeding and fasting combined with drug treatment produced different results for NPY concentrations with the most dramatic effects occurring in fasted obese and lean rats. Only hypothalamic nuclei were affected in animals given access to food. ARC, combined ARC-ME, and

VMH levels were elevated in freely fed DZ-treated obese rats compared to obese controls. Control obese rats had increased LH levels compared to leans. Lean rats only showed effects at the LH with an increase in DZ-treated rats. Again, obese rats given access to food had effects most specific to the central hypothalamus while lean rats showed trends for effects in lateral areas. The direction of effects, however, ran counter to those predicted. DZ treatment was hypothesized to decrease rather than increase NPY levels in hypothalamic nuclei, based on the assumption of increased insulin entry into the CSF. Nevertheless, these results do not necessarily contradict the hypotheses of this study. The Palkovits method (1973) used in the current study only provides information about the content of peptide present in a given nucleus. The etiology of the decrease or increase in content is not identified. It is possible that increased ARC, combined ARC-ME, and VMH levels may indicate a reduction in release at some downstream sites and a compensatory increase in intracellular peptide in those regions. The change in release of NPY at the synapse, combined with the functional role of each nucleus, may uncover the physiological purpose for the effects observed. One nucleus to which this notion applies that was affected in this study is the VMH .

The VMH is a region of the hypothalamus long associated with feeding satiety, as evidenced by an increase in feeding following lesioning (Anand & Brobeck, 1951). In addition, destruction of this region coincides with an increase in a resistance to muscle glucose uptake and increased shunting of

nutrients to fat (Penicaud et al., 1989). Alterations in VMH function may be key to normalizing metabolic status since VMH activity may be disturbed in obese rats. Transplants of fetal lean hypothalamic tissue into six-week-old obese rats decrease body weight, plasma insulin, and liver fat compared to controls or rats receiving donor transplants from other brain regions (Fukagawa, Knight, Price, Sakata, & Tso, 1996). However, insulin binding in VMH of obese and lean rats is similar indicating that a change in ARC and DMH (increased binding) may have more influence on synthesis in those regions (Wilcox et al., 1989). Kalra, Dube, Xu, and Kalra (1997) blocked neural activity in the VMH with the neurotoxin colchicine leading to an increase in feeding and body weight during a few days following treatment. NPY Y1 receptor antagonism suppressed feeding, pointing to an increase in Y1 sensitivity mediating colchicine increased feeding with suppression of synaptic NPY. To answer some of these questions with respect to the current research, *in situ* hybridization techniques could be used to assess mRNA levels and identify changes in synthesis responsible for the increased content. On the other hand, *in vivo* techniques using push-pull cannulae might be used to test for altered secretion of peptide at different nuclei.

At the same time these factors are considered, it should be recognized that physiological elevations in NPY in obese rats may not correspond to behavioral changes in feeding. Brief et al. (1992) infused NPY into the 3V of obese rats at single doses over acute periods (less than 24 hours) without affecting food intake. Alternatively, NPY antagonism has also failed to

suppress feeding in obese rats (Beck et al., 1994). McCarthy et al. (1991) infused NPY and produced a decrease in receptor binding density. These effects may point to a down regulation of receptors in obese rats resulting from chronically elevated NPY (e.g., Beck et al., 1992a). Other support for this notion comes from reduced ARC mRNA levels of NPY following chronic elevation of NPY (McMinn et al., 1998). Widdowson (1997) has shown more specifically that this may be true for the Y5 receptors in VMH, ARC, and PFH. Wyss and colleagues (1998) obtained similar findings for Y5 mediation since ICV Y5 agonist injection stimulated feeding in lean but not obese rats. Hence, the elevated nucleus content of DZ treatment may not correspond to increases in feeding behavior.

As stated previously, fasting, combined with DZ treatment, produced a more dramatic pattern of NPY results. Obese rats treated with DZ showed an increase in NPY at the PFH and LH. Rather than producing central hypothalamic effects like those in free fed drug animals or fasting controls, DZ treatment may shunt NPY to lateral areas in fasted obese animals. As an alternative, the increase in these regions may indicate a decrease in release at those sites. The importance of this shift is not clear. It should be noted that LH is a region sensitive to NPY-induced feeding (Stanley et al., 1985a) and that the PFH is proposed to be the hypothalamic site most sensitive to NPY effects on feeding (Stanley et al., 1993). By reducing release at those sites, behavioral effects of reduced feeding may occurr. McGowan, Andrews, Kelly, and Grossman (1990) showed a decrease in feeding and body weight

in animals infused chronically with insulin into the PFH and VMH with no effect at the LH. In the current research, the central insulin increase with DZ treatment may moderate reduced feeding by binding to those same insulin-sensitive regions and attenuating release of NPY.

Fasted lean rats of this study showed the most varied effects under the combined drug and feeding condition with effects exclusive to extra-hypothalamic nuclei compared to their obese counterparts. DZ decreased NPY levels of the CNA and increased BNST levels. That activity is changed in these nuclei is provocative given their presence in pathways projecting to the vagal nuclei in the brainstem (Magnuson et al., 1984). Previous DZ research has uncovered a tendency for lean animals placed on DZ to develop glucose intolerance when the same dosage of drug used in this study is continued for a longer period of time (c. 6 week duration). Immunoreactivity of the immediate early gene c-fos is elevated in the lateral central amygdaloid nucleus and brain stem nuclei more when food is present than when food is not available following ICV NPY infusion. Based on these feeding differences, the authors describe a potential role for such nuclei in postabsorptive actions like increased insulin secretion (Li, Xu, Rowland, & Kalra, 1994). The association of the CNA and BNST with vagal outflow may suggest a role for a central change in vagus control of pancreatic insulin secretion that precedes glucose intolerance in lean rats. The mild state of fasting in this study may have served to unmask this tendency.

Although increases rather than decreases in NPY were found in this study, events underlying the origin of the content changes are not clear. As stated previously, changes in synthesis, transport, or release underlie the content distributions. It was hypothesized that insulin would suppress central NPY. However, the results contradict this hypothesis. It should be noted that other studies involving insulin modification have produced findings similar to those in the current research. Malabu and colleagues (1993) infused insulin into the jugular vein of fasted rats while maintaining normal glucose levels in order to examine changes in central NPY of rats with peripheral hyperinsulinemia. Hyperinsulinemia led to an increase in ARC-ME compared to controls. Those authors hypothesized that a decrease in transport of NPY as an initial response to the acute (2.5 hour) manipulation led to an accumulation of NPY near the site of synthesis. Other evidence supports this explanation. Rouru et al. (1995) obtained similar effects when they chronically administered metformin, an insulin sensitizing drug, to obese Zucker rats. Metformin reduced feeding, weight gain, and insulin levels throughout treatment. In addition, metformin elevated PVN NPY and led to a trend for increased ARC content. However, NPY synthesis did not appear to be affected as evidenced by a failure of metformin to increase prepro-NPY mRNA. These data suggest that elevated content within the ARC-PVN system with metformin treatment may be due to suppressed release and compensatory intracellular accumulation. Similarly, Malabu et al. (1994) administered a drug "mimicing" insulin's action, vanadate, to rats and found elevated NPY in the MPO, PVN, and ARC-ME of nondiabetic animals to

levels found in rats with experimentally-induced diabetes while decreasing food intake in both groups. Thus, increased peripheral or central insulin levels do not necessarily correlate with a decrease in central hypothalamic NPY.

Schwartz and colleagues (1992a) described the insulin-NPY model on which the current research was based. Because CSF insulin was not assessed in the current study, its relationship to NPY content is only speculative. The possibility that other circulating indices of nutrition contribute to the NPY changes of this study should not be dismissed. In fact, the potential that DZ itself may have direct central effects should be considered. Although some researchers argue that DZ does not cross the blood brain barrier freely (Zivkovic et al., 1995), DZ may be transported into the CSF. The presence of K+ATPase, having properties very similar to or very different from those of pancreatic beta cells, in hypothalamic regions (e.g., VMH, Sellers, Boden, & Ashford, 1992) could provide a mechanism by which DZ directly modulates NPY. However, the direction of effects on NPY is difficult to predict as K+ATPase channel characteristics are only currently being worked out (e.g., Routh, McArdle, & Levin, 1997). One way to rule out DZ as a direct effector of NPY would be through the use of chromatographic techniques like HPLC to see if CSF-DZ is detectable following drug treatment. Another test to determine if insulin is the primary effector of NPY with DZ treatment might include use of peripheral insulin clamp along with DZ treatment to see if NPY levels resist modification when insulin levels are

maintained constant via exogenous insulin infusion (personal communication, M. Zemel).

