National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Canada

ROLE OF SENSORY NERVES IN GROWTH AND THERMOGENESIS OF BROWN ADIPOSE TISSUE

by

Jingying Cui

A thesis submitted to the School of Graduate Studies
of the University of Ottawa
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Department of Biochemistry
Faculty of Medicine
University of Ottawa
Ottawa, Ontario
Canada K1H 8M5

ISBN 0-315-80051-8

UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

ABSTRACT

The original objective of work described in this thesis was to study neural control of diet-induced thermogenesis in BAT. The hypothesis was that impairment of central warmth perception by capsaicin-desensitization (CAP-DES) would diminish central suppression of BAT thermogenesis by heat generated as a consequence of food ingestion, and thus enhance sympathetic-mediated diet-induced hypertrophy of BAT. Unexpectedly, CAP-DES caused atrophy of BAT and prevented diet-induced thermogenesis in BAT. This novel finding led to a modification of objectives to find out why CAP-DES induces atrophy of BAT and to study further the consequences for the CAP-DES rat of the atrophied state of its BAT.

Atrophy of BAT in the CAP-DES rat involved loss of total protein, loss of mitochondrial proteins and loss of cells. BAT of the CAP-DES rat was unresponsive to stimulation by diet and cold-induced growth was retarded. The most extensive destruction of BAT occurred at 1 day, gradually reversed over the next 28 days, then reappeared at 8 months. A preliminary study in which a neurotransmitter of sensory nerves, calcitonin gene-related peptide (CGRP) was infused for several days suggested that the effect of CAP-DES might be due to removal of a trophic influence of neuropeptides of sensory nerves, known to be depleted in BAT of the CAP-DES rat. The association between atrophied BAT and obesity in several different animal models suggested that CAP-DES rats might become obese. However, CAP-DES rats fed an obesity-inducing diet (high fat) for 10 weeks did not become any more obese than control rats eating the same diet and aging-induced obesity was reduced in the CAP-DES rat.

Conclusions: Neuropeptides of sensory nerves play an important role in control of BAT thermogenic capacity, supplementing that of noradrenaline. The relation of atrophied BAT to energy balance in the CAP-DES rat remains to be elucidated.

DEDICATION

This thesis is dedicated to Limin and Jia.

ACKNOWLEDGMENT

I wish to express my deepest gratitude and appreciation to my thesis supervisor, Dr. Jean Himms-Hagen. No amount of words would suffice to thank her for her constant guidance, encouragement and support which she has generously given me during my research program.

I would like to thank Dr. P.R. Larsen for the method for purifying T_4, and for T_4 and T_3 antisera, Dr. Gloria Zaror-Behrens for preparation of UCP and antisera and for writing programs used in some of the calculations, and Dr. Anna-Lisa Kates for helping me with the statistical analysis.

I would also like to thank Dr. D.O. Foster and the National Research Council of Canada for the use of the oxygen uptake equipment and the computer program for its operation.

A special note of thanks goes to Ms. Gisele Larose for excellent care of the animals and help with the surgery and assays.

Finally, I wish to thank my colleagues who worked and/or are working in this Lab: Dr. Gloria Zaror-Behrens, Dr. Anna-Lisa Kates, Dr. Lynn Sigurdson, Dr. Mary-Ellen Harper, Ms. Christiane Villemure, Ms. Mahin Baqi, Ms. Anna Melnyk and Dr. Joan Triandafillou for all the interesting discussions and pleasant memories we share as well as for their help and friendship.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADP	adenosine diphosphate
ANOVA	analysis of variance
ATP	adenosine triphosphate
BAT	brown adipose tissue
BSA	bovine serum albumin
BW	body weight
cAMP	cyclic adenosine monophosphate
CAP-DES	capsaicin-desensitization or capsaicin-desensitized
CGRP	calcitonin-gene-related-peptide
COX	cytochrome oxidase
CRF	corticotropin-releasing factor
DNA	deoxyribonucleic acid
EDTA	ethylenediamine tetraacetic acid
GDP	guanosine diphoshate
GTP	guanosine triphoshate
HEPES	hydroxyethylpiperazine-N'-2-ethane sulfonic acid
HPLC	high pressure liquid chromatography
kcal	kilocalorie
kD	kilodalton
KOH	potassium hydroxide
LH	lateral hypothalamus
MOPS	(3-(N-morpholino)propane sulfonic acid
NaCl	sodium chloride
NaOH	sodium hydroxide
Na_2SO_4	sodium sulfate
NPY	neuropeptide Y
PBS	phosphate buffered saline
PCA	perchloric acid
PPO	2,5-diphenyloxazole
PVN	paraventricular nucleus
RIA	radioimmunoassay
s.c.	subcutaneous
SEM	standard error of the mean
SDS	sodium dodecyl sulfate
SP	substance P
T_3	triiodothyronine
T_4	thyroxine
T5'D	thyroxine 5'-deiodinase
TCA	trichloroacetic acid
TES	Tris(hydroxymethyl)-2-aminoethane sulfonic acid
TRIS	Tris(hydroxymethyl) aminomethane
Tween-20	polyoxyethylene sorbitan monolaurate

UCP	uncoupling protein
VMH	ventromedial hypothalamus
WAT	white adipose tissue

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Brown adipose tissue (BAT) is a specialized adipose tissue. It is found in all hibernators and in many nonhibernators, like rats and mice (Johansson, 1959). The function of BAT was first associated with arousal from hibernation. Thereafter, the specific function of thermogenesis in BAT was described (Smith and Horwitz, 1969; Smith and Hock, 1963).

During exposure to cold, there is an increase in the metabolic rate of BAT which is referred to as cold-induced nonshivering thermogenesis (Himms-Hagen, 1984, 1986). The contribution of cold-induced nonshivering thermogenesis to the cold-induced increase in metabolic rate is distinct from that part due to muscle activity (shivering thermogenesis). Cold-induced nonshivering thermogenesis is the dominant pathway of thermoregulatory heat production in small mammals. It accounts for 60-70% of the increase in metabolic rate caused by acclimation of rats to cold (Foster and Frydman, 1978a, b, 1979).

Thermogenesis in BAT is also increased by ingestion of a palatable "cafeteria" diet and this is referred to as diet-induced thermogenesis (Rothwell and Stock, 1979). Food itself has a thermic effect, which results in an immediate increase in heat production associated with the ingestion, digestion, absorption and initial processing of the components of the food. Diet-induced thermogenesis is distinct from the thermic effect of food. Cold-induced nonshivering thermogenesis and diet-induced thermogenesis are two major aspects of BAT thermogenesis.

1.2 Categories of Thermogenesis

Thermogenesis is a physiological process that accompanies all metabolic reactions that maintain the body in a living state. Thermogenesis can be classified into two major categories: obligatory thermogenesis and facultative thermogenesis. Obligatory thermogenesis accompanies those processes essential to keep the body in a basal metabolic state. The reactions occur in every organ of the body and are primarily controlled by hormones. Changes in obligatory thermogenesis are usually small and slow. Energy expenditure involved in ingestion, digestion, absorption and storage of food, as well as energy spent for other physiological situations such as growth, pregnancy and lactation are also included in the obligatory category.

Facultative thermogenesis is different from the obligatory category in that it can be rapidly turned on or off by the nervous system, depending on the needs of the animal. Facultative thermogenesis mainly occurs in two organs, skeletal muscle and BAT. In skeletal muscle it is associated with physical activity (exercise) or with thermoregulatory shivering thermogenesis at low environmental temperatures. Facultative thermogenesis in skeletal muscle is controlled by the central nervous system via the motor nerves. In BAT, facultative thermogenesis is associated with thermoregulatory thermogenesis (nonshivering thermogenesis) or with diet-induced thermogenesis, both mediated by noradrenaline secreted from the sympathetic nerves to BAT. Diet-induced thermogenesis in BAT can be further divided into a cephalic phase and a postprandial phase (Himms-Hagen, 1989). Since BAT thermogenesis represents an auxiliary heat source and is controlled in relation to thermoregulation, any heat-load on the body, such as that imposed by exercise, tumour

growth or the stable hyperthermia of fever, can suppress BAT thermogenesis.

1.3 Brown Adipose Tissue Thermogenesis

a. Location and Morphology

Brown adipose tissue is a distinct organ which is distributed in several discrete depots in the interscapular, cervical, intercostal, pericardial and perirenal regions of the body (Smith and Horwitz, 1969). The relative proportions of total BAT in these different deposits vary from species to species. In rats and mice, the interscapular deposit is a major one, and comprises one third of the total BAT, whereas in other species, such as primates, the axillary deposit is the most abundant one (Lean et al., 1986; Lean and Trayhurn, 1987) and very little BAT is found in the interscapular area.

BAT contains, in addition to endothelial cells, four major cell types. They are mature brown adipocytes, preadipocytes, protoadipocytes and interstitial stem cells (Bukowiecki et al., 1982, 1986; Géloën et al., 1988, 1990). Proliferation and /or differentiation of the last three occur when the BAT is stimulated. Unlike white adipocytes, brown adipocytes are multilocular, and normally contain numerous small triacylglycerol droplets, as well as large mitochondria and other organelles such as smooth endoplasmic reticulum, peroxisomes, lysosomes, free ribosomes and Golgi apparatus (Smith and Horwitz, 1969; Néchad, 1986).

The other important component of BAT is endothelial cells, which proliferate when the vasculature grows (Bukowiecki et al., 1982, 1986). BAT has an extensive vasculature and also possesses arteriovenous anastomoses (Nnodim and Lever, 1988). This morphological basis can readily supply a large amount of substrate and oxygen needed to support the high

metabolic rate of brown adipocytes during stimulated thermogenesis. In addition to supplying metabolic requirements, the vascular pathways of BAT also act as a convector system for the capture and evacuation of the heat generated during stimulated thermogenesis (Smith and Roberts, 1964).

b. Innervation and Neuropeptides

Brown adipocytes and BAT blood vessels receive a direct sympathetic innervation. The neurotransmitter, noradrenaline, plays a primary role in the control of BAT thermogenesis and of BAT growth in most species (Himms-Hagen, 1991). The interscapular BAT of the rat is the most studied BAT depot and consists of two symmetrical pads, each receiving five intercostal nerves as well as a nerve which runs along the thoracodorsal blood vessels (Foster et al., 1982 a,b; Néchad, 1986; Girardier and Seydoux, 1986). The origin of these nerves is diffuse, arising from the middle and inferior cervical ganglia and the first five thoracic ganglia (Girardier and Seydoux, 1986). Many studies have demonstrated that there is no cross-innervation between the symmetrical pads (Foster et al., 1982 a,b; Park and Himms-Hagen, 1988; Himms-Hagen, 1989). Nerves which supply BAT blood vessels contain noradrenaline and neuropeptide Y (NPY) whereas those on cells contain only noradrenaline (Cannon et al., 1986; Norman et al., 1988). Noradrenergic nerves also supply the arteriovenous anastomoses (Nnodim and Lever, 1988). Recently, nerves containing other neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP) have also been found to be present in BAT (Norman et al., 1988; Lever et al., 1988, 1989; Mukherjee et al., 1989). These nerves are presumed to be sensory nerves, known to be

characterized by the possession of these peptides (Holzer, 1988). The role of sympathetic nerves or sensory nerves in BAT metabolism can be studied independently. Evidence for the predominant role of the sympathetic innervation in control of BAT thermogenesis and growth is based on several experimental approaches: effect of nerve stimulation, effect of surgical denervation, effect of neuropharmacological manipulation with guanethidine or 6-hydroxydopamine, effect of acute or chronic administration of noradrenaline on BAT and measurement of noradrenaline turnover. For the study of the function of sensory nerves, a specific chemical compound, capsaicin, can be used as a neuropharmacological tool. The use of capsaicin is discussed further in chapter 1.6.

c. Hypothalamus and Control of Sympathetic Nervous System Activity in Brown Adipose Tissue

The hypothalamus is a control centre for the sympathetic nervous system. It has been reported that there is a sympathetic nerve-mediated connection between the hypothalamus and BAT (Saito et al., 1987). Evidence has been derived from studies of hypothalamic stimulation, hypothalamic lesions and hypothalamic injection of various substances. Electrical stimulation of the ventromedial hypothalamus (VMH) in rats causes a marked increase in lipid metabolism and heat production in BAT (Shimazu and Takahashi, 1980) and in noradrenaline turnover in BAT, which is commonly used as a index of sympathetic nerve activity in the tissue. Electrical stimulation of the VMH can also increase the rate of glucose uptake in BAT of anaesthetized rats without detectable changes in plasma insulin levels (Sudo et al., 1991). Evidence for the participation of specific neurons of the VMH is

provided by the observations that thermogenesis and glucose uptake of BAT can be stimulated by microinjection of glutamate into the VMH (Amir, 1990; Sudo et al., 1991). Glutamate is known as an excitatory neurotransmitter in the central nervous system (Goodchild et al., 1982). The other strong evidence is that stimulation of VMH increases blood flow of BAT in rats (Iwai et al., 1987). The effects of VMH stimulation on BAT is most probably mediated by the sympathetic nerve activity because the VMH is regarded as a hypothalamic component of the sympathetic nervous system (Saito et al., 1987, 1989), and these effects are abolished after severing surgically the sympathetic nerves of the BAT. Unlike stimulation of the VMH, electrical stimulation of the lateral hypothalamus (LH) has no appreciable effects on noradrenaline turnover and lipid metabolism in BAT. There is no significant effect on glucose uptake in BAT after stimulation of LH (Sudo et al., 1991). In some studies, BAT is also activated when the paraventricular nucleus (PVN) is stimulated (Freeman and Wellman, 1987; Holt et al., 1987; Holt and York, 1988). Studies of the effect of cooling the preoptic area of the hypothalamus or the skin suggest that this pathway is involved in thermoregulatory control of nonshivering thermogenesis in BAT (Imai-Matsumura et al., 1988; Imai-Matsumura and Nakayama, 1987; Morimoto and Murakami, 1985).

Studies of the effects on BAT after specific brain areas have been damaged indicate that VMH lesions decrease sympathetic activity in BAT (Sakaguchi et al., 1988a, b). GDP binding, the means of assessing BAT thermogenic state, was reduced in both warm- and cold-acclimated VMH-lesioned rats (Seydoux et al., 1982a, Hogan et al., 1982). Many studies reported that noradrenaline turnover in BAT of VMH-lesioned rats was decreased (Vander

Tuig et al., 1985, 1982); except one by Yoshida and Bray (1984) in which noradrenaline turnover in BAT of VMH-lesioned rats was increased. Lesions of the LH result in an increase in noradrenaline turnover in BAT (Yoshida et al., 1983) and in GDP binding (Holt and York, 1988; Lupien et al., 1986; Park et al., 1986). From the above, it is possible to postulate a role for the VMH or LH in the regulation of thermogenesis in BAT. It seems likely that the VMH exerts an excitatory influence on BAT, whereas the LH exerts a suppressive influence. Lesions of PVN have no effect on BAT (Yoshida and Bray, 1984; Yoshida et al., 1983, 1989; Tokunaga et al., 1986; Sakaguchi et al., 1988c). Lesions of other brain areas, like lesion to the nucleus tractus solitarius in the rat, also reduce the functional capacity of BAT (Fyda et al., 1991). All these findings suggested that BAT thermogenesis is regulated by hypothalamus via its sympathetic innervation.