Even though the peripheral index of focus in this study was insulin, work with the recently identified *ob* gene product leptin may implicate its role in the anorectic effects of DZ treatment. DZ treatment decreases plasma leptin in addition to insulin as body weights fall in obese rats (Alemzadeh & Holshouser, in press). Leptin is an adipose tissue byproduct that circulates as an index of fat mass (review, Caro et al., 1996) and enters the brain via a saturable mechanism (Banks, Kastin, Huang, Jaspan, & Maness, 1996). Not surprisingly, obese Zucker rats have elevated leptin levels compared to leans, corresponding to increased fat mass (review, Rohner-Jeanrenaud, Cusin, Sainsbury, Zakrzewska, & Jeanrenaud, 1996). These elevated levels are coupled with a mutated leptin receptor (Chua et al., 1996). In nonobese rats, ICV leptin decreases feeding, increases fat liberation, reduces NPY in the ARC, PVN, and DMH, and decreases NPY synthesis as measured with decreased hypothalamic NPY mRNA (Wang et al., 1997). The presence of leptin receptor expressing mRNA in the medial hypothalamus suggests a direct route by which leptin (Hakansson, Hulting, & Meister, 1996), similar to insulin (Havrankova & Roth, 1978), can bind to alter cellular physiology and possibly NPY. This negative feedback system of adiposity with central NPY may be disturbed for obese rats similar to the proposed disruption of the insulin-NPY system of obese rats (Rohner-Jeanrenaud et al., 1996). Cusin, Rohner-Jeanrenaud, Stricker-Krongrad, and Jeanrenaud (1996) report that

ICV leptin decreases body weight of lean and obese rats over six days following treatment, with feeding decreasing during the first few days, implying that central leptin resistance may be one element of elevated body weights. These doses parallel a decrease in NPY in ARC and PVN of both groups. It should be mentioned that the most effective dose in obese rats was about ten times greater than that for leans. However, Seeley et al. (1996) report that ICV leptin reduces acute and chronic feeding in lean, non-Zucker rats, but fails to reduce acute food intake in obese Zucker rats. Further, Spanswick, Smith, Groppi, Logan, and Ashford (1997) have identified hypothalamic cells in non-obese animals that respond electrophysiologically to leptin via K+ATPase sensitive channels. When they applied the same techniques to obese Zucker cells, electrophysiological changes were blocked, an effect most likely due to receptor mutations. Other studies point to possible pharmacological manipulations of interactions between insulin, leptin, and K+ATPase channels (Harvey & Ashford, 1998) and provide a potential electrophysiological basis for leptin-NPY inhibition (Glaum et al., 1996). How this model accounts for effects observed in the Zucker rats of our study remains unclear given the conflicting findings of leptin receptor responsiveness previously outlined. The property of DZ as a K+ATPase modulator, coupled with the role of leptin in affecting K+ATPase channels, suggests a different avenue for anti-obesity effects of DZ than originally outlined in this study. This relationship provides one line of explanation by which peripheral endogenous and exogenous signals may regulate DZ-induced weight loss.

In conclusion, DZ treatment reduced body weight gain, improved glucose uptake, and modified central NPY levels of both free fed and fasted obese rats. Mild, overnight fasting led to differing effects of DZ on NPY content with a shift from medial increases to lateral hypothalamic increases compared to free fed rats. Few effects were found for changes in NPY in lean rats. However, when those changes occurred, they tended to lie outside of the hypothalamus. Future research uncovering the interaction of NPY, leptin, K+ATPase channels, and DZ may elucidate some of the current findings. For the current study, DZ was hypothesized to reduce hypothalamic and extra-hypothalamic NPY levels. Given the observed elevation in NPY levels of animals undergoing DZ treatment, additional research extending the previous studies of plasma leptin and DZ treatment may uncover a more specific role for leptin in DZ-induced reductions in feeding and concomitant weight loss. In addition, combinations of these treatments with the recent development of an NPY "knock-out" mouse (e.g., Erickson, Clegg, & Palmiter, 1996) will more fully elucidate the mechanisms of NPY in weight loss due to DZ treatment. Nevertheless, if NPY is indeed a factor in DZ-induced changes in weight gain and feeding, future studies should examine the behavioral changes, nutrient preferences, and metabolic expenditures modified with DZ treatment. These explorations may give a more complete picture of the potential benefits of DZ-induced weight loss.

REFERENCES

REFERENCES

Abe, M., Saito, M., & Shimazu, T. (1990). Neuropeptide Y in the specific hypothalamic nuclei of rats treated neonatally with monosodium glutamate. Brain Research Bulletin, 24, 289-291.

Adrian, T.E., Allen, J.M., Bloom, S.R., Ghatei, M.A., Rossor, M.N., Roberts, G.W., Crow, T.J., Tatemoto, K., & Polak, J.M. (1983). Neuropeptide Y distribution in human brain. Nature, 306, 584-586.

Akabayashi, A., Zai, C.T.B.V., Silva, I., Chae, H.J., & Leibowitz, S.F. (1993). Neuropeptide Y in the arcuate nucleus is modulated by alterations in glucose utilization. Brain Research, 621, 343-348.

Alemzadeh, R., & Holshouser, S. (1999). Effect of diazoxide on brain capillary insulin receptor binding and food intake in hyperphagic obese Zucker rats. Endocrinology, in press.

Alemzadeh, R., Jacobs, W., & Pitukcheewanont, P. (1996). Antiobesity effect of diazoxide in obese Zucker rats. Metabolism, 45, 334-341.

Alemzadeh, R., Slonim, A.E., Zdanowicz, M.M., & Maturo, J. (1993). Modification of insulin resistance by diazoxide in obese Zucker rats. Endocrinology, 133, 705-712.

Allen, J., Novotny, J., Martin, J., & Heinrich, G. (1987). Molecular structure of mammalian neuropeptide Y: Analysis by molecular cloning and computer-aided comparison with crystal structure of avian homologue. Proceedings of the National Academy of Sciences, 84, 2532-2536.

Allen, J.M., & Balbi, D. (1993). Structure and expression of the neuropeptide Y gene. In W.F. Colmers & C. Wahlestedt (Eds.), The biology of neuropeptide Y and related peptides (pp. 43-64). Totowa, NJ: Humana Press.

Allen, J.M., McGregor, G.P., Woodhams, P.L., Polak, J.M., & Bloom, S.R. (1984a). Ontogeny of a novel peptide, neuropeptide Y (NPY) in rat brain. Brain Research, 303, 197-200.

Allen, R., Boublik, J., Hauger, R.L., Scott, N., Rivier, J., & Brown, M.R. (1991). Neuropeptide Y radioimmunoassay: Characterization and application. Clinical and Experimental Pharmacology and Physiology, 18, 825-833.

Allen, Y.S., Adrian, T.E., Allen, J.M., Tatemoto, K., Crow, T.J., Bloom, S.R., & Polak, J.M. (1983). Neuropeptide Y distribution in the rat brain. Science, 221, 877-879.

Allen, Y.S., Roberts, G.W., Bloom, S.R., Crow, T.J., & Polak, J.M. (1984b). Neuropeptide Y in the stria terminalis: Evidence for an amygdalofugal projection. Brain Research, 321, 357-362.

Anand, B.K., & Brobeck, J.R. (1951). Hypothalamic control of food intake in rats and cats. Yale Journal of Biology and Medicine, 24, 123-140.

Bai, F.L., Yamano, M., Shiotani, Y., Emson, P.C., Smith, A.D., Powell, J.F., & Tohyama, M. (1985). An arcuato-paraventricular and dorsomedial hypothalamic neuropeptide Y-containing system which lacks noradrenaline in the rat. Brain Research, 331, 172-175.