Injection of glucose into the VMH increases the firing rate of the sympathetic nerves to BAT (Sakaguchi and Bray, 1987a). Insulin, however, suppresses the activity of BAT sympathetic nerves when injected into the VMH (Sakaguchi and Bray, 1987b; Amir et al., 1989; Sakaguchi et al., 1988b). Injections of glucose, serotonin, CRF into the PVN stimulate the function of BAT (Sakaguchi and Bray 1988c, 1989; LeFeuvre et al., 1987). Insulin injected into the PVN inhibits activity of sympathetic nerves (Sakaguchi and Bray, 1988). In contrast, glucose injected into the LH suppresses BAT while injection of insulin into the LH increases the firing rate of the sympathetic nerves to BAT (Sakaguchi et al., 1988a). Similar to injection of glucose into VMH or PVN, intravenous injection of glucose increases the activity of sympathetic nerves to BAT, but the effect is inhibited by VMH lesion (Niijima, 1986; Sakaguchi and Bray, 1990). Injection of insulin intravenously has the opposite effect

(Sakaguchi and Bray, 1987a, b and 1990). Thus, it is possible that both glucose and insulin are involved in responses of BAT to carbohydrate ingestion. In other studies, a high fat diet (Mercer and Trayhurn, 1984a, 1987) and a protein-deficient diet (Johnston and Balachandran, 1987; Vander Tuig and Romsos, 1984) can also bring about activation of BAT. But, in these cases, the regulation by the hypothalamus is still not clear.

It is known that there is a cephalic phase to food ingestion associated with the sight, smell, taste and sensory qualities of food. The cephalic phase of feeding is associated with sympathetic activation and neurally-mediated insulin secretion (Berthoud et al., 1981; Diamond and LeBlanc, 1987a, b; Steffens et al., 1986). Therefore, it can be presumed that part of the neurally-mediated response of BAT to diet is a cephalic phase response dependent on the sensory qualities of the food. The effect of a cafeteria diet to induce BAT thermogenesis is a typical example. Evidence of a connection between hypothalamus and a gustatory neurons has been provided by some researchers (Berthoud et al., 1981). Recently, it is reported that intake of palatable food increases sympathetic nervous system activity in BAT and that this effect is largely cephalic (Griggio et al., 1991).

d. Mechanism of Thermogenesis

The thermogenic mechanism of BAT mitochondria involves a unique proton conductance pathway which permits uncoupling of electron transport from oxidative phosphorylation. This uncoupling is accomplished by increasing proton permeability, thereby dissipating the proton electrochemical gradient (Nicholls, 1976). As a result, the electron transport system is freed from respiratory control. This allows for maximum rates of electron

transport and conversion of energy to heat (Nicholls, 1983). The uncoupling protein (UCP), located in the BAT inner mitochondrial membrane (Nicholls, 1976), serves as a proton translocator (Nicholls et al., 1986; Klingenberg and Winkler, 1986). When UCP is functioning the rapid dissipation of the proton gradient leads to a speeding up of electron transport and of substrate oxidation, thus overall heat production increases. When heat production is no longer required, the proton conductance pathway is blocked by binding of purine nucleotides to UCP (Nicholls, 1976). UCP is unique to BAT and undetectable in liver, white adipose tissue and muscle (Cannon et al., 1982; Afong et al., 1985). The capacity of BAT cells for thermogenesis is determined by the concentration of UCP (Hansen and Knudsen, 1986; Rafael et al., 1986).

The UCP of BAT mitochondria has been purified and sequenced. The gene sequence has been determined as well. It is a small protein of 306 amino acids and a molecular weight of about 32 kD (Aquila et al., 1985; Bouillaud et al., 1986; Kozak et al., 1988). The UCP completely traverses the inner mitochondrial membrane several times and has a C-terminal region that projects from the outer surface of the membrane (Aquila et al., 1985; Eckerskorn and Klingenberg, 1987). Each transmembrane domain is coded for by a different exon in the UCP gene (Kozak et al., 1988; Bouillaud et al., 1988). The control of function appears to be mediated through the external C-terminal region which contains a nucleotide binding site (Kopecky et al., 1987), one site per molecule of UCP (French et al., 1988). The C-terminal region is also the site of the antigenic determinant. This is of practical important when raising antisera to UCP. A standard technique to measure thermogenic capacity in BAT is the radioimmunoassay of UCP.

BAT thermogenesis is primarily controlled by the hypothalamus via its sympathetic innervation and noradrenaline. Activation of thermogenesis is mediated by both ß- and α-adrenergic receptors located on brown adipocytes (Mohell et al., 1983; Schimmel et al., 1983; Himms-Hagen, 1989). The neurotransmitter, noradrenaline activates the lipolytic cascade. Interaction of noradrenaline with ß-adrenergic receptors results in activation of adenylate cyclase and consequent increased production of cyclic adenosine 5'-monophosphate (cAMP). cAMP activates a protein kinase that phosphorylates hormone-sensitive lipase and produces the active form of this enzyme (Bukowiecki, 1984). Then, the rate of intracellular lipolysis increases and fatty acid levels rise. The fatty acids can serve both as the signal for increased operation of the proton-translocating UCP and as the initial fuel for the increase in mitochondrial oxidations. It is believed that most (about 80%) of the consequent increase in BAT thermogenesis is secondary to the increase in fatty acid level (Mohell et al., 1983; Schimmel et al., 1983). The action of noradrenaline to activate adenylate cyclase is inhibited by adenosine (Schimmel et al., 1987). Moreover, the stimulation by noradrenaline of uncoupled respiration results in large changes in relative concentrations of the adenine nucleotides such that ATP levels decline and AMP levels increase (Lanoue et al., 1986; Ma and Foster, 1984). The active 5'-nucleotidase in BAT then converts some of the AMP to adenosine, thus restraining over-stimulation of the tissue by noradrenaline (Schimmel et al., 1987).

Interaction of noradrenaline with α_1-adrenergic receptors in BAT results in an increased operation of the phosphatidylinositol bisphosphate (PIP_2) cycle. This, in turn increases production of two second messengers, inositol trisphosphate (IP_3) and

diacylglycerol (DAG) (Mohell et al., 1984, 1987; Nanberg and Putney, 1986; Schimmel et al., 1986). IP_3 increases Ca^{++} release from intracellular stores (Connolly et al., 1984; Nedergaard et al., 1986). The source of Ca^{++} may be endoplasmic reticulum or mitochondria (Nedergaard et al., 1986). The Ca^{++} mobilization is a Na^+-dependent process (Nanberg and Nedergaard, 1987). The increase in Ca^{++} activates a Ca^{++}-dependent K^+ channel which caused a transient efflux of K^+ (Nanberg et al., 1985; Nedergaard et al., 1986; Sciemen and Reuhl, 1987). During noradrenaline stimulation, Na^+ influx also occurs, increasing intracellular Na^+ concentration (Nedergaard et al., 1986). The increase in Na^+ influx and K^+ efflux results in plasma membrane depolarisation. In order to restore the normal distribution of these ions, Na^+, K^+-ATPase is promoted to function, pumping ions with increased utilization of ATP. An increase in Na^+ K^+-ATPase activity is a part of the α_1-adrenergic agonist thermogenic response in BAT (Mohell et al., 1987; Nanberg et al., 1984). However, this plays a relatively minor role in the thermogenic response of isolated cells to noradrenaline (about 20%) (Mohell et al., 1983).

Two pathways can control the proton-translocating function of the UCP. One, mentioned in the preceding paragraph is the binding of purine nucleotides to UCP which inhibits its proton translocating function. The binding of these nucleotides is pH dependent; a high pH induces a reduced affinity and ready loss of the bound nucleotides (Klingenberg, 1984, 1988). It has been shown that the thermogenic effect of isoproterenol (ß-agonist) can be enhanced by a high intracellular pH (Giovannini et al., 1988). The other pathway involves the intracellular concentration of fatty acids which is regulated by the activity of hormone-sensitive lipase. Fatty acids can interact with UCP in such a way as to promote its

proton-translocating activity (Bukowiecki et al., 1986; Nicholls et al., 1986; Cunningham et al., 1986). The sensitivity of the fatty acid modulation of ion permeability is dependent on chain length, extent of unsaturation and pH (Rial et al., 1983). However, direct proof of the existence of the putative fatty acid binding site on UCP is still lacking. Fatty acids do not bind at the purine nucleotide-binding site on UCP because the effect of their removal on H^+ transport is preserved when the nucleotide binding is prevented by chemical modification (Kopecky et al., 1987).

The direct effect of noradrenaline on the blood vessels of BAT is vasoconstriction (Foster, 1986, Foster and Depocas, 1980). However, blood flow through BAT is directly related to the state of thermogenesis and increases dramatically during stimulated thermogenesis, like cold-induced nonshivering thermogenesis (Foster, 1986). Arteriovenous anastomoses are directly responsible for the control of local blood flow through lobules of BAT (Nnodim and Lever, 1988). Under basal conditions, patent arteriovenous anastomoses allow arterial blood to be partially shunted into the venous system at a precapillary level. Under stimulation, noradrenaline discharged from sympathetic nerve endings causes a constriction of regional arteriovenous anastomoses, thereby reducing shunting and enabling a greater fraction of the arterial blood supply to reach the parenchymal capillary bed. In addition, it is likely that the action of an endogenous vasodilator substance is involved (Ma and Foster 1984; Foster and Depocas, 1980).

The identity of the putative vasodilator substance, which is not noradrenaline itself, but some product of noradrenaline-stimulated thermogenesis in BAT, remains obscure. One of the sensory neuropeptides, calcitonin-gene related peptide (CGRP) is a potent vasodilator

(Brain et al., 1985, Holzer, 1988) and it is known that sensory nerves also innervate BAT in addition to the sympathetic nervous system. Hence, it is possible that CGRP is involved in the vasodilation that occurs in stimulated BAT. Whether CGRP is the unidentified substance needs to be further studied.

There are two ways, direct or indirect, to measure BAT thermogenesis. Direct measurement of BAT thermogenesis can quantitate BAT thermogenesis contribution to overall energy expenditure. At present, there is only one method to measure BAT thermogenesis directly. That is, measurement of blood flow through BAT and its oxygen consumption at the same time in conscious, unrestrained animals. But, the technique is not easy to perform and is not applicable to all the different deposits of BAT in the body.

An indirect measurement of the capacity of BAT thermogenesis is the size of the increase in metabolic rate generated by infusion of noradrenaline, which is known to stimulate BAT thermogenesis. In order to keep BAT thermogenesis at a minimum before noradrenaline is infused, animal must be at thermoneutrality and in a resting state.

Measurement of temperature of BAT itself and colonic temperature is another commonly used qualitative method for assessment of BAT function in intact animals.

As mentioned earlier, the UCP has a specific site that binds purine nucleotides and this site is probably involved in the regulation of the proton conductance pathway. However, the extent to which isolated mitochondria bind purine nucleotides is not directly related to the concentration of UCP, but to the extent to which the UCP is operating in the intact animal. Thus, rapid "unmasking" of binding sites occurs in acute exposure to cold, preceding the increase in UCP concentration (Trayhurn and Milner, 1989; Milner and Trayhurn, 1988;

Peachey et al., 1988; Desautels et al., 1978); this "unmasking" is reflected in an increase in GDP binding which persists in the isolated mitochondria. If the animals have been sufficiently stressed to stimulated sympathetic nervous system activity in their BAT, for example by acute exposure to cold, the maximum of "unmasking" of binding sites will be reached. Under this condition, the level of GDP binding relates closely to the concentration of UCP.

T_4 5'-deiodinase is other important component involved in the thermogenic and trophic response of BAT. This enzyme converts thyroxine (T_4) to triiodothyronine (T_3), providing a major source of T_3 in BAT.

1.4 Brown Adipose Tissue Growth

a. Measurement of BAT growth

In in vitro studies, many methods can be used to assess the amount of BAT present in an animal and BAT function and trophic state. The wet weight of BAT only gives a rough idea of the true size of this tissue because of wide variations in fat content. The functional indices of BAT thermogenic capacity include total protein, UCP, cytochrome oxidase (COX), DNA content and total T_4 5'-deiodinase as well as GDP binding. Measurements of UCP and COX in both tissue homogenate and isolated mitochondria are measures of mitochondrial mass. The amount of UCP reflects potential thermogenic capacity. COX is an independent marker of mitochondrial protein. Measurement of DNA content provides an index of BAT cellularity. Since only about 40% of BAT cells are brown adipocytes, interpretation of DNA results should consider other measurements like total protein and

UCP content. T_4 5'-deiodinase activity is a marker of BAT trophic response to stimulation. This enzyme is fully controlled by sympathetic nervous system. GDP binding is used as an index of the thermogenic state of BAT mitochondria.

b. Hypertrophy

BAT also exhibits a trophic response concurrent with the thermogenic response induced by chronic stimulation by cold and by a cafeteria diet. This growth results from the formation of new brown adipocytes from interstitial cells and from brown preadipocytes, as well as from the division of precursor endothelial cells of the vasculature (Bukowiecki et al., 1982, 1986; Géloën et al., 1988). Total tissue protein and mitochondrial mass increase and the composition of the mitochondria is altered. There is a specific increase in certain components involved in the thermogenic and trophic response of BAT to cold and diet: mitochondria UCP, lipoprotein lipase (LPL) and T_4 5'-deiodinase.

The increase in UCP is associated with an increase in the amount of $mRNA_{UCP}$ (Ricquier et al., 1984, 1986; Freeman et al., 1988). The synthesis of the mRNA is increased rapidly after cold exposure. It can be detected in the nucleus within 15 min (Ricquier et al., 1986; Bianco et al., 1988). Its maximum concentration is reached within 12-24 hours (Jacobsson et al., 1985; Ricquier et al., 1986). The level of mRNA encoding for UCP is also increased by a cafeteria diet (Falcou et al., 1985). A similar increase in $mRNA_{UCP}$ can be induced by noradrenaline (Bouillaud et al., 1984) and by the novel ß-agonist, BRL 26830A (Ricquier et al., 1986) suggesting that synthesis of the UCP is essentially controlled by the activity of sympathetic fibers releasing noradrenaline acting on BAT ß-receptors.

Similar to UCP, lipoprotein lipase activity is markedly increased after cold exposure. The maximum activity can be reached within 1-3 days (Carneheim et al., 1984). The effect can be mimicked by noradrenaline and by the ß-agonist isoprenaline, whereas the α-agonist phenylephrine has no effect. The ß-antagonist propranolol inhibits the cold-induced increase in lipoprotein lipase activity (Carneheim et al., 1984). It is concluded that a ß-adrenergic mechanism is responsible for the rapid recruitment of lipoprotein lipase during cold exposure. Cold-induced recruitment of lipoprotein lipase in BAT is due to increased lipoprotein lipase mRNA (Carneheim et al., 1988). In some studies, diet also increased lipoprotein lipase activity (Carneheim and Alexson, 1989).

In response to cold-exposure, there is a very large and rapid increase in T_4 5'-deiodinase activity which results in increased T_3 production within BAT. Increased synthesis of T_4 5'-deiodinase is mediated by the action of noradrenaline primarily on α_1-adrenergic receptors (Silva and Larsen, 1983, 1985). Measurable activity of this enzyme disappears completely after denervation of BAT (Park and Himms-Hagen, 1988). The increase in T_3 production is sufficient to saturate the nuclear T_3 receptors (Silva and Larsen, 1985; Bianco and Silva, 1987). The effect of T_3 is to amplify the noradrenaline-induced increase in synthesis of the $mRNA_{UCP}$ (Bianco and Silva, 1987; Silva, 1988), but, T_3 itself does not exert any effect on $mRNA_{UCP}$ in denervated BAT (Bianco et al., 1988). Thus, UCP gene is primarily regulated by noradrenaline, but it can also secondarily be controlled by T_3.

c. Atrophy

As opposed to hypertrophy, BAT atrophies when sympathetic tone to this tissue is

absent or very low. The atrophy of BAT is characterized by rapid loss of total protein, loss of mitochondrial mass, a selective decrease in the concentration of mitochondrial UCP and loss of T_4 5'-deiodinase activity and lipoprotein lipase. This atrophy is produced in associated with the reduction in sympathetic nervous system activity caused by denervation, by fasting, by lactation, by diabetes and by exercise. These different treatments produce similar patterns of atrophy as described below.

Surgical denervation of BAT reduces its total protein and total mitochondrial mass. The degree of reduction after surgical denervation depends on the environment temperature. When rats live at a warm environmental temperature (24-26°C) or at room temperature (20-22°C), surgical denervation has no or a small effect on BAT content of protein (Himms-Hagen et al., 1990; Park and Himms-Hagen, 1988; Rothwell and Stock, 1984a; Gong et al., 1990; Desautels and Dulos, 1988), UCP (Himms-Hagen et al., 1990; Géloën and Trayhurn, 1990a), $mRNA_{UCP}$ (Silva, 1988; Bianco et al., 1988), lipoprotein lipase (Bartness and Wade, 1984; Billington et al., 1987), T_4 5'-deiodinase (Himms-Hagen et al., 1990; Park and Himms-Hagen, 1988), and total mitochondrial mass (Desautels and Dulos, 1988; Himms-Hagen et al., 1990; Park and Himms-Hagen, 1988; Billington et al., 1987). The change is much more marked when previous hypertrophy has been brought about, for example, by acclimation to cold. There is then a very large and rapid loss of T_4 5'-deiodinase activity (Park and Himms-Hagen, 1988), indicating the activity of T_4 5'-deiodinase is totally dependent upon the innervation of BAT. There is selective reduction of UCP concentration in the mitochondria after a lag of about one day (Desautels et al., 1986; Park and Himms-Hagen 1988). The lipoprotein lipase activity is also decreased (Bartness and Wade 1984;

Billington et al., 1987). Mitochondrial GDP binding decreases much more rapidly than UCP concentration in the mitochodria, probably because of masking of binding sites (Park and Himms-Hagen, 1988). Denervation does not change DNA content (Desautels et al, 1986).

In the fasting state, there is loss of total protein, mitochondrial protein and succinate dehydrogenase (Desautels et al., 1986; Desautels and Dulos, 1988; Trayhurn and Jennings 1986, 1988). In some studies, a selective decrease in mitochondrial UCP concentration also occurred (Trayhurn and Jennings, 1986, 1988).

Lactation and diabetes also produce atrophy of BAT because the sympathetic nervous activity is weak. Changes in BAT include reduction of total protein, mitochondrial protein, GDP binding, mitochondrial UCP concentration and decrease enzyme activity such as T_4 5'-deiodinase and lipoprotein lipase. They do not change the DNA content of BAT.

However, the atrophy produced by return of a cold-acclimated animal to thermoneutrality is different. In this case, there is a substantial loss of DNA besides loss of mitochondrial UCP, total protein and T_4 5'-deiodinase activity (Desautels et al., 1986; LeBlanc and Diamond, 1988). The loss of cells at thermoneutrality may be due to disappearance of a maintenance factor at thermoneutrality. The nature of this factor is not known.

d. Control of Brown Adipose Tissue Growth

Sympathetic nervous system

It is generally agreed that sympathetic nerves play the major role in regulation of BAT growth. There are two major experimental approaches which provide evidence for the

predominance of the sympathetic innervation in the control of BAT thermogenesis and growth. The first approach involves the study of the effect of surgical denervation on the pattern of BAT growth in response to stimulation. The second approach involves the study of the effect of acute or chronic administration of noradrenaline on BAT (Himms-Hagen, 1989).

Surgical denervation of BAT provide the best studied example of atrophy. This atrophy reduces both BAT total metabolic mass and BAT mitochondria mass as described before. The greatest effect can be seen when the BAT has already been hypertrophied by acclimation to cold. Surgical denervation also prevents the cold-induced increase in BAT blood flow (Foster et al., 1982a) and the effect of cold-acclimation to increase the responsiveness of adenylate cyclase of BAT to noradrenaline (Granneman et al., 1985). Surgical denervation also markedly reduces growth and thermogenic activation of BAT in rats on a palatable cafeteria diet (Himms-Hagen and Park, 1984; Rothwell and Stock, 1984a).

The effect of noradrenaline administration on BAT growth includes BAT hyperplasia (Géloën et al., 1988) and an increase in the level of $mRNA_{UCP}$ (Bouillaud et al., 1984; Ricquier et al., 1984, 1986). Chronic administration results in a selective increase in UCP level in BAT mitochondria as well as hypertrophy of the BAT (Mory et al., 1984; Cunningham and Nicholls, 1987). The response is exerted mainly via ß-adrenergic receptors (Ricquier et al., 1984, 1986). Noradrenaline administration also increase the level of T_4 5'-deiodinase activity (Silva and Larsen, 1983, 1986).

However, some findings are not consistent with noradrenaline being the sole

mediator of stimulated BAT growth. Surgically denervated BAT still retains some capacity to grow in response to chronic stimulation by cold (Park and Himms-Hagen, 1988). The growth in BAT induced by cold-acclimation can not be mimicked at 100% by the effect of continuous infusion of noradrenaline (Géloën et al., 1988), particularly the increase in concentration of UCP (Desautels and Dulos, 1988). The growth that occurs in response to cafeteria feeding, and response to cold after surgical denervation of BAT is not associated with an increase in T_4 5'-deiodinase. The cold-induced increase in $mRNA_{UCP}$ synthesis is detectable in the nucleus at a very early state of cold-induced hypertrophy (Ricquier et al., 1986) whereas the increase in T_4 5'-deiodinase activity occurs only after a lag phase (Kopecky et al., 1986). It seems likely that UCP concentration and T_4 5'-deiodinase are regulated independently, but, the mechanism of this regulation is not clear. It is possible that factors other than noradrenaline could be involved in the control of the trophic response of BAT.

Hormones

As discussed above, growth of BAT is regulated mainly by activation of the sympathetic nervous system, mediated by the neurotransmitter noradrenaline. There is evidence that numerous other hormones play a permissive or modulating role in the regulation of thermogenesis in BAT by the nervous system.

Insulin plays two major roles in the control of BAT function. One is a direct effect on BAT to influence the capacity for glucose utilization. Insulin increases glucose utilization by BAT both in vivo (Cooney et al., 1985, 1987) and in vitro (Ebner et al., 1987; Saggerson

et al., 1988). In the cold exposed or acclimated state, the responsiveness of BAT to insulin is greater and more sensitive (Vallerand et al., 1987, 1990). There is a selective increase in concentration of glucose transporters in BAT in the cold acclimated state (Greco-Perotto et al., 1987a, b). A high carbohydrate diet enhances basal glucose utilization by BAT and increases its capacity to use glucose in response to insulin (Kraegen et al., 1986; Storlien et al., 1986). The other direct effect of insulin on BAT is a central effect to inhibit sympathetic nervous system activity in BAT (Sakaguchi and Bray, 1987b). In addition, specific roles of insulin in the control of BAT hypertrophy and atrophy are suggested. BAT has small thermogenic activity or is atrophied in the absence of insulin (Jamal and Saggerson, 1987, 1988a, b; Seydoux et al., 1983, 1984). For example, in streptozotocin-diabetic rats, the basal and noradrenaline-stimulated total heat production rate and GDP binding were significantly decreased. Insulin administration reversed these changes (Jamal and Saggerson, 1988a, b). Insulin also has a regulatory effect on the level of UCP and on the tissue mitochondrial protein content of BAT in both rats and mice (Géloën and Trayhurn, 1990a, b). The amount of UCP is decreased in streptozotocin-diabetic animals, insulin replacement to diabetic animals leads to a dose-dependent restoration of the protein (Géloën and Trayhurn, 1990a). Denervation of interscapular BAT inhibits the recovery of the amount of UCP in rats with different doses of insulin replacement (Géloën and Trayhurn, 1990b). This clearly suggests that regulation of the UCP by insulin involves sympathetic mediation. The involvement of insulin in the increased metabolic activity of BAT in both diet and cold-induced thermogenesis has been reported (Agius and Williamson 1981; Rothwell and Stock, 1981). Cafeteria feeding failed to produce an increase in resting metabolic rate or in the response

to noradrenaline in diabetic animals. Diabetic rats cannot maintain body temperature when exposed to cold (Rothwell and Stock, 1981). Therefore, there is an insulin requirement for BAT thermogenesis.

Thyroid hormone is required for the acute thermogenic response of BAT to ß-adrenergic stimulation (Seydoux et al., 1982a). Animals with severe hypothyroidism can not survive in cold environment and humans with hypothyroidism are cold intolerant and develop hypothermia. In addition, T_3 is also required for the initiation of the trophic response, including the large increase in synthesis of UCP that results in a selective increase in the concentration of this protein in the mitochondria (Bianco and Silva, 1988). During cold exposure, T_4 5'-deiodinase is increased rapidly, which generate T_3 from T_4. Then, increased intracellular T_3 can saturate T_3 receptors (Silva and Larsen 1985; Bianco and Silva, 1987) for the optimal expression of the BAT UCP gene (Silva, 1988; Bianco and Silva, 1987).

T_3 itself does not induce thermogenesis in BAT or synthesis of the $mRNA_{UCP}$. But, it is necessary for activation of a thermogenic response in BAT. T_3 plays a role in the enhancement of the noradrenaline-induced increase in synthesis of the $mRNA_{UCP}$ (Bianco and Silva 1987; Silva, 1988). Thus, it can be concluded that thyroid hormones act in a permissive manner in BAT metabolism (Himms-Hagen, 1989; Park et al., 1988).

Glucagon is another hormone involved in hypertrophy of BAT. The level of glucagon is markedly increased in cold exposed and cold acclimated rats and it is suggested that glucagon play an important role in control of BAT growth (Kuroshima et al., 1984). Indeed, chronic administration of glucagon to rats induces a hyperplastic growth in BAT. The

hypertrophy is associated with an increase in mitochondrial mass, protein, DNA, GDP binding and lipoprotein lipase activity (Billington et al., 1987) and can be induced by physiological doses of glucagon (Billington et al., 1991). Surgically denervated interscapular BAT retains its ability to respond to exogenous glucagon (Billington et al., 1987), suggesting that the trophic response of BAT induced by glucagon is not dependent on the sympathetic innervation of BAT.

Glucocorticoids cause suppression of BAT thermogenesis and may result in atrophy of BAT (Galpin et al., 1983; Tokuyama and Himms-Hagen, 1987). Glucocorticoids inhibit production of corticotropin-releasing factor (CRF) in the hypothalamus (Levin et al., 1988). CRF is known to stimulate activity of sympathetic nervous system in BAT (Arase et al., 1988; LeFeuvre et al., 1987). Removal of glucocorticoids, as in the rat after adrenalectomy, can improve BAT thermogenic responsiveness in old and obesity-prone rats (Fukushima et al., 1985; Marchington et al., 1986; Mercer and Trayhurn, 1986).

1.5 BAT thermogenesis in relation to thermal and energy balance.

Total energy expenditure is the sum of many different metabolic processes, occurring in different organs and regulated in different ways. The thermal balance is established by balancing facultative thermogenic mechanisms and heat loss mechanisms. An imbalance results in hypothermia or hyperthermia. Energy balance is established by balancing food intake and thermogenesis. An imbalance results in leanness or in accumulation of energy stores, i.e., in obesity. In thermal balance, body temperature is usually stringently regulated within a rather narrow range. In contrast, there is no specific mechanism for regulation of

energy balance. The size of the energy stores is dependent on separate controls of food intake and of thermogenesis.

BAT thermogenesis is a facultative component of overall thermogenesis. It plays an important role in thermoregulation during cold exposure. This fact is well documented using a blood-flow analysis (Foster and Frydman, 1978b) and the biochemical assessment of the thermogenic activity and capacity of brown adipose tissue. The diet-induced thermogenesis in BAT has also been well described. Cafeteria diet induce an increase of the UCP, $mRNA_{UCP}$, (Falcou et al., 1985) and GDP binding (Fisler et al., 1987; Marchington et al., 1986). The development of obesity has generally been ascribed to defective diet-induced thermogenesis. However, the use of blood flow measurements has resulted in the questioning of the role of BAT in energy balance during overeating. Direct measurements of the O_2 consumption of BAT in cafeteria diet-fed, hyperphagic rats have shown no greater thermogenesis in BAT of cafeteria-fed rats than control rats at either 28°C (thermoneutrality) or 24°C (room temperature) (Ma et al., 1988). Since partial hepatectomy has largely reduced the resting oxygen consumption of cafeteria-fed rats, liver as the major (70-100%) effector of the diet-induced thermogenesis of cafeteria-fed rats is suggested (Ma, et al., 1987). Nevertheless, in most cases, factors involving thermogenesis and energy balance are related to the regulation of BAT thermogenesis.

Many studies have demonstrated that BAT is in a thermogenically quiescent and relatively atrophic state in most types of obese animals. The inactivity of BAT results in a deficit in energy expenditure for thermogenesis and is believed to contribute to the high metabolic efficiency and obesity of these animals (Himms-Hagen, 1989). Two major

categories of obese animals in which BAT is deranged can be discerned among the many types studied. In the first category, thermogenesis in BAT can not be induced by diet; such animals may or may not be hyperphagic. Examples of this category include the rat with hypothalamic parasagittal knife-cuts, the rat with ventromedial hypothalamic lesions, the adult genetically obese, fa/fa, fatty rat and the genetically obese, cp/cp, corpulent rat. All of these animals are capable of activating their BAT in the cold but not of activating their BAT in response to diet. When they live in a warm environment, the lack of diet-induced thermogenesis in their BAT contributes to their high metabolic efficiency (Himms-Hagen, 1989 for review).

In the second category, the animals tend to thermoregulate at a low body temperature for all or part of time. This results in reduced energy expenditure in all organs as well as suppressed thermogenesis in BAT. These animals may or may not also exhibit defective diet-induced thermogenesis in their BAT (Himms-Hagen, 1989). Examples of the second category include the genetically obese, ob/ob, mouse, the genetically obese-diabetic, db/db, mouse and the newborn fatty, fa/fa, rat.

It should also be mentioned that the ability to regulate body temperature diminishes with age. In the rat, the metabolic capacity and the amount of lean body mass were significantly decreased with age (McDonald et al., 1987). There are age-associated decreases in ß-adrenergic receptors and adenylate cyclase activity in BAT (Scarpace et al., 1988). These biochemical alterations with age may contribute to the inability of older animals to thermoregulate when exposure to cold. However, some studies have demonstrated increased GDP binding and oxygen consumption in older rats after cold exposure. The response in

older rats is not different from that of younger rats (McDonald et al., 1987, 1989a, b). Diet-induced thermogenesis in BAT is defective in old, obesity-prone rats (Marchington et al., 1986; Rothwell and Stock, 1988). Thus, these animal can be classed into the first category.

1.6 Capsaicin and the sensory nervous system

a. Sensory Nervous System

In a recent review of the efferent function of sensory nerves, Maggi (1991) states "that capsaicin can be viewed as a new member of that club of "elected pharmacological tools" (e.g. atropine and guanethidine) which are commonly used to dissect out specific components in the response of peripheral organs to drug action." Because the principal subject of this thesis is a study in which capsaicin is used to elucidate the role of sensory nerves in BAT, a review of the function of sensory nerves and of the neuropharmacology of capsaicin is presented here.

Sensory nerves are classified as the afferent branch of the autonomic nervous system (Lefkowitz et al., 1990). The efferent branch is made up of the sympathetic outflow and the parasympathetic outflow, which supply virtually all organs of the body. Skeletal muscle, in addition, is supplied by motor somatic nerves. While sensory nerves have in the past been regarded as having a solely afferent function, as their name implies, recent evidence indicates that sensory nerves can have an efferent function that involves local release of their neurotransmitters in the innervated organ (Holzer, 1988; Maggi and Meli, 1988; Maggi, 1991; Chahl, 1988). Such a local effector role indicates that "antidromic" stimulation of the peripheral stump of transected dorsal roots or sensory nerves induces vasodilatation and

other signs of inflammation in the skin, involved in regulating blood flow, vascular permeability, trophic and immunologic processes, activity of autonomic ganglia and visceral smooth muscle.