Baker-Norton Pharmaceuticals, Inc. (1995). Proglycem [Drug Insert]. Miami, FL.

Banks, W.A., Kastin, A.J., Huang, W., Jaspan, J.B., & Maness, L.M. (1996). Leptin enters the brain by a saturable system independent of insulin. Peptides, 17, 305-311.

Bard, J.A., Walker, M.W., Branchek, T.A., & Weinshank, R.L. (1995). Cloning and functional expression of a human Y4 subtype receptor for pancreatic polypeptide, neuropeptide Y, and peptide YY. Journal of Biological Chemistry, 270, 26762-26765.

Baskin, D.G., Porte, D., Guest, K., & Dorsa, D.M. (1983). Regional concentrations of insulin in the rat brain. Endocrinology, 112, 898-903.

Beck, B., Burlet, A., Bazin, R., Nicolas, J.-P., & Burlet, C. (1993). Elevated neuropeptide Y in the arcuate nucleus of young obese Zucker rats may contribute to the development of their overeating. Journal of Nutrition, 123, 1168-1172.

Beck, B., Burlet, A., Nicolas, J.-P., & Burlet, C. (1990a). Hypothalamic neuropeptide Y (NPY) in obese Zucker rats: Implications in feeding and sexual behaviors. Physiology and Behavior, 47, 449-453.

Beck, B., Burlet, A., Nicolas, J.-P., & Burlet, C. (1992a). Unexpected regulation of hypothalamic neuropeptide Y by food deprivation and refeeding in the Zucker rat. Life Sciences, 50, 923-930.

Beck, B., Jhanwar-Uniyal, M., Burlet, A., Chapleaur-Chateau, M., Leibowitz, S.F., & Burlet, C. (1990b). Rapid and localized alterations of neuropeptide Y in discrete hypothalamic nuclei with feeding status. Brain Research, 528, 245-249.

Beck, B., Stricker-Krongrad, A., Musse, N., Nicolas, J.-P., & Burlet, C. (1994). Putative neuropeptide Y antagonist failed to decrease overeating in obese Zucker rats. Neuroscience Letters, 181, 126-128.

Beck, B., Stricker-Krongrad, A., Nicolas, J.P., & Burlet, C. (1992b). Chronic and continuous intracerebroventricular infusion of neuropeptide Y in Long-Evans rats mimics the feeding behavior of obese Zucker rats. International Journal of Obesity, 16, 295-302.

Becker, E.E., & Grinker J.A. (1977). Meal patterns in the genetically obese Zucker rat. Physiology and Behavior, 18, 685-692.

Ben-Ari, Y., Krnjevic, K., & Crepel, V. (1990). Activators of ATP-sensitive K+ channels reduce anoxic depolarization in CA3 hippocampal neurons. Neuroscience, 37, 55-60.

Billington, C.J., Briggs, J.E., Harker, S., Grace, M., & Levine, A.S. (1994). Neuropeptide Y in hypothalamic paraventricular nucleus: A center coordinating energy metabolism. American Journal of Physiology, 266, R1765-R1770.

Bjork, E., Berne, C., Kampe, O., Wibell, L., Oskarsson, P., & Karlsson, F.A. (1996). Diazoxide treatment at onset preserves residual insulin secretion in adults with autoimmune diabetes. Diabetes, 45, 1427-1430.

Black, J. (1968). Diazoxide and the treatment of hypoglycemia: An historical review. Annals of New York Academy of Sciences, 150, 194-203.

Braitman, L.E., Adlin, E.V., & Stanton, J.L. (1985). Obesity and caloric intake: The national health and nutrition examination survey of 1971-1975 (HANES I). Journal of Chronic Disease, 38, 727-732.

Brief, D.J., Sipols, A.J., & Woods, S.C. (1992). Intraventricular neuropeptide Y injections stimulate food intake in lean, but not obese Zucker rats. Physiology and Behavior, 51, 1105-1110.

Broberger, C., Johansen, J., Schalling, M., & Hokfelt, T. (1997). Hypothalamic neurohistochemistry of the murine anorexia (anx/anx) mutation: Altered processing of neuropeptide Y in the arcuate nucleus. Journal of Comparative Neurology, 387, 124-135.

Broberger, C., Landry, M., Wong, H., Walsh, J.N., & Hokfelt, T. (1997). Subtypes Y1 and Y2 of the neuropeptide Y receptor are respectively expressed in pro-opiomelanocortin- and neuropeptide Y-containing neurons of the rat hypothalamic arcuate nucleus. Neuroendocrinology, 66, 393-408.

Brunani, A., Invitti, C., Dubini, A., Piccoletti, R., Bendinelli, P., Maroni, P., Pezzoli, G., Ramella,G., Calogero, A., & Cavagnini, F. (1995). Cerebrospinal fluid and plasma concentrations of SRIH, beta-endorphin, CRH, NPY, and GHRH in obese and normal weight subjects. International Journal of Obesity and Related Metabolic Disorders, 19, 17-21.

Buchberger, P., & Schmidt, I. (1994). The earliest stages of excess fat deposition in fatty Zucker rat pups are not associated with changed drive for food intake. International Journal of Obesity, 13, 129. [Abstract]

Calza, L., Giardino, L., Battistini, N., Zanni, M., Galetti, S., Protopapa, F., & Velardo, A. (1989). Increase of neuropeptide Y-like immunoreactivity in the paraventricular nucleus of fasting rats. Neuroscience Letters, 104, 99-104.

Card, J.P., & Moore, R.Y. (1989). Organization of lateral geniculate-hypothalamic connections in the rat. Journal of Comparative Neurology, 284, 135-147.

Caro, J.F., Sinha, M.K., Kolaczynski, J.W., Zhang, P.L., & Considine, R.V. (1996). Leptin: the tale of an obesity gene. Diabetes, 45, 1455-1462.

Castonguay, T.W., Hartman, W.J., Fitzpatrick, E.A., & Stern, J.S. (1982). Dietary self-selection and the Zucker rat. Journal of Nutrition, 11, 796-800.

Centers for Disease Control (CDC). (1997). Update: Prevalence of overweight among children, adolescents, and adults--United States 1988-1994. Morbidity and Mortality Weekly Report, 46, 199-202.

Chronwall, B.M., DiMaggio, D.A., Massari, V.J., Pickel, V.M., Ruggiero, D.A., & O'Donohue, T.L. (1985). The anatomy of neuropeptide Y-containing neurons in rat brain. Neuroscience, 15, 1159-1181.

Chua, S.C., Chung, W.K., Wu-Peng, S., Zhang, Y., Liu, S.M., Tartaglia, L., & Leibel, R.L. (1996). Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. Science, 271, 994-996.

Clark, J.T., Kalra, P.S., Crowley, W.R., & Kalra, S.P. (1984). Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. Endocrinology, 115, 427-429.

Cusin, I., Rohner-Jeanrenaud, F., Stricker-Krongrad, A., Jeanrenaud, B. (1996). Weight-reducing effect of intracerebroventricular bolus injection of leptin in genetically obese *fa/fa* rats: Reduced sensitivity compared to lean animals. Diabetes, 45, 1446-1451.

Dawbarn, D., Hunt, S.P., & Emson, P.C. (1984). Neuropeptide Y: Regional distribution chromatographic characterization and immunohistochemical demonstration in post-mortem human brain. Brain Research, 296, 168-173.

DeQuidt, M.E., & Emson, P.C. (1986). Distribution of neuropeptide Y-like immunoreactivity in the rat central nervous system--II. Immunohistochemical analysis. Neuroscience, 18, 545-618.

Doods, H.N., Wieland, H.A., Engel, W., Eberlein, W., William, K.D., Entzeroth, M., Wienan, W., & Rudolf, K. (1996). BIBP 3226, the first neuropeptide Y1 receptor antagonist: Review of its pharmacological properties. Regulatory Peptides, 65, 71-77.