At the light microscopic level, sensory neurons are divided into two types. (1) A-type neurons which have large light somata. (2) B-type neurons which have small dark somata. It is widely assumed that size of the cell bodies is related to the diameter of the fibers. With respect to fiber diameter, sensory neurons can be further separated into three groups: (1) large myelinated, (2) small myelinated and (3) small unmyelinated fibers. This morphological heterogeneity of afferent fibers is closely paralleled by a functional heterogeneity with regard to projection into different layers of the spinal dorsal horn, conduction velocity and sensory modality.

The cell bodies of sensory neurons are generally located in the spinal (dorsal root) or cranial sensory ganglia and send fibres in both the central and peripheral directions. The endings of the fibers may be either receptors themselves or connected to special sensory structures. Many studies using anatomical, histochemical and immunological methods have revealed the presence of a variety of peptides in sensory nerves, including substance P (SP), calcitonin-gene related peptide (CGRP), arginine vasopressin (AVP), CRF, bombesin, neurokinin A (NKA), neuropeptide K (NPK). From functional evidence, some of these substance play a mediator or transmitter role. SP was the first biologically active peptide demonstrated in primary sensory neurons (Von Euler and Gaddum 1931; Pernow 1983). SP has been implicated in the transmission of nociceptive information by unmyelinated primary afferent neurons that terminate in the dorsal portion of the spinal

cord. SP can be released by antidromic nerve stimulation from sensory nerves as an excitatory transmitter of primary sensory neurons and the mediator of antidromic vasodilatation and neurogenic plasma extravasation. SP is synthesized in dorsal root ganglia and is an 11 amino acid peptide. It is axoplasmically transported toward the terminal regions of the peripheral sensory nerve branches and is stored in nerve endings (Payan, 1989).

CGRP is another neuropeptide present in sensory nerves. It is a recently discovered 37 amino acid neuropeptide and is produced by alternative tissue-specific RNA processing of the calcitonin gene (Amara et al., 1982; Rosenfeld et al., 1983). The calcitonin gene consists of six exons, stretching over about 6.5 kilobases of DNA. The first three exons are common to both calcitonin and CGRP sequence. Exon IV contains the calcitonin-coding sequence and a sequence encoding its C-terminal flanking peptide. This is followed by an untranslated sequence, at the end of which is the usual polyadenylation signal AATAAA, used for generating calcitonin mRNA. Exon V encodes the CGRP sequence. The sixth exon, which is also part of the CGRP transcript, is not translated. It ends with an alternative polyadenylation signal ATTAAA. It has been shown that cells synthesizing calcitonin splice together exons I-IV to form mature mRNA, whereas cells which produce CGRP splice together exons I-III, V and VI. The calcitonin mRNA predominates in the thyroid C cells while the CGRP-specific mRNA appears to predominate in the nervous system (Rosenfeld et al., 1983; Zaidi et al., 1987) .

CGRP is widely distributed in both the central and peripheral nervous system. Localization of CGRP in nerves is associated with the smooth muscle of blood vessels and is seen to play a role in regulation of local blood flow (Rosenfeld et al., 1983). It has been

reported that CGRP is the most powerful vasodilator (Brain et al., 1985). In addition, CGRP was shown to act on heart (Etienne et al., 1984; Tippins et al., 1984), to inhibit gastric acid secretion in rats and dogs (Lenz et al., 1984, 1985; Tache et al., 1984), to stimulate noradrenergic sympathetic outflow if administered intracerebroventricularly (Fisher et al., 1983), and to function in nociception, ingestive behaviour and modulation of the endocrine system (Rosenfeld et al., 1983). Centrally administered CGRP appears to modulate food intake in animals (Krahn et al., 1984). Recently, it was demonstrated that plasma CGRP concentration was significantly increased in obese women and fat intake may be associated with increased CGRP secretion (Zelissen et al., 1990). The levels of CGRP both in plasma and in the thyroid gland are increased with age (Wimalawansa, 1991). The role of CGRP in these two latter cases is unknown.

SP and CGRP are two major neuropeptides in sensory nerves and they are both involved in neurogenic inflammation and thermal pain sensation (Chahl, 1988). Both SP and CGRP can be released from the sensory nerves by capsaicin treatment (Geppetti et al, 1988; Chahl, 1988; Buck and Burks, 1986). CGRP but not SP can mimic capsaicin-induced coronary vasodilation (Franco-Cereceda et al., 1987) indicating an important function for CGRP rather than SP in regulation of coronary blood flow. In some other tissue, sensory nerves are known to exert local trophic effects (Kjartansson et al., 1986; Baker, 1988; Laufer and Changeux, 1989; Denis-Donini, 1988; Ekstrom et al., 1989; Mansson et al., 1990). There is evidence that the sensory neuropeptide, SP, can stimulate DNA synthesis in cultured arterial smooth muscle cells and human skin fibroblasts (Nilsson et al., 1985). CGRP has been demonstrated to stimulate proliferation of endothelial cells in culture (Haegerstrand

et al., 1990). CGRP can also increase survival area of ischaemic tissue (Kjartansson and Dalsgaard, 1987) and regulate muscle acetylcholine receptor synthesis (New and Mudge, 1986). The functions of other peptides presented in sensory nerves are less well understood. The role of sensory nerves in BAT thermogenesis and growth is unknown.

b. Sources and Chemical Properties of capsaicin

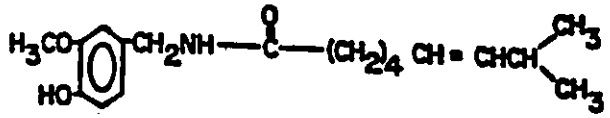
Capsaicin is the pungent ingredient in a wide variety of red peppers of the genus capsicum. Chemically, it is a derivative of vanillyl amine, 8-methyl-N-vanillyl-6-nonenamide (Fig. 1) and has a molecular weight of 305.42. The capsaicin content of hot red-pepper varieties ranges from 0.12 to 0.53%. Capsaicin is readily soluble in organic solvents such as alcohol, ether, hot carbon disulfide and fatty oils. Capsaicin is weakly acidic and contains a phenol group. It is not precipitated, or only very slightly, by the usual reagents which precipitate alkaloids. The phenol group can be methylated to form methyl capsaicin, a derivative with much less pungency than the parent compound. In addition to capsaicin, four naturally occurring derivatives have been isolated and identified (Fig.1).

c. Relationship of Capsaicin and Sensory Nerves:

Capsaicin has well documented pharmacological effects on the sensory nervous system. Effects of capsaicin on certain sensory processes was first studied by Nicholas Jancso in the late 1940s. These studies, lasting about 20 years, revealed that most of the biological effects of capsaicin resulted from an initial intense excitation of certain sensory nerves that was followed by a prolonged period of insensitivity to physicochemical stimuli, including the

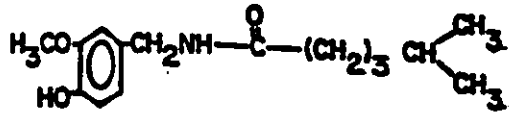
Capsaicin

(MW= 305) N-(3-methoxy-4-hydroxybenzyl)-8-methylnon trans-6-enamide



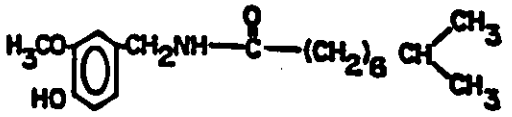
Norhydrocapsaicin

(MW=293) 7-methyl-octanoic acid vanillylamide



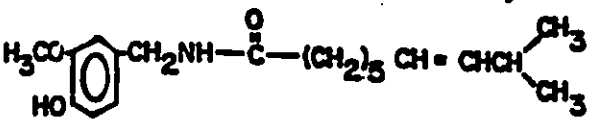
Dihydrocapsaicin

(MW=307) 8-methylnonanoic acid vanillylamide



Homocapsaicin

(MW=319) 9-methyldec-trans-7-enoic acid vanillylamide



Homodihydrocapsaicin

(MW=321) 9-methyl-decanoic acid vanillylamide

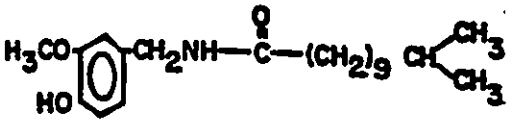


Fig. 1. Structures of naturally occurring capsaicin and its derivatives. (Modified from Masada et al, J. Food Sci.,36, 858, 1971.)

natural environmental stimuli for the sensory nerve endings (Buck and Burks, 1986). A surge of new interest in capsaicin among neuroscientists began in 1978 with the reported by Jessell, et al., (1978) that radioimmunoassay and immunohistochemical methods could measure SP. SP was reduced in the dorsal horn of the spinal cord of adult rats treated systemically with capsaicin.

Owing to its neuropharmacological effects, capsaicin has gained a special position in the investigation of primary afferent neurons. Capsaicin is now widely used as a probe for sensory neuronal mechanisms in various tissues.

Most studies with capsaicin have been carried out in small rodents like rats, mice, and guinea-pigs. It is important to note that the effects of low doses of capsaicin differ quantitatively and qualitatively from those of high doses. At low doses (in the μg/kg range) or concentrations (in the nM-μM range), capsaicin exerts a powerful excitatory effect on peripheral sensory nerve endings, this effect being apparently confined to unmyelinated afferent (C-fibres). Excitation, however, is soon followed by desensitization to the drug and by a block of nerve conduction. Systemic administration of high doses (in the mg/kg range) has a definitely neurotoxic effect on a population of sensory neurons, the extent of damage depending on the dosage, route of administration, animal species and age of the animals. The most extensive and consistent lesions are produced by the systemic treatment of newborn rats: a single dose of 50 mg/kg capsaicin results in a permanent loss, by degeneration, of 50-90% of all unmyelinated afferent fibres with no significant change in the myelinated afferent fibres (Holzer, 1988). The treatment is known as capsaicin-desensitization.

Electron microscopic examinations of rats peripheral nerves have also confirmed the loss of unmyelinated fibers from these nerves after capsaicin treatment (Holzer, 1988; Jancso et al., 1977). The loss of unmyelinated fibers is associated with the degeneration of cell bodies from the spinal and cranial sensory ganglia. This morphological change in sensory neurons is reflected by a depletion of SP and CGRP in spinal cord, sensory ganglia and peripheral targets of sensory neurons. It can also be reflected by functional deficits. Capsaicin treatment produces damage in both afferent functions and local effector functions. The former include impairment of warmth reception, thermoregulation, and nociception. The latter include impairment of vasodilatation, increase in vascular permeability, changes in the activity of cardiac and visceral muscle. Some functional deficits produced by capsaicin in the adult rat are reversible while permanent in the neonatal rat (Holzer, 1988, 1991 for reviews). Capsaicin-desensitization can also induce lesions in the central nervous system, both in neonatal (Ritter and Dinh, 1990) and adult (Ritter and Dinh, 1988) rats, in areas not known to be linked to the sensory nervous system. But, the ability of capsaicin desensitized animals to detect and discriminate food odours and taste are normal (Silver et al., 1985) although effect of capsaicin-desensitization is widespread. There is one recent report of a damaging effect of capsaicin on certain non-neuronal cells (platelets, mast cells, erythrocytes) but not on others (lymphocytes, intestinal mucosa cells) (Meddings et al., 1991). Whether it could have such an effect on brown adipocytes is not known.

The mechanism of action of capsaicin on sensory neurons is not clear. Capsaicin is a relatively lipophilic molecule, and its dissolution in lipid structures probably contributes to its action on sensory neuron membranes. The specific action of capsaicin has been

revealed by a combination of electrophysiological and ion-flux studies. The compound presumably affects membrane fluidity and/or ion permeability of the plasma membrane. There is a resulting change in the permeability such that Ca^{++} and possibly other cations stream across the membrane (Marsh et al., 1987; Bevan and Szolcsanyi, 1990; Maggi and Pierau, 1991). Ruthenium red, an inorganic dye which blocks transmembrane calcium fluxes in neural tissue, prevents capsaicin-induced neurotoxic action on sensory nerves (Maggi et al., 1988a,b; Jin et al., 1989; Maggi et al., 1989; Maggi, 1991). The protective action of ruthenium red is concentration-dependent (30nM-3μM). Ruthenium red antagonizes the action of capsaicin by blocking the release of neuropeptides from the peripheral endings of sensory nerves (Maggi et al., 1988a, b).

The early entry of Ca^{++} produced by capsaicin at the free ending of sensory nerves results in initiation of an impulse, the release of SP, CGRP and ultimately the acute painful, burning sensation associated with exposure to capsaicin (Buck and Burks, 1986). In addition to these acute effects of capsaicin on sensitive sensory neurons, the compound also results in a long-lasting plasma membrane perturbation that locks the membrane in a depolarized state or otherwise prevents subsequent depolarization from being initiated and propagated. It is this membrane effect that produces the sensory deficits in the treated animals. There is also a disruption of the sensory neuron microtubule system (Buck and Burks, 1986) and axoplasmic transport (Gamse et al., 1982) including transport of nerve growth factors (Miller et al., 1982), which renders the neuron unable to replenish the depleted peripheral and central SP, CGRP stores (Buck and Burks, 1986). Ultrastructural impairment of axonal profiles contribute to the mechanisms of capsaicin desensitization as well (Kiraly et al.,

1991).

In the present study of BAT thermogenesis, capsaicin has been used to investigate the link between sensory nerves and BAT metabolism, which are reported in this thesis.

CHAPTER 2

SUMMARY OF OBJECTIVES

The *original long-term objective* of the work described in this thesis was to study the neural control of diet-induced thermogenesis in BAT.

The *first short-term original objective* was to study cephalic phase activation of BAT thermogenesis by the use of a palatable non-nutritive sweetener (saccharin) in the drinking water. This study is described in Chapter 4.

The *second short-term original objective* was to try to enhance diet-induced thermogenesis in BAT by preventing warmth reception, and hence possible thermoregulatory suppression of BAT thermogenesis, by treatment of rats with capsaicin. This study is described in Chapter 5. However, because capsaicin-desensitization not only did NOT enhance diet-induced thermogenesis in BAT but actually induced atrophy of BAT and prevented diet-induced activation, a novel and unexpected finding, objectives were modified.

The *modified long-term objective* was to elucidate the mechanism of the unexplained atrophy of BAT in the capsaicin-desensitized rat and to study the implications of the atrophy for the animal.

The *first modified short-term objective* was to characterize further the atrophy

of BAT. The atrophy induced by capsaicin-desensitization was compared with that induced by surgical denervation, already known to induce atrophy and to prevent growth of BAT because of the removal of transmitter of the sympathetic nerves, noradrenaline, to see whether the pattern was similar. This is described in Chapter 6. The time-course of the atrophy was studied in order to find out whether it was rapid, slow, or occurred only after a lag period. In addition, other organs were assessed to find out whether the atrophy was specific to BAT. This study is described in Chapter 7.

The *second modified short-term objective* was to find out why capsaicin-desensitization induced atrophy of BAT. One hypothesis was that overheating of BAT as an initial response to capsaicin might damage the tissue. Therefore, a study of the temperature of BAT of anaesthetized rats after injection of capsaicin was done. This study is described in Chapter 8. Another hypothesis was that capsaicin-desensitization removed the trophic influence of one or more neuropeptides characteristic of the sensory nerves that innervate BAT. Therefore, one of these neuropeptides, CGRP, was infused continuously over a period of two weeks to see whether it would reverse the atrophy in capsaicin-desensitized rats. This study is described in Chapter 9.

The *third modified short-term objective* was to study the implications of the atrophy of BAT for the physiological function of the animal. Because some of the earlier studies had involved exposing capsaicin-desensitized rats to cold and showed the retention of a thermogenic and trophic response, albeit somewhat attenuated,

further studies concentrated on the implications for energy balance. Based upon the well established atrophied state of BAT in obese animals and the current belief that this atrophied state leads to a deficit in energy expenditure and thus contributes to the obesity, the hypothesis was that capsaicin-desensitized rats would be more susceptible to obesity. The initial studies had included feeding a cafeteria diet for a short period and had not disclosed any tendency of the capsaicin-desensitized rats to obesity. Therefore, a longer study was done in which a high-fat diet was fed for 10 weeks in order to provoke obesity and to see whether capsaicin-desensitized rats became any more obese than control rats. In addition, the well-known development of obesity with aging was studied in capsaicin-desensitized rats to see whether they might be more susceptible to this form of obesity. Both of these studies are described in Chapter 10.

CHAPTER 3

MATERIAL AND METHODS

3.1 Animals

Male Sprague-Dawley rats were used. They were obtained from Charles River Laboratories at 5 weeks of age. Rats were housed in individual plastic cages at 24-26°C. In cafeteria feeding and high fat diet feeding experiments, rats were maintained in wire-mesh cages individually.The light cycle was 12 hours light/ 12 hours dark with lights on at 0700 hours. They had free access to food and water. After one week under these conditions animals were weighed and randomly assigned to groups. In some experiments, rats had access to both 0.15% saccharin solution and water. Some other rats were fed with cafeteria food or a high fat diet (see 3.2 below). At the end of the experiment animals were decapitated between 07:30 and 08:30h.

3.2 Diets

1. Cafeteria Diet: In some experiments, rats were fed a palatable diet which consisted of three kinds of food: nuts, cookies and meat. The type of these ingredients were changed daily based on three different menus:

Menu I: nuts (almonds), cookies (shortbread), meat (pastrami).

Menu II: nuts (cashews), cookies (chocolate chip), meat (salami).

Menu III: nuts (walnuts), cookies (oreo), meat (hotdog).

2. High fat diet: This diet is lard-based and consisted of 63% fat, 20% protein and 17% carbohydrate. It is 5.3 kcal/g. The composition is shown as below.

The Composition of High Fat Diet

Component	percentage % (by weight)
Lard	31.0
Casein	27.0
Sucrose	20.0
Corn oil	7.0
Corn starch	3.1
Alphacel™	4.5
AIN vitamin mix	1.6
AIN mineral mix	5.2
D, L-methionine	0.3
Choline bitartrate	0.2
D, L-α-tocopherol	0.1

3. Laboratory chow: Rat chow used in the experiment was obtained from Agway company (RMH 4020). It is 3.5 kcal/g, 14.5% fat, 24% protein and 61.5% carbohydrate. In the case of cafeteria feeding or high fat diet feeding, laboratory chow was also provided to the animals at the same time. Thus, animals could choose the type of food.

3.3 Measurement of saccharin and water intake

Twenty-four hours saccharin solution (0.15%) and water intake were determined by

weighing the bottles (contained saccharin solution or water) to be given to the rats. Control group rats had access to bottles which only contained tap water. Saccharin group rats had two bottles, one containing 0.15% saccharin solution, the other containing tap water. This allowed the rats to select a highly-palatable saccharin solution or water. All bottles were weighed and given to the rats at the same time. After twenty-four hours, bottles were weighed again. The differences between the weights were used to calculate saccharin solution and/or water intake per day. The total amount of fluid drunk divided by the amount of saccharin solution drunk was referred to as percentage of preference for saccharin. Saccharin (sodium salt hydrate) was purchased from Terochem Laboratories Ltd.

3.4 Measurement of body weight and food intake

In most experiments, body weights were determined just before decapitation between 07:30 -08:30h. In some cafeteria feeding experiments and long term experiments, body weights were measured twice per week. Differences between initial body weight and final body weight are referred to as body weight gains. Changes in body weight were determined to characterize the over-all effects of the experimental manipulations. After capsaicin injection or surgery, body weights were also measured.

Food intake was determined once per week. Food to be given to the rats was weighed. After 24 hours, food remaining was weighed. In cafeteria and high fat diet feeding experiments, food intake was measured twice per week. Paper towel was placed beneath each cage to collect fragments of uneaten food that spilled out of the wire-mesh cages. Correction was made for cafeteria food which had dried in the animal room by leaving a

piece of the same food in a empty cage. Energy intake and composition (fat, protein and carbohydrate) were calculated from published analyses (Pennington, 1989).

3.5 Capsaicin-desensitization (CAP-DES)

At 6 weeks of age rats were anaesthetized with halothane and injected either with capsaicin (12.5 mg/kg s.c.) or with an equal volume of the vehicle in which the capsaicin was dissolved (saline 80%, Tween 80 10%, ethanol 10%). They remained anaesthetized for approximate 5 min following the injection. Injections of capsaicin or of vehicle were repeated twice the next day (the dose of capsaicin was 25 mg/kg s.c. each time) and once the day after (62.5 mg/kg s.c.). Rats were not anaesthetized during these later injections. Dosage and schedule are as described by Castonguay and Bellinger (1987). Capsaicin was obtained from Fluka and contained 65% capsaicin and 35% dihydrocapsaicin.

3.6 Experimental conditions

On the day of the experiment, rats were transferred in their cages to the preparation room. The rats were weighed and killed by decapitation immediately thereafter. Blood was collected immediately in 42 ml centrifuge tubes with the aid of a plastic funnel and kept on ice. Rectal temperature was then determined with a BAT-8 digital thermometer equipped with a flexible Teflon-sheathed probe inserted to a depth of 6 cm. The interscapular BAT depot was quickly removed and placed in ice cold isolation medium (0.25 sucrose (BDH), 1.0 mM N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES) (Sigma), 0.2 mM ethylenediamine tetraacetic acid (EDTA) (Sigma), potassium salt, at pH 7.2). Epididymal

and retroperitoneal white adipose tissues were removed and placed in a small plastic container until they were cleaned and weighed. In some experiments liver, kidney and heart were also removed and weighed. Tissues, blood and rat carcasses were transferred to the laboratory for further processing. Usually twelve rats were killed on each preparation day.

3.7 Preparation of serum

After animals were killed, trunk blood was collected and kept on ice for approximately 20 minutes. The samples were centrifuged at 3000 rpm, 1,500 x g at 4°C for 15 minutes. Serum was then transferred to Eppendorf centrifuge tubes and frozen in liquid nitrogen. Frozen serum was stored at -80°C for subsequent assays of hormones.

3.8 Preparation of BAT homogenates

BAT was dissected free of adhering white adipose tissue and muscle on ice. The tissue was then blotted dry, weighed and finely minced with scissors. Homogenization was done in a small volume of isolation medium using a tightly fitting Teflon plunger in a glass homogenizer. The homogenate was made to 8ml, then 600 μl samples were removed and further homogenized by hand with a glass-glass homogenizer for tissue protein, UCP, thyroxine 5'-deiodinase, DNA and cytochrome oxidase (COX) determination. The remaining BAT homogenate was used to isolate mitochondria.

3.9 Isolation of BAT mitochondria

Mitochondria were isolated by the method described by Slinde et al (1975).

Homogenates of BAT remaining after samples were taken were made up to 42 ml with isolation medium in Sorvall centrifuge tubes. Homogenates were centrifuged at 4°C for 10 min (1,500 x g) at 3000 rpm in a Sorvall RC-5B or RC-2B centrifuge with brake off. The pellets were resuspensed in 42 ml of isolation medium and centrifuged at high speed (16,000 x g) 10,000 rpm at 4°C for 15 mins. Mitochondria were washed two more times using high speed (16,000 x g) centrifugation. The final mitochondrial pellet was resuspended in 150-600 μl of isolation medium. Usually, 150-200 μl of isolation medium was used to resuspend mitochondria from capsaicin treated rats while 400-600 μl was used for vehicle treated and cold-acclimated rats. Mitochondrial suspensions were kept on ice for immediate use in the GDP-binding assay. In some experiments, mitochondrial suspensions were saved for estimation of protein, UCP and COX.

3.10 GDP Binding

The purine nucleotide binding assay was as described by Nicholls (1976) as modified by Desautels et al., (1978). I used this method to measure binding of ^{3}H-GDP to BAT mitochondria. Fresh mitochondrial suspensions (150-300μg protein) were incubated in the presence or absence of 1 mM ADP (Sigma) in a mixture which contained 1 mM EDTA (disodium salt) (Sigma), 10 mM choline chloride (Sigma), 20 mM tris(hydroxymethyl)-2-aminoethane sulfonic acid (TES) (Sigma), 100 mM sucrose (BDH), 5 μM rotenone (Sigma), 100 μM atractyloside (sodium salt) (Sigma), 0.1 μCi/ml [^{14}C] sucrose (Amersham) and 0.78 μCi/ml [^{3}H]-GDP (Amersham) with a final concentration of 10 μM GDP (Sigma), pH 7.1. ADP was present to correct for non-specific binding of GDP. [^{14}C] Sucrose was used to

estimate the amount of water trapped in the pellet. After 2 min incubation at room temperature, samples were centrifuged for 2 min at 12,000 rpm in an Eppendorf microcentrifuge (#3200). The supernatant was aspirated and mitochondrial pellets were dissolved by incubation with 1 ml NCS tissue solubilizer (Amersham) at 55°C overnight. When pellets were dissolved, 50 μl of 10% ascorbic acid (BDH) followed by 10 ml of toluene containing (2,5-diphenyloxazole) 0.7% PPO (Sigma) was used for scintillation counting. The samples were counted with a Beckman LS6800 liquid scintillation counter. The binding of ^{3}H GDP was calculated by subtracting non-specific binding from the total binding measured. Results are expressed as pmol GDP bound per mg mitochondrial protein.

3.11 Protein assay

Protein concentration was determined in BAT homogenate and BAT mitochondria suspensions using Lowry's method (1951) as modified by Schacterle and Pollack (1973). 10 μl BAT homogenate or 5 μl BAT mitochondria sample were added to 0.5 N NaOH (Fisher) to make 1 ml. Then 1 ml alkaline copper reagent was added. After 10 min incubation at room temperature, 4 ml Folin's phenol solution was added and mixed. All sample tubes were placed into a waterbath (55°C) for 5 min. Then samples were removed and kept on ice. The absorbance was read at 650 nm with a Bausch and Lomb Spectronic 20. A series of standards (15-120 μg) were prepared for each assay from a stock solution of 10 mg/ml bovine serum albumin (BSA)(Sigma). Protein concentration was calculated from the standard curve. Each sample was done in duplicate.

3.12 Uncoupling protein solid phase radioimmunoassay (RIA)

BAT homogenate and BAT mitochondrial UCP were determined using a solid phase RIA. The assay was developed based on Lean's method (1983). Some modifications were made in this lab.

UCP purification

Uncoupling protein (UCP) was derived from cold-acclimated rat BAT mitochondria. Purification of UCP was performed by a procedure based on the method of Lin and Klingenberg (1980, 1982) except that the sucrose density gradient centrifugation step was omitted. The method is outlined as follows:

BAT mitochondria were isolated by differential centrifugation in isolation medium (see 3.9 above). The final pellet was resuspended at a concentration of 1 mg protein/ml in a "standard buffer" (20 mM 3-(N-morpholino) propane sulfonic acid (MOPS) (Sigma), 1 mM EDTA, 20 mM Na_2SO_4 (Analar), pH 6.7) frozen in liquid nitrogen, and stored at -80°C.

Next day, the suspension was incubated with 3.2% Lubrol WX for 30 minutes at 0°C to remove soluble and peripheral membrane proteins. This suspension was centrifuged at 100,000 x g for 30 minutes at 4°C, then the pellet was washed once in ice-cold sucrose medium (300 mM sucrose, 10 mM tris (hydroxymethy) aminomethane (Tris) (Sigma), 2 mM EDTA, pH 7.2) and resuspended again in standard buffer containing 5% Triton X-100 (BDH). After 30 minute incubation at 0°C, the suspension was centrifuged (100,000 x g). The supernatant contained UCP-Triton micelles.

The major step of UCP purification is chromatography on hydroxylapatite. The UCP-

Triton extract was applied to a 3x10 cm hydroxylapatite column that had been equilibrated with standard buffer. The pass-through fractions were collected at room temperature and UCP peak was determined by a modified method of Lowry (Peterson, 1977) in the presence of 1% SDS (sodium dodecyl sulfate) (Bio-Rad). The peak fractions were then pooled. The major contaminant, the ADP/ATP carrier protein, is not stable at room temperature and is adsorbed onto the hydroxylapatite. The amount of Triton in the purified UCP was determined spectrophotometrically at 276 nm using Triton solution as standards.

The purity of the UCP was examined using SDS-polyacrylamide gel electrophoresis. It was done two ways at the same time. One gel was stained with 0.05% Coomassie Blue (R-250, Bio-Rad) and the other was electrophoretically transferred to nitrocellulose membrane (0.2-0.4 pore size, Bio-Rad). The transfer was run in the 4°C cold room at 60V for 3 hours with mixing using a magnetic stirrer. The nitrocellulose membrane was washed in PBS (phosphate buffered saline containing 5 mM K_2HPO_4 (BDH), 1.5 mM KH_2PO_4 (BDH) 137mM NaCI (BDH), 2.7 mM KCI (BDH) pH 7.4)+0.05% Tween 20 for 30 minutes at room temperature in a shaking water bath. Nitrocellulose membrane was then transferred to 100 ml PBS-Tween containing anti-hamster UCP antibody and incubated for 2 hours at room temperature in a shaking waterbath. After 4x30 min washes of 300 ml PBS-Tween, the nitrocellulose membrane was incubated in 100 ml PBS-Tween containing 100,000 cpm/ml [^{125}I] protein A (ICN Radiochemicals 30-70 μCi/μg) for 1 hour at room temperature in a shaking water bath, then washed with 300 ml PBS-Tween three times for 30 minutes and a fourth time overnight. The blot was dried between sheets of filter paper and subjected to autoradiography overnight at -80°C using Kodak X-ray film. Both the stained gel and the

western blot showed only one band. Purified UCP was used for UCP solid phase radioimmunoassay.

Preparation of UCP Antibody

Hamster UCP antibodies were raised in rabbits as described by Fernandez et al (1987).

Pre-immune serum was collected from rabbits before they were immunized. Next day, rabbits were injected with 100 μg of purified UCP with Freund's complete adjuvant in 2 intramuscular and 2 subcutaneous sites. The following week 100 μg of UCP with Freund's incomplete adjuvant was injected subcutaneously at several sites in the back. After 1 week, a booster injection was given by injecting 100 μg UCP in a total volume of 500 μl of saline intravenously via the ear vein. The following week the rabbits were bled into polycarbonate tubes and the blood was left on ice at 4°C overnight. On the second day, the blood was centrifuged at 2500 x g for 30 minutes. Supernatant collected was centrifuged again at the same speed for 15 minutes. The serum was then diluted 1:1 with glycerol and stored at -80°C. Further dilution of antibody was needed for UCP determination.

UCP Assay

In order to increase protein binding to the plates and reduce variability in binding, 96-well microtitre plates were washed to remove residues from the manufacturing process. The plates were soaked in Pierce RBS 35 detergent diluted with hot tap water for 2 hours. Then plates were rinsed 6 times with cold tap water followed 3 times with distilled water.

After drying at room temperature, plates were ready to use.