Dryden, S., Pickavance, L., Frankish, H.M., & Williams, G. (1995). Increased neuropeptide Y secretion in the hypothalamic paraventricular nucleus of obese *fa/fa* Zucker rats. Brain Research, 690, 185-188.

Dube, M.G., Sahu, A., Kalra, P.S., & Kalra S.P. (1992). Neuropeptide Y release is elevated from the microdissected paraventricular nucleus of food-deprived rats: An in vitro study. Endocrinology, 131, 684-688.

Dumont, Y., Fournier, A., & Quirion, R. (1998). Expression and characterization of the neuropeptide Y5 receptor subtype in the rat brain. Journal of Neuroscience, 18, 5565-5574.

Erickson, J.C., Clegg, K.E., & Palmiter, R.D. (1996). Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. Nature, 381, 415-418.

Eva, C., Keinanen, K., Monyer, H., Seeburg, P., & Sprengel, R. (1990). Molecular cloning of a novel G protein-coupled receptor that may belong to the neuropeptide receptor family. FEBS Letters, 271, 81-84.

Flood, J.F., & Morley, J.E. (1991). Increased food intake by neuropeptide Y is due to an increased motivation to eat. Peptides, 12, 1329-1332.

Fukagawa, K., Knight, D.S., Price, H.V., Sakata, T., & Tso, P. (1996). Transplantation of lean fetal hypothalamus restores hypothalamic function in Zucker obese rats. American Journal of Physiology, 271, R55-R63.

Gehlert, D.R. (1998). Multiple receptors for the pancreatic polypeptide (PP-fold) family: Physiological implications. Proceedings of the Society for Experimental Biology and Medicine, 218, 7-22.

Gehlert, D.R. (1994). Subtypes of receptors for neuropeptide Y: Implications for the targeting of therapeutics. Life Sciences, 55, 551-562.

Gerald, C., Walker, M.W., Criscione, L., Gustafson, E.L., Batzl-Hartmann, C., Smith, K.E., Vaysse, P., Durkin, M.M., Laz, T.M., Linemeyer, D.L., Schaffhauser, A.O., Whitebread, S., Hofbauer, K.G., Taber, R.I., Branchek, T.A., & Weinshank, R.L. (1996). A receptor subtype involved in neuropeptide-Y-induced food intake. Nature, 382, 168-171.

Glaum, S.R., Hara, M., Bindokas, V.P., Lee, C.C., Polonsky, K.S., Bell, G.I., & Miller, R.J. (1996). Leptin, the obese gene product, rapidly modulates synaptic transmission in the hypothalamus. Molecular Pharmacology, 50, 230-235.

Gorsky, R.D., Pamuk, E., & Williamson, D. (1996). The 25-year health care costs of women who remain overweight after 40 years of age. American Journal of Preventive Medicine, 12, 388-394.

Gray, T.S., O'Donohue, T.L., & Magnuson, D.J. (1986). Neuropeptide Y innervation of amygdaloid and hypothalamic neurons that project to the dorsal vagal complex in rat. Peptides, 7, 341-349.

Gray, T.S., O'Donohue, T.L., Watson, S.J., & Magnuson, D.J. (1984). Pro-opiomelanocortin and neuropeptide Y projections from arcuate and peri-arcuate hypothalamic areas to the nucleus tractus solitarius-dorsal vagal complex. Society for Neuroscience Abstracts, 10, 432.

Greenwood, R.H., Mahler, R.F., & Hales, C.N. (1976). Improvement in insulin secretion in diabetes after diazoxide. Lancet, 1, 444-446.

Grey, N., & Kipnis, D.M. (1971). Effect of diet composition on the hyperinsulinemia of obesity. New England Journal of Medicine, 285, 827-831.

Grundemar, L., Sheikh, S.P., & Wahlestedt, C. (1993). Characterization of receptor types for neuropeptide Y and related peptides. In W.C. Colmers & C. Wahlestedt (Eds.), The biology of NPY and related peptides, (pp. 197-239). Totowa, NJ: Humana.

Guyton, A.C., & Hall, J.E. (1996). Textbook of Medical Physiology (9th ed.). Philadelphia: W.B. Saunders Co.

Hakansson, M.L., Hulting, A.L., & Meister, B. (1996). Expression of leptin receptor mRNA in the hypothalamic arcuate nucleus relationship with NPY neurons. NeuroReport, 7, 3087-3092.

Harvey, J., & Ashford, M.L.J. (1998). Insulin occludes leptin activation of ATP-sensitive K+ channels in rat CRI-G1 insulin secreting cells. Journal of Physiology, 511.3, 695-706.

Havrankova, J., & Roth, J. (1978). Insulin receptors are widely distributed in the central nervous system of the rat. Nature, 272, 827-829.

Havrankova, J., Schmechel, D., Roth, J., & Brownstein, M. (1978). Identification of insulin in rat brain. Proceedings of the National Academy of Sciences, 75, 5737-5741.

Haynes, A.C., Arch, J.R.S., Wilson, S., McClue, S., & Buckingham, R.E. (1998). Characterization of the neuropeptide Y receptor that mediates feeding in the rat: A role for the Y5 receptor? Regulatory Peptides, 75-76, 355-361.

Heilig, M. (1993). Neuropeptide Y in relation to behavior and psychiatric disorders: Some animal and clinical observations. In W.F. Colmers & C. Wahlestedt (Eds.), The biology of neuropeptide Y and related peptides (pp. 511-545). Totowa, NJ: Humana.

Henquin, J.C. (1987). Regulation of insulin release by ionic and electrical events in B cells. Hormone Research, 27, 168-178.

Higuchi, H., Yang, H.Y.T., & Sabol, S.L. (1988). Rat neuropeptide Y precursor gene expression: mRNA structure, tissue distribution, and regulation by glucocorticoids, cyclic AMP, and phorbol ester. Journal of Biological Chemistry, 262, 6288-6295.

Ikeda, H., West, D.B., Pustek, J.J., Figlewicz, D.P., Greenwood, M.R.C., Porte, D., & Woods, S.C. (1986). Intraventricular insulin reduces food intake and body weight of lean but not obese Zucker rats. Appetite, 7, 381-386.

Jahng, J.W., Houpt, T.A., Kim, S.J., Joh, T.H., & Son, J.H. (1998). Neuropeptide Y mRNA and serotonin innervation in the arcuate nucleus of anorexia mutant mice. Brain Research, 790, 67-73.

Jhanwar-Uniyal, M., & Chua, S.C. (1993). Critical effects of aging and nutritional state on hypothalamic neuropeptide Y and galanin gene expression in lean and genetically obese Zucker rats. Molecular Brain Research, 19, 195-202.

Kalra, S.P., Dube, M.G., Fournier, A., & Kalra, P.S. (1991a). Structure-function analysis of stimulation of food intake by neuropeptide Y: Effects of receptor agonists. Physiology and Behavior, 50, 5-9.

Kalra, S.P., Dube, M.G., Sahu, A., Phelps, C.P., & Kalra, P.S. (1991b). Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. Proceedings of the National Academy of Sciences, 88, 10931-10935.

Kalra, P.S., Dube, M.G., Xu, B., & Kalra, S.P. (1997). Increased receptor sensitivity to neuropeptide Y in the hypothalamus may underlie transient hyperphagia and body weight gain. Regulatory Peptides, 72, 121-130.

Kanatani, A., Ishihara, A., Asahi, S., Tanaka, T., Ozaki, S., & Ihara, M. (1996). Potent neuropeptide Y Y1 receptor antagonist, 1229U91: Blockade of neuropeptide Y-induced and physiological food intake. Endocrinology, 137, 3177-3182.

Karam, J.H., Grodsky, G.M., & Forsham, P.H. (1963). Excessive insulin response to glucose in obese subjects as measured by immunochemical assay. Diabetes, 12, 197-204.

Kawano, H., & Masuko, S. (1993). Synaptic inputs to neuropeptide Y-immunoreactive noradrenergic nerve terminals to neurons in the nucleus preopticus medianus which project to the paraventricular nucleus of the hypothalamus of the rat: A combined immunohistochemical and retrograde tracing method. Brain Research, 600, 74-80.