Assay plates were coated with rat UCP at 10μg/ml with 50μl/well. Non-specific binding was estimated with PBS+0.1% Triton. The plates were incubated at 25°C for 2 hours. Then solution in the plates was removed by "slapping" on 5 layers of Kleenex tissue until they were dried. The plates were washed with 200μl PBS+1% BSA+0.1% sodium azide (BDH) twice using a multipipetter. During a third washing, the plates were incubated at 25°C for 10 mins. This step is to block sites not bound to UCP. Then plates were washed 3 times more with PBS+1% BSA+0.1% sodium azide, slapping well to dry plates between each wash. After the last wash plates were allowed to drain in the inverted position for at least 5 min.

BAT homogenate and BAT mitochondria were prepared as described above. If protein concentration was higher than 5mg/ml, samples were diluted to 2mg/ml with PBS first. During the 2 hours coating incubation described above, samples were extracted, taking 12.5μl samples and 5μl of PBS containing 5% Triton into Eppendorf centrifugation tubes which contain 32.5 μl PBS to make 50μl. After 30 min incubation at room temperature, 200μl PBS was added to the samples, and they were mixed and centrifuged at maximum speed for 2 minutes in an Eppendorf microcentrifuge (#3200). The supernatants were decanted and kept on the ice. The supernatants were further diluted to give a final protein concentration of 5μg/ml. 40μl of 5μg/ml containing 0.2 μg original protein was used for each well in the assay. If protein concentrations were lower than 5mg/ml, samples were diluted to 0.5 mg/ml with PBS buffer. The remaining steps were the same as described above and 0.2 μg original protein was used for each well.

A standard curve was prepared for each plate. The standard solutions were made from purified rat UCP (382 μg/ml) in the range 0.1-2.38 μg/ml. Each standard and samples were assayed in triplicate. Some wells were used for total and non-specific binding. 40 μl of samples, each standard or PBS+0.1% Triton (for non-specific binding) were added to the wells. Then 10 μl of rabbit anti-hamster UCP antiserum (diluted 1: 1600 in PBS) was added to all wells. The plates were covered with parafilm and incubated at 4°C overnight.

The next day plates were washed 5 times with PBS+1%BSA+0.1% sodium azide as the day before (twice with the multipipetter and 3 times with a siphon). ^{125}I-protein A (ICN specific activity 30-70 μCi/ug) was diluted in PBS. 50 μl of ^{125}I-protein A (60000-70000 cpm/well) was added to all wells. After incubation for 1.5 hours at room temperature, plates were washed another 5 times with the same solution. The plates were dried in air and the wells cut out into 12x75 mm plastic tubes and counted in a gamma counter (Beckman #5500). Results expressed as μg UCP/mg protein in homogenate or in mitochondria or as μg UCP per total tissue. Calculations were made using an IBM Lotus 1-2-3 package.

3.13 Measurement of DNA

DNA content was measured in BAT, heart, kidney and liver homogenates. The method was adapted from Downs and Wilfinger (1983). Homogenate containing 5mg tissue was sonicated (15sec) and added into 1 ml AT (ammonium hydroxide-Triton X-100) extraction solution (1N NH_4OH (BDH), 0.2% Triton x-100 (BDH)). Samples were incubated in a waterbath at 37°C for 10 min. Then samples were further diluted to 1 mg/ml tissue homogenate with assay buffer (100mM NaCl (BDH), 10mM EDTA, 10mM Tris Base

(Sigma)). Then samples were centrifuged using Beckman J6B 2500 x g at 4°C for 30 minutes. 100 μl supernatant was placed into a disposable cuvette (Sarstedt) which contained 3 ml Hoechst 33258 buffer (2mM EDTA, 50 mM Na_2HPO_4 (BDH), 2M NaCl and 1 μg/ml Hoechst 33258 dye (Sigma)). DNA standard curve (range from 0.5-5μg) was performed each time. Concentration of DNA (Sigma calf thymus type I) stock solution was 0.5 mg/ml. Before each assay, 200 μl of DNA stock solution was added into 1 ml AT solution and diluted with assay buffer to give final DNA concentration 50 μg/ml. Both the standard and the unknown samples were placed on ice and then measured by spectrofluorometer (Turner Model 430) with the excitation and emission wavelengths set at 350 and 455 nm. Standard DNA concentrations were verified by spectrophotometry (Beckman DU-50) at 260nm. DNA contents of samples were determined in duplicate.

3.14 Measurement of cytochrome oxidase (COX)

COX was determined in BAT homogenates and BAT mitochondria using a spectrophotometric assay. The principle of this assay is that COX oxidizes its substrate, reduced cytochrome c, and this can be measured through the rate of disappearance of reduced cytochrome c. Both BAT homogenate and BAT mitochondria samples were prepared as described above and stored in -80°C refrigerator. On the day of the assay, samples were diluted to a protein concentration of 0.5 mg/ml with 0.1M phosphate buffer (K_2HPO_4) (BDH), pH 7.0 and then treated with Lubrol WX 0.3% (1 μl Lubrol/μg protein) and kept on ice for 1.5 hours. During this time, fresh 200 μM cytochrome c (Sigma, horse heart type III) solution was made. Cytochrome c was dissolved in 10% ascorbic acid (BDH)

in phosphate buffer. Rate of reaction was linear up to 16 μg protein in both BAT homogenate and mitochondria samples. The other homogenates (heart, kidney and liver) can be used up to 80 μg protein. 8 μg protein was used for each BAT sample while 50-60 μg heart, kidney and liver protein were used for the assay. Samples were assayed in duplicate with 3 different concentration of cytochrome c (20, 30 and 40 μM) in order to obtain a Lineweaver-Burk plot and get Vmax for each sample. Samples were added into disposable spectrophotometric cuvettes (Sarstedt) containing 3 ml phosphate buffer with the different concentrations of reduced cytochrome c. Absorbency was measured immediately for 2 mins at 550 nm in a spectrophotometer (Beckman DU-50) using the kinetic soft-pac module (program kindata). The Lineweaver-Burk plot was done by plotting the reciprocal of the initial velocity vs the reciprocal of the substrate concentration, used to determine the initial velocity and calculate the Vmax.

3.15 Measurement of thyroxine 5'-deiodinase (T_4 5'D)

The method used is based on the release of free iodide from T_4 by the enzyme T_4 5'D. Some modifications were made to the basic method (Visser et al., 1982; Leonard et al., 1983; Silva and Larsen, 1983).

Substrate, ^{125}I-T4 (Amersham) (> 1200 μCi/μg) was purified first since autoradiolysis occurs spontaneously. Purification was done according to Larsen's method. A column of 2 ml was made with LH-20 Sephadex (bead size:25-100μ) (Sigma) and washed 5 times with water and 2 times with 0.01M potassium phosphate buffer pH 7.0. Then ^{125}I-T_4 was diluted 1:5 in potassium phosphate buffer and added onto the column. The eluate collected in test

tubes contained free ^{125}I and was discarded. Column was washed 2 times with 2 ml distilled water. The pure ^{125}I-T_4 was obtained by adding 2 ml ethanol:H_2O (70:30) to the column and collecting the eluate. The solution was concentrated by gently blowing nitrogen gas into the tube.

Samples of homogenate (stored at -80°C) were diluted to a protein concentration of 1mg/ml. 50 μl sample was incubated with assay mixture (10 mM dithiothreitol (Sigma), 2.57nM unlabelled T_4 (Sigma), 0.15 nM ^{125}I T_4 and 1 nM 6-propyl-2 thiouracil (PTU) (Sigma) in 150 μl of 0.1 M potassium phosphate-1.0 mM EDTA buffer, pH 7.0. The reaction was stopped by the addition of thyroid hormone-free normal human serum (AMF Biological Diagnostics Co.) which contains T_4 and T_3 binding proteins. Next, 350 μl 12.5% trichoroacetic acid (TCA) was added to precipitate T_4-and T_3-protein complexes and all other proteins. Samples were centrifuged at maximum speed for 2 mins in a bench top Eppendorf centrifuge (#3200). 500 μl supernatant was passed through a column of 2.5 ml containing Dowex AG 50 W-X2 100-200 mesh cation exchange resin (Bio-Rad) in acetic acid:H_2O (1:10) which bound any excess ^{125}I-T_4 that might not have been bound by the protein and the column was washed 3 times with 1 ml acetic acid:H_2O. The total eluate containing free iodide was counted for 2 mins in a Beckman gamma 5500 counter. Assay was done in triplicate for all samples.

3.16 Measurement of serum thyroid hormones

Larsen's method (1976) was used to determine T_4 and T_3 in the serum. Both T_4 and T_3 standard solutions were prepared by diluting a solution made of 40μg/ml T_4 (Sigma) or

40μg/ml T_3 (Sigma) in 0.05 N NaoH+0.1% BSA (Sigma, RIA grade) in thyroid hormone-free rat serum. The solutions of T_4 and T_3 were standardized by reading the absorption at 325 nm for T_4 and 320 nm for T_3 in a Gilford 2400-2 spectrophotometer. Concentration of T_4 or T_3 was calculated by knowing the molar extinction coefficient (T_4 = 6207, T_3 = 651.01). Concentration of T_4 = A325/6207 x 776.93. Concentration of T_3 = A320/4658 x 651.01. The range of standards covered was 0.25-8.00 μg/dl for T_4 and 10-300 ng/dl for T_3.

Thyroid hormone free rat serum was prepared by incubating the serum with 50000 cpm/ml $^{125}T_4$ or $^{125}T_3$ (Amersham) and 0.023g/ml Norit A Charcoal (Bio-Rad). The mixture was constantly stirred overnight at 4°C and centrifuged at 100,000g for 30 minutes at 4°C in a Beckman L8-55M ultracentrifuge with a Ti55 rotor. A sample, 100μl serum, was counted before and after stripping of hormones. When serum contained less than 5% of the counts that were put in at the beginning, it could be considered free of thyroid hormones. Thyroid hormone free serum was stored at -80°C until needed.

Antisera for T_4 and for T_3 were provided by Dr. Larsen. For T_4 determination, 5μl sample serum or each standard with 95 μl working buffer (glycine-acetate buffer pH 8.6) were added into 900 μl assay buffer (0.2 M glycine (Sigma), 0.2 M sodium acetate (BDH Analar), 0.02g/ml sodium salicylate (Fisher), 0.2 mg/ml BSA (Sigma, RIA grade)) which contain ^{125}I-T_4 (Amersham) and T_4 antibody (1:10000 dilution). After incubation at 4°C overnight, antibody-bound T_4 was separated from free T_4 by adding 1 ml of suspension of 0.04% Norit A charcoals (Bio-Rad) and 0.004% dextran T 70 (Sigma). For the total count, tubes contained 900 μl assay buffer only and non-specific binding was performed at same time. All tubes were incubated 45 min at 4°C and centrifuged at 1500 x g for 20 min in a

Beckman J6B centrifuge. Supernatants were transferred to new tubes and counted for 2 minutes in a Beckman gamma 5500 counter. For the T_3 determination, a similar procedure was followed, but the amount of samples measured, antibody diluted and incubation time were different. 15 μl samples were used and optimum dilution of T_3 antibody was 1:25000. Samples were incubated with T_3 antibody at 4°C for 24 hours. The ^{125}I T_3 (Amersham) was added and samples incubated again at 4°C for another 24 hours. Calculations of both T_4 and T_3 were made using linear regression from an IBM Lotus 1-2-3 package.

3.17 Denervation of interscapular BAT

Denervation was performed unilaterally. The rats were anaesthetized lightly with halothane and fur was cut off in an area of about 4x3 cm in the interscapular region. Incision of 1.5-2 cm in the skin was made and the tissue was carefully separated with forceps to expose interscapular BAT. By blunt dissection, the right side of the BAT pad was lifted and the five major nerves innervating the BAT could be seen. The five nerves were cut with surgical scissors and the BAT pad replaced to the normal position. The left side of the BAT pad remain intact as a control. Incisions were closed with surgical clips. Rats were kept warm until they awakened. After returning to animal room, all rats had normal food intake and body weight.

3.18 Implantation of mini pumps into rats

Mini osmotic pumps were obtained from Alzet (200 μl, 0.5 μl/h #2002). Pumps with cannulas needed for implantation were filled on the day before surgery. The procedure of

preparation followed the instruction providing by Alzet exactly. On the day of the surgery, animals were anaesthetized using halothane and the skin in the back was cleaned. Then a 1.5-2 cm horizontal incision was made and a small pocket was made in the connecting tissue beneath the skin of the flank. The mini pump was implanted into this pocket with cannula directed towards the interscapular BAT. Half of the animals had pumps containing CGRP as treatment. The other half of the animals had pumps filled with saline as control. All animals recovered within one day after operation. The CGRP dose used was $3x10^{-10}$ mol/kg/min, which has been shown to reduce the mean arterial pressure of rats significantly (Marshall et al, 1987). The advantage of the mini-osmotic pump is that it can deliver substance continuously and maintain the concentration of CGRP in BAT at continuously high levels.

3.19 Measurement of oxygen consumption

Oxygen consumption was measured with the open circuit system previously described by Foster (1974). The system consisted of (a) either a small glass hood fitted over the head of an anaesthetized rat or a water-jacketed metabolic chamber with an internal volume of 1.5 L in which an unanaesthetized rat was placed, (b) a Beckman Model 755 oxygen analyzer, (c) air drying system including both drying air and removing CO_2, (mixtures used for drying air: magnesium perchlorate/Drierite 10-20 mesh indicating and for removing CO_2: soda lime 6-12 mesh/Ascarite II 8-12 mesh), (d) an air pump and (e) flowmeter and a recording barometer. The temperature of the water circulated through the jacket was maintained at 28°C. Fresh dried air was drawn into the chamber and then, after water and

CO_2 were removed, drawn through the oxygen analyzer. Air flow was regulated at 1000 ml per minute with a mass flowmeter (Brooks Instruments Division, Emerson Electric. Co.). Data recording and calculation were done by the computer connected with this equipment and expressed as ml O_2 per minute. Usually 2 rats were studied per day between 0900 and 1300h. The resting value of VO_2 is obtained from the O_2 consumption recorded over a period of 60 min after the rat is accustomed to the measurement condition (45-60 min). Results are expressed in terms of total body weight, per 100g of body weight, per $100cm^2$ of body surface (12.5 x body weight$^{0.60}$) (Lee, 1929) and in terms of metabolic mass ($kg^{0.75}$).

For the measurement of the acute effect of capsaicin injection, rats were anaesthetized with sodium pentobarbital (50 mg/kg sc). Thermistor probes were placed underneath the interscapular BAT (YSI probe 427), through a incision in the skin above the scapulae, and inserted 6 cm into the rectum (two YSI 402 probes). Temperature of the body was controlled at 36^0C with a YSI proportional temperature controller (model 72) with heat supplied by two 60-W incandescent light bulbs, one above and one below the rat, which was lying on a open-mesh grid. Rectal and BAT temperature were monitored at 1-5 minute intervals using a YSI telethermometer (model 43) and a YSI switch box (model 4002). The rat's head was inserted into a small glass hood through which dried room air was draw at 1000 ml per minute. Pentobarbital injections were repeated, approximately hourly, for the duration of the experimental period (up to 4 hours). Data recording and calculation were made by the computer.

3.20 Preparation of heart, kidney and liver homogenates

After BAT was dissected, heart, kidney and liver were removed from the carcass and cleaned, weighed and stored at -80°C. Later, these organs were homogenized in isolation medium using a Polytron (PT 10/30). The homogenates were made to 25 ml, 50 ml and 100 ml for heart, kidney and liver respectively. Only a small portion (2 ml) of each organ homogenate was kept. These samples were stored in a -80°C refrigerator until needed for the assays.

3.21 Body composition analysis

(1) EM-SCAN analyzer

EM-SCAN is a recently developed small research animal body composition analyzer (EM-SCAN^{INC}, Springfield, IL, 62707, U.S.A.). It can be used to estimate fat content of small rodents by measuring total body electrical conductivity (TOBEC). This technique is based on the widely differing electrical conductivities of lean tissue and body fat. The electrical conductivity of fat is only about 4-5% that of lean tissue, body fluid and bone (Walsberg, 1988). The TOBEC uses an electromagnetic coil surrounding the measurement chamber to induce a current throughout the body. The magnitude of the current is proportional to the animal's lean body mass. Body fat content is calculated from the difference between the lean body mass and body weight.

Each rat was anaesthetized with sodium pentobarbital (35 mg/100g sc) and placed in the measurement chamber of EM-SCAN^{TM} SA-1 analyzer. The reading appeared on the screen immediately. Readings were obtained in triplicate for each rat and used to calculate the lean body mass (Walsberg, 1988). To minimize measurement error, the rat was always

kept in the prone position and in the centre of the cylinder chamber. It took about two minutes to finish the measurements for each rat. Body weights of rats were 350-450 g.

(2) Carcass lipid extraction

Rats were killed by decapitation and BAT and organs (heart, kidney and liver) were removed for later studies. The remaining carcass was then stored at -20°C. For lipid extraction, each carcass, excluding tail, paws and skin, was homogenized in distilled water, using a Polytron (Brinkman) PT 45 with PT 45/2k generator. Homogenate was mixed well and 25 ml used for each extraction. Samples were placed in cellulose thimbles, dried, weighed and extracted in duplicate with 250 ml petroleum ether (35-60°C fraction) using a soxhlet apparatus. After 3 hours extraction, thimbles containing fat-free samples were dried and weighed. The remaining solvent was evaporated and extracted lipid was weighed. The amount of fat in 25 ml homogenate was obtained by averaging loss of fat from samples and extracted lipid weights. The total lipid content of each carcass was calculation from the volume of the total homogenate.

3.22 Statistical analysis of the results

Experimental data was analyzed using a SAS statistics program on the main frame computer at University of Ottawa. One way or two way ANOVA was performed as appropriate. In some case, student's t-test was also performed. Differences were considered to be statistically significant when $P < 0.05$.

CHAPTER 4

ACTIVATION OF BROWN ADIPOSE TISSUE BY SACCHARIN

Background:

The thermogenic effect of food has two parts: an obligatory increase in heat production associated with the absorption, processing and storage of the components of the food itself and a facultative increase associated with activation of the sympathetic nervous system. The latter can be divided into a cephalic phase, and an absorptive phase. The cephalic phase is primarily caused by gustatory, olfactory and cognitive stimulation and is considerably reduced when food is given by gavage. The heat production during the absorptive phase is directly proportional to the amount of food ingested (Diamond et al., 1985; LeBlanc and Diamond, 1986). Numerous studies performed in humans and animals have indicated an important cephalic activation of the sympathetic nervous system following food intake that is of importance in energy balance. Cephalic phase thermogenesis can be avoided by tube-feeding the animals (Rothwell and Stock, 1984) and inhibited by atropine treatment (Diamond and LeBlanc, 1987). It has been suggested that the palatability of cafeteria diets is at least in part responsible for the activation of BAT thermogenesis in rats (Allard and LeBlanc, 1988; LeBlanc et al., 1986) and mice (Ciriggio et al., 1991).

Saccharin is a non-nutritive artificial sweetener. Saccharin solution has been used to study various aspects of ingestive behaviour in animals for many years. It has been shown that rats prefer to drink a highly-palatable saccharin solution (Nissenbaum and Sclafani, 1987) and that saccharin can elicit cephalic-phase insulin secretion which is regulated by

autonomic nervous system (Berthoud et al., 1981). This insulin response occurs in the absence of any significant change of glycemia (Berthoud et al., 1981). Because the cephalic responses represent the earliest physiological reactions when an individual is exposed to food and may be of importance in energy balance, it is important to know how cephalic phase thermogenesis occur. BAT is a major thermogenic organ and its functional status is directly related to thermal and energy balances. Whether saccharin induces a cephalic phase thermogenic response in BAT is unknown.

Objectives:

The objective of the experiment was to find out whether drinking saccharin solution would activate BAT thermogenesis and have any effect on body weight gain. Saccharin was chosen as the non-nutritive sweetener because rats do not seem to taste the more commonly used aspartame in human diets as sweet (Sclafani and Abrams, 1986).

Methods:

Sprague-Dawley male rats arrived at 5 weeks of age. They were housed in individual plastic cages with free access to chow and water and a 12 hour light/dark cycle. The room temperature was 24°C. After 1 week, rats were divided into two groups. Half of the animals were allowed to access to both tap water and 0.15% saccharin solution for 1, 3, 7, 35 days and 3 months. The another half of the animals were given tap water only.

Twenty-four hour fluid intake of all rats was measured twice a week by weighing both kinds of bottles before and after 24 hours. The difference gave fluid intake per day.

Saccharin preference was assessed by calculating the percentage of saccharin solution intake of total fluid intake. Food intake and body weight were also measured along with fluid intake measurements. At selected times, rats were killed by decapitation between 07:30 and 08:30 hours. After rats were killed, rectal temperature was measured and blood was collected immediately. The epididymal white fat was removed and weighed. The interscapular BAT depot was quickly removed, cleaned, weighed and homogenized. Samples of homogenate were taken and stored at -80°C for protein and T_4 5'-deiodinase assay. The remaining homogenate was used to isolate mitochondria for GDP binding assay. The method used was exactly as described in chapter 3 (3.9). Each sample was assayed in duplicate for protein and in triplicate for T_4 5'-deiodinase and GDP binding. Data was analyzed by student t-test in this experiment. A significant difference was indicated when $P<0.05$.

Results:

After 1, 3, 7, 35 days and 3 months of drinking 0.15% saccharin solution, there was no effect of saccharin on body weight, body temperature, BAT weight and white fat weight (Table 1). Both saccharin and control group rats had a normal food intake (Table 2). Control rats drank 35-50 ml water per day while saccharin group rats drank much less water (Table 2). Total fluid intake of saccharin rats was 80-90 ml, twice of that of control rats. These rats selected 80-90% of their total fluid intake as saccharin solution (Table 2). After 3 and 7 days of saccharin intake, BAT mitochondrial GDP binding was significantly increased when compared with control rats (Fig. 2). After 35 days of saccharin, BAT

TABLE 1
EFFECT OF SACCHARIN ON BODY WEIGHT, BODY TEMPERATURE, BROWN ADIPOSE TISSUE WEIGHT AND WHITE FAT WEIGHT AT VARIOUS TIMES

	1 day		3 days		7 days		35 days		84 days	
	water (30)	sacc (30)	water (6)	sacc (6)	water (5)	sacc (5)	water (6)	sacc (6)	Water (8)	Sacc. (8)
Body Weight(g)	243.9 ±3.2	243.3 ±0.27	218.6 ±3.8	220.0 ±4.8	251.0 ±3.4	256.0 ±1.7	440.5 ±9.0	445.8 ±13.2	571.4 ±13.9	591.7 ±3.1
Body Temperature	37.1 ±0.07	37.1 ±0.08	37.2 ±0.3	37.5 ±0.06	37.2 ±0.2	37.2 ±0.2	37.9 ±0.2	37.8 ±0.2	37.3 ±0.2	37.2 ±0.1
WAT (g)	1.77 ±0.07	1.78 ±0.07	1.30 ±0.1	1.40 ±0.1	---	---	5.61 ±0.3	5.97 ±0.9	13.5 ±1.4	11.8 ±1.0
BAT(mg)	339.8 ±13.2	325.6 ±12.6	257.2 ±12.8	306.8 ±22.6	316.7 ±15.9	271.9 ±18.1	695.5 ±36.9	649.7 ±40.4	1146.8 ±94.8	1200.7 ±62.5

TABLE 2
FOOD INTAKE, WATER INTAKE AND SACCHARIN INTAKE AT VARIOUS TIMES

	1 day		3 days		7 days		35 days		84 days	
	water (30)	sacc (30)	water (6)	sacc (6)	water (5)	sacc (5)	water (6)	sacc (6)	Water (8)	Sacc. (8)
food intake (g/day)	22.7 ±0.9	24.4 ±1.5	22.8 ±0.5	24.0 ±0.7	22.4 ±0.7	23.0 ±0.5	27.3 ±0.7	27.9 ±0.9	28.8 ±1.0	31.2 ±0.5
water intake (ml/day)	40.2 ±1.9	9.1* ±1.2	36.5 ±0.7	16.6* ±8.4	37.5 ±2.2	8.1* ±2.2	44.1 ±3.1	8.7* ±1.4	50.7 ±1.4	6.6* ±0.5
sacc. intake (ml/day)	---	70.3 ±6.6	---	86.0 ±9.0	---	57.7 ±12.9	---	84.0 ±10.0	---	88.0 ±8.0
% sacc.	---	84.9 ±2.86	---	84.8 ±6.32	---	84.8 ±5.89	---	90.6 ±1.22	---	93.0 ±0.67

*Significant difference between control and saccharin rats, comparing animals studied at the same time.

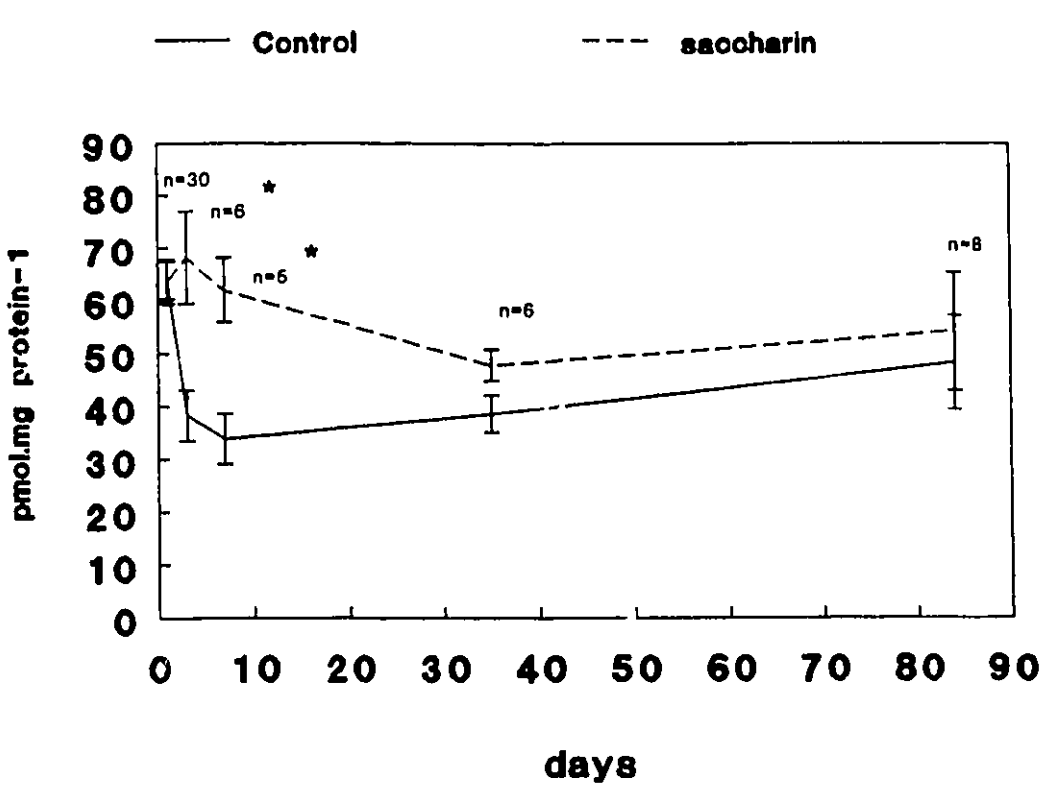


Fig. 2 GDP binding of BAT mitochondria after 1, 3, 7, 35 and 84 days of drinking 0.15% saccharin solution.

Values are means±SEM for the number (n) of rats per group. The symbol * indicates a significant effect of saccharin. A significant difference is indicated when $p<0.05$.

mitochondrial GDP binding was no longer increased. 3 months later, GDP binding also remained unchanged. There was no effect of saccharin on BAT mitochondrial GDP binding at 1 day since GDP binding in control rats was relatively high (Fig. 2). Protein content of BAT did not change throughout the experiment (Fig. 3). T_4 5'-deiodinase activity (Fig. 4) and serum T_4 (Fig. 5), T_3 (Fig. 6) concentrations did not show any significant difference between the two groups at 3, 35 and 84 days after drinking saccharin solution.

Discussion:

Diet-induced thermogenesis in BAT is associated with an increase in the activity of the mitochondrial proton conductance pathway, which can be assessed from GDP binding. GDP binding is an index of the thermogenic state of BAT mitochondria. It varies directly with the extent to which the UCP is operating, independently of any changes in UCP concentration (Peachey et al., 1988), which usually take a much longer period to occur. The major finding from this experiment is that saccharin has an acute stimulatory effect on BAT thermogenesis at 3 and 7 days, as indicated by increased GDP binding after drinking saccharin. Saccharin, unlike glucose ingestion, does not create a postabsorptive state. It can be used to stimulate cephalic phase thermogenesis. In this experiment, rats showed a high saccharin preference. They selected 80-90% of their total fluid intake as saccharin. This finding is consistent with other observations (Nissenbaum and Sclafani, 1987). It can be suggested that the palatability of saccharin plays a role in the early postprandial thermogenesis, associated with sensory feeding inputs. Anatomical studies have showed that hypothalamic neurons innervate brain stem regions which receive afferent gustatory

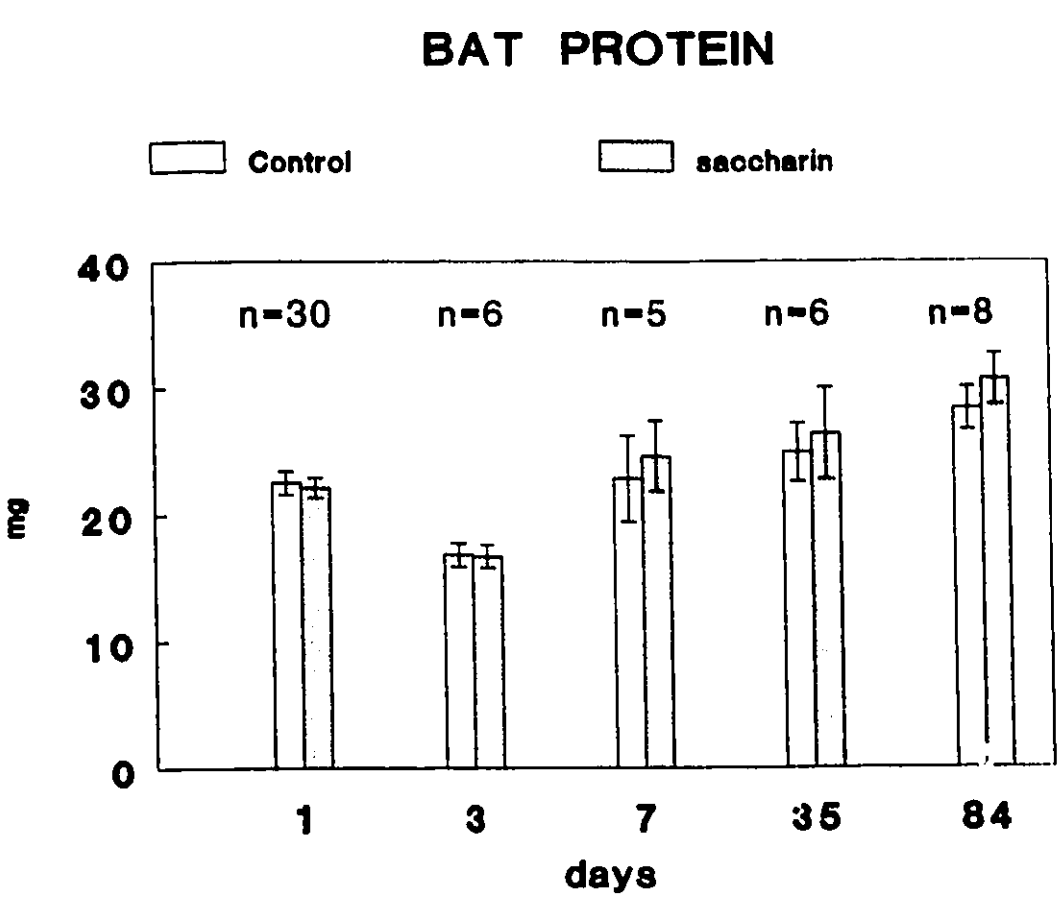


Fig. 3 BAT protein content after drinking saccharin solution for various times.