Kaye, W.H., Berrettini, W., Gwirtsman, H., & George, D.T. (1990). Altered cerebrospinal fluid neuropeptide Y and peptide YY immunoreactivity in anorexia and bulimia nervosa. Archives of General Psychiatry, 47, 548-556.

Kaye, W.H., Berrettini, W.H., Gwirtsman, H.E., Gold, P.W., George, D.T., Jimerson, D.C., & Ebert, M.H. (1989). Contribution of CNS neuropeptide (NPY, CRH, and Beta-endorphin) alterations to psychophysiological abnormalities in anorexia nervosa. Psychopharmacology Bulletin, 25, 433-438.

Keesey, R.E., & Powley, T.L. (1975). Hypothalamic regulation of body weight. American Scientist, 63, 558-565.

Kerkerian, L., & Pelletier, G. (1986). Effects of monosodium L-glutamate administration on neuropeptide Y-containing neurons in the rat hypothalamus. Brain Research, 369, 388-390.

Krause, J., Eva, C., Seeburg, P.H., & Sprengel, R. (1992). Neuropeptide Y1 subtype pharmacology of a recombinantly expressed neuropeptide receptor. Molecular Pharmacology, 41, 817-821.

Lantos, T.A., Gorcs, T.J., & Palkovits, M. (1995). Immunohistochemical mapping of neuropeptides in the premammillary region of the hypothalamus in rats. Brain Research Reviews, 20, 209-249.

Larhammar, D., Ericsson, A., & Persson, H. (1987). Structure and expression of the rat neuropeptide Y gene. Proceedings of the National Academy of Sciences, 84, 2069-2072.

Larhammar, D., Soderberg, C., & Blomqvist, A.G. (1993). Evolution of the neuropeptide Y family of peptides. In W.F. Colmers & C. Wahlestedt (Eds.), The biology of neuropeptide Y and related peptides (pp. 1-41). Totowa, NJ: Humana.

Leahy, J.L., Bumbalo, L.M., & Chen, C. (1994). Diazoxide causes recovery of B-cell glucose responsiveness in 90% pancreatectomized diabetic rats. Diabetes, 43, 173-179.

Lebrun, P., Antoine, M.-H., & Hercheulz, A. (1992). Minireview: K+ channel openers and insulin release. Life Sciences, 51, 795-806.

Leibowitz, S.F., & Alexander, J.T. (1991). Analysis of neuropeptide Y-induced feeding: Dissociation of Y1 and Y2 receptor effects on natural meal patterns. Peptides, 12, 1251-1260.

Levine, A.S., & Morley, J.E. (1984). Neuropeptide Y: A potent inducer of consummatory behavior in rats. Peptides, 5, 1025-1029.

Li, B.H., Xu, B., Rowland, N.E., & Kalra, S.P. (1994). c-fos expression in the rat brain following central administration of neuropeptide Y and effects of food consumption. Brain Research, 665, 277-284.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., & Randall, R.J. (1951). Protein measurement with the folin phenol reagent. Journal of Biological Chemistry, 193, 265-275.

Lynch, W.C., Grace, M., Billington, C.J., & Levine, A.S. (1993). Effects of neuropeptide Y on ingestion of flavored solutions in nondeprived rats. Physiology and Behavior, 54, 877-880.

Maggio, C.A., & Vasselli, J.R. (1990). Insulin hypersecretion contributes to hyperphagia and obesity in the Zucker rat. FASEB Journal, 5, A511. [Abstract]

Magnuson, D.J., O'Donohue, T.L., & Gray, T.S. (1984). Neuropeptide "Y" innervation of hypothalamic and amygdala neurons projecting to the nucleus tractus solitarius/dorsal vagal nuclei. Society for Neuroscience Abstract, 10, 432.

Malabu, U.H., Cotton, S.J., Kruszynska, Y.T., & Williams, G. (1993). Acute hyperinsulinemia increases neuropeptide Y concentration in the hypothalamic arcuate nucleus of fasted rats. Life Sciences, 52, 1407-1416.

Malabu, U.H., Dryden, S., McCarthy, H.D., Kilpatrick, A., & Williams, G. (1994). Effects of chronic vanadate administration in STZ-induced diabetic rat: The antihyperglycemic action of vanadate is attributable entirely to its suppression of feeding. Diabetes, 43, 9-15.

Maltais, L.J., Lane, P.W., & Beamer, W.G. (1984). Anorexia, a recessive mutation causing starvation in preweanling mice. Journal of Heredity, 75, 468-472.

Marks, J.L., Li, M., Schwartz, M., Porte, D., & Baskin, D.G. (1992). Effect of fasting on regional levels of neuropeptide Y mRNA and insulin receptors in the rat hypothalamus: An autoradiographic study. Molecular and Cellular Neurosciences, 3, 199-205.

Martel, J.C., St. Pierre, S., & Quirion, R. (1988). Comparative distribution of neuropeptide Y immunoreactivity and receptor autoradiography in rat forebrain. Peptides, 9, 15-20.

Martel, J.C., St. Pierre, S., & Quirion, R. (1986). Neuropeptide Y receptors in rat brain. Autoradiographic localization. Peptides, 7, 55-60.

Martin, R.J., Harris, R.B.S., & Jones, D.D. (1986). Evidence for a central mechanism of obesity in the Zucker fatty rat (*fa/fa*). Proceedings of the Society for Experimental Biology and Medicine, 183, 1-10.

Martin, S., Housley, K., McCoy, H., Greenhouse, P., Stigger, F., Kenney, M.A., Shoffner, S., Fu, V., Korslund, M., Ercanli-Huffman, F.G., Carter, E., Chopin, L., Hegsted, M., Clark, A.J., Disney, G., Moak, S., Wakefield, T., & Stallings, S. (1988). Self-esteem of adolescent girls as related to weight. Perceptual and Motor Skills, 67, 879-884.

McCarthy, H.D., McKibbin, P.E., Holloway, B., Mayers, R., & Williams, G. (1991). Hypothalamic neuropeptide Y receptor characteristics and NPY-induced feeding responses in lean and obese Zucker rats. Life Sciences, 49, 1491-1497.

McGowan, M.K., Andrews, K.M., Kelly, J., & Grossman, S.P. (1990). Effects of chronic intrahypothalamic infusion of insulin on food intake and diurnal meal patterning in the rat. Behavioral Neuroscience, 104, 373-385.

McKibbin, P.E., Cotton, S.J., McMillan, S., Holloway, B., Mayers, R., McCarthy, H.D., & Williams, G. (1991). Altered neuropeptide Y concentrations in specific hypothalamic regions of obese (fa/fa) Zucker rats: Possible relationship of obesity and neuroendocrine disturbances. Diabetes, 40, 1423-1429.

McMinn, J.E., Seeley, R.J., Wilkinson, C.W, Havel, P.J., Wood, S.C., & Schwartz, M.W. (1998). NPY-induced overfeeding suppresses hypothalamic NPY mRNA expression: Potential roles of plasma insulin and leptin. Regulatory Peptides, 75-76, 425-431.

Melnyk, R.B. (1987). Decreased binding to hypothalamic insulin receptors in young genetically obese rats. Physiology and Behavior, 40, 237-241.

Menendez, J.A., McGregor, I.S., Healey, P.A., Atrens, D.M., & Leibowitz, S.F. (1990). Metabolic effects of neuropeptide Y injections into the paraventricular nucleus of the hypothalamus. Brain Research, 516, 8-14.

Michel, M. (1991). Receptors for neuropeptide Y: Multiple subtypes and multiple second messengers. Trends in Pharmacological Sciences, 12, 389-294.

Mikkelsen, J.D., & Larsen, P.J. (1992). A high concentration of NPY (Y1)-receptor mRNA-expressing cells in the rat arcuate nucleus. Neuroscience Letters, 148, 194-198.

Moltz, J.H., & McDonald, J.K. (1985). Neuropeptide Y: Direct and indirect action on insulin secretion in the rat. Peptides, 6, 1155-1159.

Morley, J.E., Levine, A.S., Gosnell, B.A., Kneip, J., & Grace, M. (1987). Effect of neuropeptide Y on ingestive behaviors in the rat. American Journal of Physiology, 252, R599-R609.

Nakajima, T., Yashima, Y., & Nakamura, K. (1986). Quantitative autoradiographic localization of neuropeptide Y receptors in the rat lower brainstem. Brain Research, 380, 144-150.

Palkovits, M. (1973). Isolated removal of hypothalamic or other brain nuclei of the rat. Brain Research, 59, 449-450.

Paxinos, G., & Watson, C. (1997). The rat brain in stereotaxic coordinates (3rd ed.). New York: American Press.

Pelletier, G. (1990). Ultrastructural localization of neuropeptide Y in the hypothalamus. Annals of the New York Academy of Sciences, 611, 232-246.

Pelletier, G., Desy, L., Kerkerian, L., & Cote, J. (1984). Immunocytochemical localization of neuropeptide Y (NPY) in the human hypothalamus. Cell Tissue Research, 238, 203-205.

Penicaud, L., Kinebanyan, M.F., Ferre, P., Morin, J., Kande, J., Smadja, C., Marfaing-Jallat, P., & Picon, L. (1989). Development of VMH obesity: In vivo insulin secretion and tissue insulin sensitivity. American Journal of Physiology, 257, E255-E260.

Pesonen, U., Huupponen, R., Rouru, J., & Koulu, M. (1992). Hypothalamic neuropeptide expression after food restriction in Zucker rats: Evidence of persistent neuropeptide Y gene activation. Molecular Brain Research, 16, 255-260.

Pierson, A.M., Williams, G., Jones, P.M., Ball, J.A., Legon, Dixon, J.E., & Bloom, S.R. (1988). Hypothalamic neuropeptide Y mRNA is increased in streptozotocin-diabetic (STZ-DM) rats. Diabetic Medicine, 5, 19A. [Abstract]

Pi-Sunyer, F.X. (1993). Medical hazards of obesity. Annals of Internal Medicine, 119, 655-660.

Pronicka, E., Gruszczynska, B., Wozniewicz, B., Cedro, A., Kaminski, W., & Lesniewska, A. (1995). Pancreatic glucagon levels in infants and children with hyperinsulinemia. Journal of Pediatrics, 126, 948-951.

Rabinowitz, D., & Zierler, K.L. (1962). Forearm metabolism in obesity and its response to intraarterial insulin. Characterization of insulin resistance and evidence for adaptive hyperinsulinism. Journal of Clinical Investigation, 41, 2173-2181.

Ratzmann, K.P., Ruhnke, R., & Kohnert, K.D. (1983). Effect of pharmacological suppression of insulin secretion on tissue sensitivity to insulin in subjects with moderate obesity. International Journal of Obesity, 7, 453-458.

Roche, C., Boutin, P., Dina, C., Gyapay, G., Basdevant, A., Hager, J., Guy-Grand, B., Clement, K., & Froguel, P. (1997). Genetic studies of neuropeptide Y and neuropeptide Y receptors Y1 and Y5 in morbid obesity. Diabetologia, 40, 671-675.

Rodin, J. (1981). Current status of the internal-external hypothesis for obesity: What went wrong? American Psychologist, 36, 361-372.

Rodin, J., Wack, J., Ferrannini, E., & DeFronzo, R.A. (1985). Effect of insulin and glucose on feeding behavior. Metabolism, 34, 826-831.

Rohner-Jeanrenaud, F. (1995). A neuroendocrine reappraisal of the dual centre hypothesis: Its implications for obesity and insulin resistance. International Journal of Obesity, 19, 517-534.

Rohner-Jeanrenaud, F., Cusin, I., Sainsbury, A., Zakrzewska, K.E., & Jeanrenaud, B. (1996). The loop system between neuropeptide Y and leptin in normal and obese rodents. Hormone and Metabolic Research, 28, 642-648.

Rohner-Jeanrenaud, F., Ionescu, E., & Jeanrenaud, B. (1983). The origins and role of efferent vagal nuclei in hyperinsulinemia in hypothalamic and genetically obese rodents. Journal of the Autonomic Nervous System, 9, 173-184.

Rose, P.M., Fernandes, P., Lynch, J.S., Frazier, S.T., Fisher, S.M., Kodukula, K., Kienzle, B., & Seethala, R. (1995). Cloning and functional expression of a cDNA encoding a human type 2 neuropeptide Y receptor. Journal of Biological Chemistry, 270, 22661-22664.

Rouru, J., Pesonen, U., Koulu, M., Huupponen, R., Santti, E., Virtanen, K., Rouvari, T., & Jhanwar-Uniyal, M. (1995). Anorectic effect of metformin in obese Zucker rats: Lack of evidence for the involvement of neuropeptide Y. European Journal of Pharmacology, 273, 99-106.

Routh, V.H., McArdle, J.J., & Levin, B.E. (1997). Phosphorylation modulates the activity of the ATP-sensitive K+ channel in the ventromedial hypothalamic nucleus. Brain Research, 778, 107-119.

Rubin, A.A., Roth, F.E., Winbury, M.M., Topliss, J.B., Sherlock, M.H., Sperber, N., & Black J. (1961). New class of antihypertensive agents. Science, 133, 2067.

Rubin, A.A., Zitowitz, L., & Hausler, L. (1963). Acute circulatory effects of diazoxide and sodium nitrite. Journal of Pharmacology and Experimental Therapeutics, 140, 46-51.

Sahu, A., Kalra, S.P., Crowley, W.R., & Kalra, P.S. (1988). Evidence that NPY-containing neurons in the brainstem project into selected hypothalamic nuclei: Implication in feeding behavior. Brain Research, 247, 376-378.

Sahu, A., Sninsky, C.A., Kalra, P.S., & Kalra, S.P. (1990). Neuropeptide-Y concentration in microdissected hypothalamic regions and in vitro release from the medial basal hypothalamus-preoptic area of streptozotocin-diabetic rats with and without insulin substitution therapy. Endocrinology, 126, 194-198.

Sahu, A., Sninsky, C.A., Phelps, C.P., Dube, M.G., Kalra, P.S., & Kalra, S.P. (1992). Neuropeptide Y release from the paraventricular nucleus increases in association with hyperphagia in streptozotocin-induced diabetic rats. Endocrinology, 131, 2979-2985.

Sainsbury, A., Cusin, I., Doyle, P., Rohner-Jeanrenaud, F., & Jeanrenaud, B. (1996). Intracerebroventricular administration of neuropeptide Y to normal rats increases obese gene expression in white adipose tissue. Diabetologia, 39, 353-356.

Sainsbury, A., Rohner-Jeanrenaud, F., Zakrzewska, K.E., Halban, P.A., Gaillard, R.C., & Jeanrenaud, B. (1997). Chronic central neuropeptide Y infusion in normal rats: Status of the hypothalamo-pituitary-adrenal axis, and vagal mediation of hyperinsulinaemia. Diabetologia, 40, 1269-1277.

Sanacora, G., Kershaw, M., Finkelstein, J.A., & White J.D. (1990). Increases hypothalamic content of prepro-neuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food deprivation. Endocrinology, 127, 730-737.

Sawchenko, P.E., & Pfeiffer, S.W. (1988). Ultrastructural localization of neuropeptide Y and galanin immunoreactivity in the paraventricular nucleus of the hypothalamus in the rat. Brain Research, 474, 231-245.

Sawchenko, P.E., Swanson, L.W., Grzanna, R., Howe, P.R.C., Bloom, S.R., & Polak, J.M. (1985). Colocalization of neuropeptide Y immunoreactivity in brainstem catecholaminergic neurons that project to the paraventricular nucleus of the hypothalamus. Journal of Comparative Neurology, 241, 138-153.

Schachter, S., Goldman, R., & Gordon, A. (1968). Effects of fear, food deprivation, and obesity on eating. Journal of Personality and Social Psychology, 10, 91-97.

Schade, D.S., & Eaton, R.P. (1975). Insulin secretion by perfused islets from the obese Zucker rat. Proceedings of the Society for Experimental Biology and Medicine, 149, 311-314.

Schwartz, M.W., Figlewicz, D.P., Baskin, D.G., Woods, S.C., & Porte, D. (1992a). Insulin in the brain: A hormonal regulator of energy balance. Endocrine Reviews, 13, 81-108.

Schwartz, M.W., Figlewicz, D.F., Kahn, S.E., Baskin, D.B., Greenwood, M.R.C., & Porte, D. (1990). Insulin binding to brain capillaries is reduced in genetically obese hyperinsulinemic Zucker rats. Peptides, 11, 467-472.

Schwartz, M.W., Figlewicz, D.P., Woods, S.C., Porte, D., & Baskin, D.B. (1993). Insulin, neuropeptide Y, and food intake. Annals of the New York Academy of Sciences, 692, 60-71.

Schwartz, M.W., Marks, J.L., Sipols, A.J., Baskin, D.B., Woods, S.C., Kahn, S.E., & Porte, D. (1991). Central insulin administration reduces neuropeptide Y mRNA expression in the arcuate nucleus of food-deprived lean (Fa/Fa) and not obese (fa/fa) Zucker rats. Endocrinology, 128, 2645-2647.

Schwartz, M.W., Sipols, A.J., Marks, J.L., Sanacora, G., White, J.D., Scheurink, A., Kahn, S.E., Baskin, D.G., Woods, S.C., Figlewicz, D.P., & Porte, D. (1992b). Inhibition of hypothalamic neuropeptide Y gene expression by insulin. Endocrinology, 130, 3608-3616.

Seeley, R.J., Payne, C.J., & Woods, S.C. (1995). Neuropeptide Y fails to increase intraoral intake in rats. American Journal of Physiology, 268, R423-R427.

Seeley, R.J., Van Dijk, G., Campfield, L.A., Smith, F.J., Burn, P., Nelligan, J.A., Bell, S.M., Baskin, D.G., Woods, S.C., & Schwartz, M.W. (1996). Intraventricular leptin reduces food intake and body weight of lean rats but not obese Zucker rats. Hormone and Metabolic Research, 28, 664-668.

Sellers, A.J., Boden, P.R., & Ashford, M.L.J. (1992). Lack of effect of potassium channel openers on ATP-modulated potassium channel recorded from rat ventromedial hypothalamic neurones. British Journal of Pharmacology, 107, 1068-1074.

Sigal, R.J., El-Hashimy, M., Martin, B.C., Soeldner, J.S., Krolewski, A.S., & Warram, J.H. (1997). Acute postchallenge hyperinsulinemia predicts weight gain. Diabetes, 46, 1025-1029.

Spanswick, D., Smith, M.A., Groppi, V.E., Logan, S.D., & Ashford, M.L.J. (1997). Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. Nature, 390, 521-525.

Stanley, B.G., Anderson, K.C., Grayson, M.H., & Leibowitz, S.F. (1989). Repeated hypothalamic stimulation with neuropeptide Y increases daily carbohydrate and fat intake and body weight gain in female rats. Physiology and Behavior, 46, 173-177.

Stanley, B.G., Chin, A.S., & Leibowitz, S.F. (1985a). Feeding and drinking elicited by central injection of neuropeptide Y: Evidence for a hypothalamic site(s) of action. Brain Research Bulletin, 14, 521-524.

Stanley, B.G., Daniel, D.R., Chin, A.S., & Leibowitz, S.F. (1985b). Paraventricular nucleus injections of peptide YY and neuropeptide Y preferentially enhance carbohydrate ingestion. Peptides, 6, 1205-1211.

Stanley, B.G., Kyrkouli, S.E., Lampert, S., & Leibowitz, S.F. (1986). Neuropeptide Y chronically injected into the hypothalamus: A powerful neurochemical inducer of hyperphagia and obesity. Peptides, 7, 1189-1192.

Stanley, B.G., & Leibowitz, S.F. (1985). Neuropeptide Y injected in the paraventricular hypothalamus: A powerful stimulant of feeding behavior. Proceedings from the National Academy of Sciences, 82, 3940-3943.

Stanley, B.G., & Leibowitz, S.F. (1984). Neuropeptide Y: Stimulation of feeding and drinking by injection into the paraventricular nucleus. Life Sciences, 35, 2635-2642.

Stanley, B.G., Magdalin, W., Seirafi, A., Nguyen, M.M., & Leibowitz, S.F. (1992). Evidence for neuropeptide Y mediation of eating produced by food deprivation and for a variant of the Y1 receptor mediating this peptide's effect. Peptides, 13, 581-587.

Stanley, B.G., Magdalin, W., Seirafi, A., Thomas, W.J., & Leibowitz, S.F. (1993). The perifornical area: The major focus of (a) patchily distributed hypothalamic neuropeptide Y-sensitive feeding system(s). Brain Research, 604, 304-317.

Stanley, C.A. (1997). Hyperinsulinism in infants and children. Pediatric Clinics of North America, 44, 363-374.

Stein, L.J., Dorsa, D.M., Baskin, D.G., Figlewicz, D.P., Ikeda, H., Frankmann, S.P., Greenwood, M.R.C., Porte, D., & Woods, S.C. (1983). Immunoreactive insulin levels are elevated in the cerebralspinal fluid of genetically obese Zucker rats. Endocrinology, 113, 2299-2301.

Stein, L.J., Dorsa, D.M., Baskin, D.B., Figlewicz, D.P., Porte, D., & Woods, S.C. (1987). Reduced effect of experimental peripheral hyperinsulinemia to elevate cerebrospinal fluid insulin concentrations of obese Zucker rats. Endocrinology, 121, 1611-1615.

Stein, L.J., West, D.B., Figlewicz, D.P., Porte, D., & Woods, S.C. (1985). Immunoreactive insulin in the cerebrospinal fluid of baboons and genetically obese rats. International Journal of Obesity, 9, A156. [Abstract]

Steinman, J.L., Gunion, M.W., & Morley, J.E. (1987). Third and fourth ventricular (3V, 4V) neuropeptide Y (NPY) stimulates feeding and drinking in rats. FASEB Abstracts, 46, 1125.

Stern, J., Johnson, P.R., Greenwood, M.R.C., Zucker, L.M., & Hirsch, J. (1972). Insulin resistance and pancreatic insulin release in the genetically obese Zucker rat. Proceedings of the Society for Experimental Biology and Medicine, 139, 66-69.

Stern, J.S., & Johnson, P.R. (1977). Spontaneous activity and adipose cellularity in the genetically obese Zucker rat (fafa). Metabolism, 26, 371-380.

Stricker-Krongrad, A., Max, J.P., Musse, N., Nicolas, J.P., Burlet, C., & Beck, B. (1994). Increased threshold concentrations of neuropeptide Y for stimulatory effect of food intake in obese Zucker rats--Changes in the microstructure of feeding behavior. Brain Research, 660, 162-166.

Strombom, U., Krotkiewski, M., Blennow, K., Mansson, J.-E., Ekman, R., & Bjorntorp, P. (1996). The concentrations of monoamine metabolites and neuropeptides in the cerebrospinal fluid of obese women with different body fat distribution. International Journal of Obesity and Related Metabolic Disorders, 20, 361-368.

Takeuchi, T., Gumucio, D.L., Yamada, T., Meisler, M.H., Minth, C.D., Dixon, J.E., Eddy, R.E., & Shows, T.B. (1986). Genes encoding pancreatic polypeptide and neuropeptide Y are on human chromosomes 17 and 7. Journal of Clinical Investigation, 77, 1038-1041.

Tatemoto, K., Carlquist, M., & Mutt, V. (1982). Neuropeptide Y--a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature, 296, 659-660.

Tenenbaum, R., Ochoa, F.Y., Sassano, M.L., Chua, S.C., Leibel, R.L., & Campfield, L.A. (1994). Altered insulin secretion from the pancreas of pre-obese Zucker rat pups: Evidence for an early and persistent genetic defect. International Journal of Obesity, 18, 67. [Abstract]

Unden, A., Tatemoto, K., & Bartfai, T. (1983). Receptors for neuropeptide Y in rat brain. Society for Neuroscience Abstracts, 9, 170.

Unden, A., Tatemoto, K., Mutt, V., & Bartfai, T. (1984). Neuropeptide Y receptor in the rat brain. European Journal of Biochemistry, 145, 525-530.

Vanhatalo, S., & Soinila, S. (1996). Pituitary gland receives both central and peripheral neuropeptide Y innervation. Brain Research, 740, 253-260.

Van Houten, M., Posner, B.I., Kopriwa, B.M., & Brawer, J.R. (1980). Insulin binding sites localized to nerve terminal in rat median eminence and arcuate nucleus. Science, 207, 1081-1083.

Van Huijsduijnen, C.B.H., Rohner-Jeanrenaud, F., & Jeanrenaud, B. (1993). Hypothalamic neuropeptide Y messenger ribonucleic acid levels in pre-obese and genetically obese (fa/fa) rats: Potential regulation thereof by corticotropin-releasing factor. Journal of Neuroendocrinology, 5, 381-386.

Vasselli, J.R., Cleary, M.P., Jen, K.L.C., & Greenwood, M.R.C. (1980). Development of food motivated behavior in free feeding and food restricted Zucker fatty (fa/fa) rats. Physiology and Behavior, 25, 565-573.

Vettor, R., Zarjevski, N., Cusin, I., Rohner-Jeanrenaud, F., & Jeanrenaud, B. (1994). Induction and reversability of an obesity syndrome by intracerebroventricular neuropeptide Y administration to normal rats. Diabetologia, 37, 1202-1208.

Wahlestedt, C., & Reis, D.J. (1993). Neuropeptide Y-related peptides and their receptors--Are the receptors potential therapeutic drug targets? Annual Reviews of Pharmacology and Toxicology, 32, 309-352.

Wahlestedt, C., Yanaihara, N., & Hakanson, R. (1986). Evidence for different pre- and post-junctional receptors for neuropeptide Y and related peptides. Regulatory Peptides, 13, 307-318.

Walker, P., Grouzmann, E., Burnier, M., & Waeber, B. (1991). The role of neuropeptide Y in cardiovascular regulation. Trends in Pharmacological Sciences, 12, 111-115.

Wang, Q., Bing, C., Al-Barazanji, K., Mossakowaska, D.E., Wang, X.-M., McBay, D.L., Neville, W.A., Taddayon, M., Pickavance, L., Dryden, S., Thomas, M.E.A., McHale, M.T., Gloyer, I.S., Wilson, S., Buckingham, R., Arch, J.R.S., Trayhurn, P., & Williams, G. (1997). Interactions between leptin and hypothalamic neuropeptide Y neurons in the control of food intake and energy homeostasis in the rat. Diabetes, 46, 335-341.

White, B.D., & Martin, R.Y. (1997). Evidence for a central mechanism of obesity in the Zucker rat: Role of neuropeptide Y and leptin. Proceedings of the Society for Experimental Biology and Medicine, 214, 222-232.

White, J.D., & Kershaw, M. (1990). Increased hypothalamic neuropeptide Y expression following food deprivation. Molecular and Cellular Neurosciences, 1, 41-48.

Widdowson, P.S. (1997). Regionally-selective down-regulation of NPY receptor subtypes in the obese Zucker rat. Relationship to the Y5 "feeding" receptor. Brain Research, 758, 17-25.

Widdowson, P.S., Upton, R., Henderson, L., Buckingham, R., Wilson, S., & Williams, G. (1997). Reciprocal regional changes in brain NPY receptor density during dietary restriction and dietary-induced obesity in the rat. Brain Research, 774, 1-10.

Wigand, J.P., & Blackard, W.G. (1979). Downregulation of insulin receptors in obese man. Diabetes, 28, 287-291.

Wilcox, B.J., Corp, E.S., Dorsa, D.M., Figlewicz, D.P., Greenwood, M.R.C., Woods, S.C., & Baskin, D.G. (1989). Insulin binding in the hypothalamus of lean and genetically obese Zucker rats. Peptides, 10, 1159-1164.

Williams, G., Cardoso, H.M., Lee, Y.C., Ball, J.M., Ghatei, M.A., Stock, M.J., & Bloom, S.R. (1991). Hypothalamic regulatory peptides in obese and lean Zucker rats. Clinical Science, 80, 419-426.

Wyss, P., Levens, N., & Stricker-Krongrad, A. (1998). Stimulation of feeding in lean but not in obese Zucker rats by a selective neuropeptide Y Y5 receptor agonist. Neuroreport, 9, 2675-2677.

York, D.A., & Bray, G.A. (1973). Genetic obesity in rats. II. The effect of food restriction on the metabolism of adipose tissue. Metabolism, 22, 443-454.

York, D.A., Steinke, J., & Bray, G.A. (1972). Hyperinsulinemia and insulin resistance in genetically obese rats. Metabolism, 21, 277-284.

Young, W.S. (1986). Periventricular hypothalamic cells in the rat brain contain insulin mRNA. Neuropeptides, 8, 93-97.

Zarjevski, N., Cusin, I., Vettor, R., Rohner-Jeanrenaud, F., & Jeanrenaud, B. (1993). Chronic intracerebroventricular neuropeptide-Y administration to normal rats mimics hormonal and metabolic changes of obesity. Endocrinology, 133, 1753-1758.

Zarjevski, N., Cusin, I., Vettor, R., Rohner-Jeanrenaud, F., & Jeanrenaud, B. (1994). Intracerebroventricular administration of neuropeptide Y to normal rats has divergent effects on glucose utilization by adipose tissue and skeletal muscle. Diabetes, 43, 764-769.

Zhang, X., Bao, L., Xu, Z.Q., Kopp, J., Arvidsson, U., Elde, R., & Hokfelt, T. (1994). Localization of neuropeptide Y Y1 receptors in the rat nervous system with special reference to somatic receptors on small dorsal root ganglion neurons. Proceedings of the National Academy of Sciences, 91, 11738-11742.

Zivkovic, I., Thompson, D.M., Bertolino, M., Uzunov, D., DiBella, M., Costa, E., & Guidotti, A. (1995). 7-chloro-3-methyl-3-4-dihydro-2H-1,2,4, benzothiadiazine S,S-dioxide (IDRA 21): A benzothiadiazine derivative that enhances cognition by attenuating DL-alpha-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic acid (AMPA) receptor desensitization. Journal of Pharmacology and Experimental Therapeutics, 272, 300-309.

Zucker, L.M. (1975). Efficiency of energy utilization by the Zucker hereditarily obese rat "fatty." Proceedings of the Society for Experimental Biology and Medicine, 148, 498-500.

Zucker, L.M., & Antoniades, H.N. (1972). Insulin and obesity in the Zucker genetically obese rat "fatty." Endocinology, 90, 1320-1330.

Zucker, L.M., & Zucker, T.F. (1961). Fatty, a new mutation in the rat. Journal of Heredity, 52, 275-278.

Zucker, T.F., & Zucker L.M. (1962). Hereditary obesity in the rat associated with high serum fat and cholesterol. Proceedings of the Society for Experimental Biology and Medicine, 110, 165-171.

VITA

Ingrid Edgemon Hensley was born in Jackson, Mississippi in April 1971. Shortly after her birth, her family moved to northwest Georgia where she grew up and attended public school. Ingrid graduated from Ringgold High School in 1989, and declaring psychology as her major, immediately enrolled in the University of Georgia (UGA). During her college studies, she became interested in the physiological aspects of behavior and emotion. She graduated summa cum laude from UGA in 1993. In the fall of 1993, she entered the experimental psychology doctoral program at the University of Tennessee, Knoxville. She began working in the lab of Dr. James Lawler, studying cardiovascular psychophysiology and specializing in animal models of environmentally-induced hypertension. For her doctoral research, she began a collaborative project between the Department of Pediatrics Research at the University of Tennessee Medical Center in Knoxville and her home lab to study changes in neurotransmitter levels of obese animals undergoing insulin-antagonized weight loss. Throughout graduate school, one of the greatest pleasures that she experienced was in teaching undergraduate courses in summer and evening school. Ingrid is currently teaching psychology in a full-time position at Tennessee Wesleyan College in Athens, Tennessee.