Values are means±SEM for the number (n) of rats per group. Statistical analyses indicate that there was no significant effect of saccharin on BAT protein content.

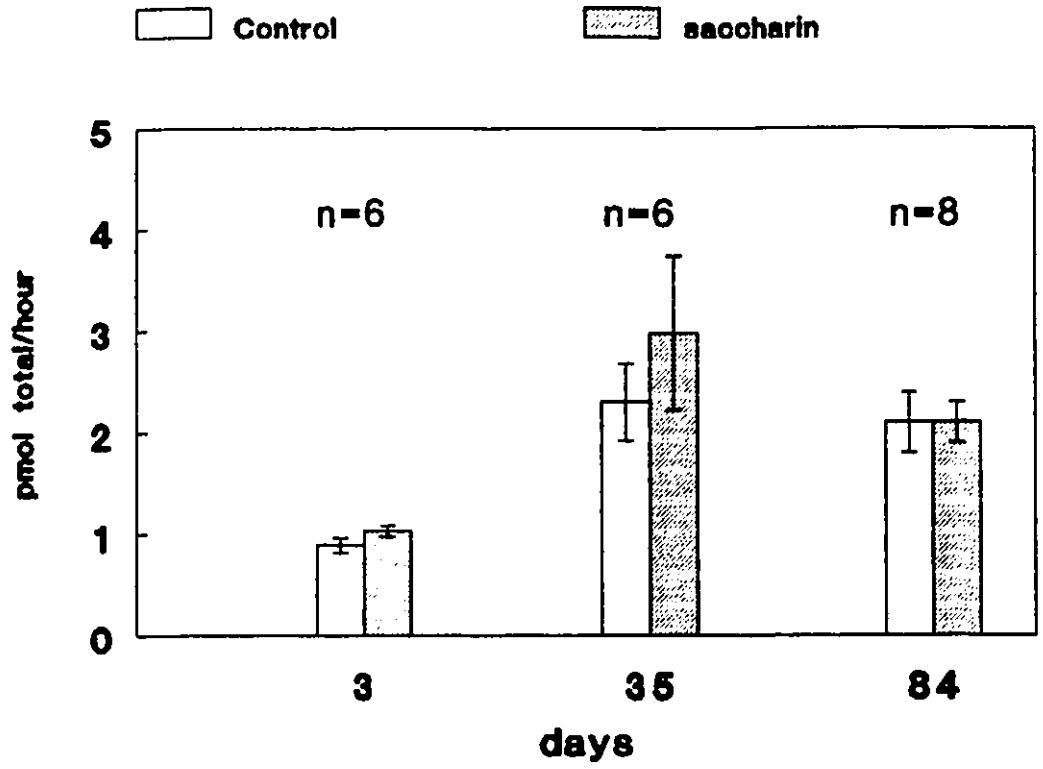


Fig. 4 T_4 5'-deiodinase activity in BAT after 3,35 and 84 days of saccharin intake.

Values are means±SEM for the number (n) of rats per group. Statistical analyses indicate that there was no significant effect of saccharin on the enzyme activity.

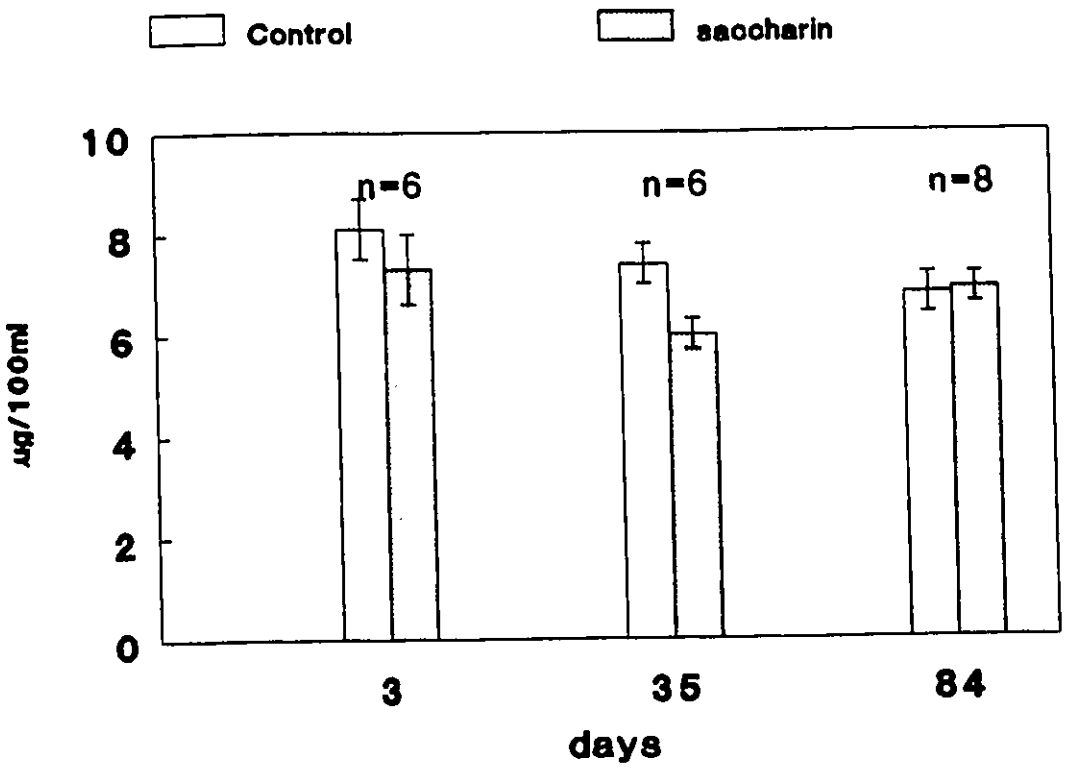


Fig. 5 Serum T_4 after 3, 35 and 84 days of saccharin intake.

Values are means±SEM for the number (n) of rats per group. Statistical analyses indicate that there was no significant effect of saccharin on the T_4 concentration.

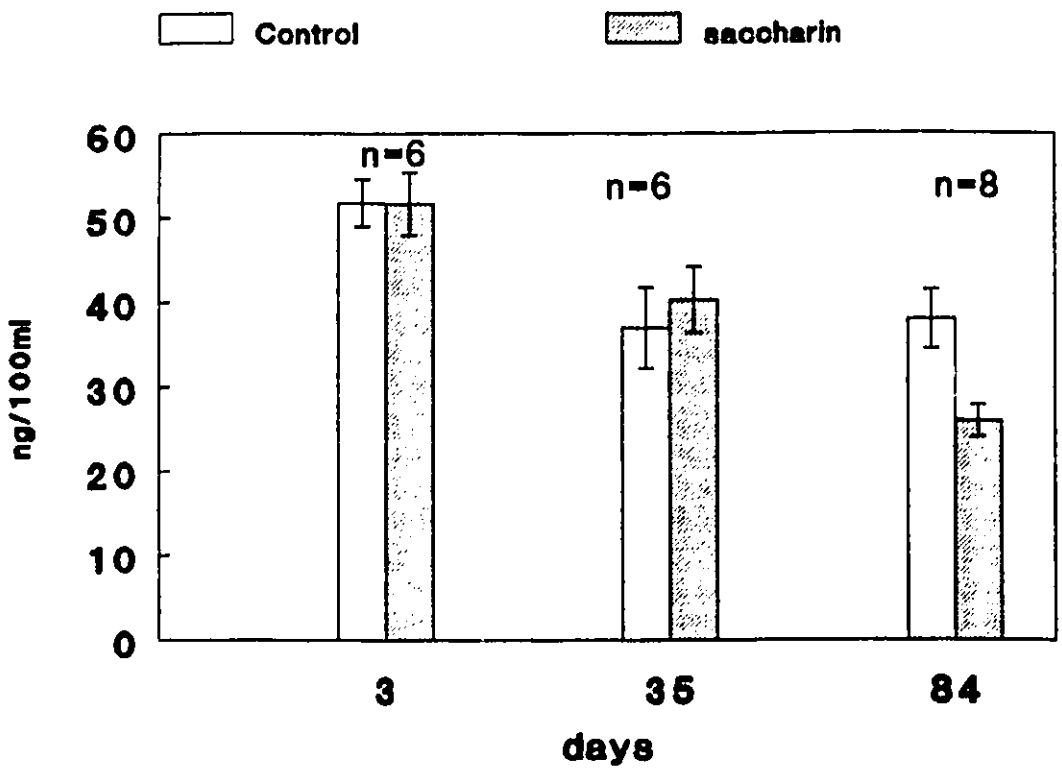


Fig. 6 Serum T_3 after 3, 35 and 84 days of saccharin intake.

Values are mean ± SEM for the number (n) of rats per group. Statistical analyses indicate that there was no significant effect of saccharin on the T_3 concentration.

information (nucleus of solitary tract) as well as brain stem nuclei which contribute efferent fibers to the vagal nerve (Berthoud et al., 1981). Therefore the gustatory-evoked vagal activity underlies the cephalic phase reflex release of insulin and perhaps also cephalic phase thermogenesis. It is now known that a sweet taste on the tongue, produced by sucrose or glucose solution in anaesthetized rats, increases the firing rate in the nerves to BAT (Niijima, 1991).

No effect of saccharin on BAT mitochondrial GDP binding was seen at one day, but the level in both saccharin and control rats was high at this time. This experiment was done twice. When the first experiment with 12 rats in each group showed the high level of GDP binding in both saccharin and control rats, it was thought that the initial stress caused by handling the rats might have been responsible. The experiment was repeated with a large number of rats (18 per group) that were handled daily for one week before. The results were the same. The results can only be explained by the housing of both groups of rats in the same room. Control rats might smell saccharin and activate cephalic phase thermogenesis due to olfactive stimulation or might respond to the behaviour of the other rats drinking the saccharin.

Recently, studies showed that oral intake of saccharin in the food for 10 days can increase thermogenic capacity in BAT of rats (Kawada et al., 1991). This finding agrees with my results that cephalic phase thermogenesis in BAT can be evoked by allowing rats to drink saccharin solution. However, the total fluid intake in the saccharin group was much higher than in the control group (Table 1). Since it is difficult to restrict rats to drink the same amount of liquid between two groups, it could not be ruled out that the thermogenesis

was induced by large amount of fluid intake, by which sympathetic nerves were activated.

There was no significant difference in T_4 5'-deiodinase activity between the two groups. Consequently, serum T_4 and T_3 remained unchanged. Protein content of BAT was also unchanged in the saccharin rats. Results suggest that saccharin can transiently induce cephalic phase thermogenesis in BAT but does not have a long term trophic effect on BAT.

Although cephalic phase thermogenic response in BAT can be induced by drinking saccharin solution, the energy balance of rat was not affected. These animals exhibited normal food intake and body weight after drinking saccharin solution. The results were consistent with the finding by others. For example, the experimental results by Kawada et al. (1991) demonstrated that after rats were fed saccharin in the food for 10 days (pair-fed to control rats), there was no effect of saccharin on body weight, despite a trophic effect on BAT (increase in concentration of UCP). In their experiments (1988), Porikos and Koopmans found that when saccharin was given in water for 8 weeks, there was no effect on body weight gain and food intake. Some other studies (Tordoff and Friedman, 1989a, b, c, d; Tordoff, 1988) showed that rats drinking saccharin solution increased their short-term food intake but there was no effect on body weight gain. Our present study did not show the long-term effect of saccharin on either food intake or body weight gain. It is therefore suggested that transient activation of BAT thermogenesis by saccharin was without effect on energy balance.

In summary, based on the results obtained, it can be concluded that the cephalic phase of diet-induced thermogenesis is associated with activation of BAT thermogenesis and that saccharin drinking may be a useful tool for the study of this phase of the response.

CHAPTER 5

EFFECT OF CAFETERIA FEEDING AND COLD EXPOSURE ON BROWN ADIPOSE TISSUE OF CAPSAICIN-DESENSITIZED (CAP-DES) RATS

Background:

Capsaicin is the pungent principle of hot chili peppers. Capsaicin has been used as a tool in the study of sensory nerve function in a variety of tissues owing to its neuropharmacological effect on these nerves (see Chapter 1). Administered at low doses, capsaicin exerts a powerful excitatory effect on peripheral sensory nerve endings, an effect that is apparently confined to unmyelinated afferents. Systemically administered at high doses, capsaicin results in a permanent loss of a majority of unmyelinated afferent fibers in newborn animals and long-lasting damage to a subpopulation of primary afferent sensory neurons in adult animals (Holzer, 1988; Buck and Burks, 1986). This treatment is called capsaicin-desensitization (CAP-DES). Many studies have shown that CAP-DES results in lesions in both the central and the peripheral nervous system which cause modification of numerous regulatory mechanisms (Szolcsanyi and Jancso Gabor, 1973; Cormarèche-Leydier, 1981; Kawada et al., 1986; Cormarèche-Leydier et al., 1985), such as thermoregulation and regulation of feeding behaviour. CAP-DES rats are unable to regulate body temperature in high ambient temperature environments and became markedly hyperthermic and die. These enhanced hyperthermic responses appeared to be due to an impairment of central and peripheral warmth receptors (Szolcsanyl and Jancso-Gabor, 1973) which influence heat-dissipating mechanisms. It has also been reported that endotoxin-induced fever can be

enhanced in CAP-DES rats in association with enhanced thermogenesis in BAT (Szekely and Szolcsanyi, 1979). However, CAP-DES rats are able to thermoregulate in the cold (Hori, 1984; Cormarèche-Leydier et al., 1985) suggesting that the cold receptors are unaffected. Food intake in CAP-DES is significantly reduced during the capsaicin injection period and rats lose weight. After 10-14 days, the amount of food eaten by the CAP-DES rats and body weight recover to the normal range. (Cormarèche-Leydier, 1981; Castonguay and Bellinger, 1987).

Diet-induced thermogenesis is defined as a regulatory, facultative component of energy expenditure, stimulated by overeating, which helps maintain energy balance. Diet-induced thermogenesis plays a important role in the regulation of energy expenditure and in the etiology of certain types of obesity. Most experiments testing the existence or the mechanism of diet-induced thermogenesis have used the cafeteria diet for the purpose of stimulating hyperphagia, a requisite for studies of diet-induced thermogenesis. Diet-induced thermogenesis in cafeteria-fed rats is proposed to result from sympathetic activation of thermogenesis in BAT (Rothwell and Stock, 1979, 1986) which allows animals to dispose of excess nutritional energy. Thermogenesis in BAT can also be increased by cold (Himms-Hagen, 1989). However, if any other category of thermogenesis is increased in the body, whether obligatory or facultative, in excess of heat loss, thermogenesis in BAT, including diet-induced thermogenesis, can be suppressed. For example, exercise-induced thermogenesis can inhibit thermogenesis in BAT during cold exposure (Arnold et al., 1986; Arnold and Richard, 1987).

In the first experiment, I studied the effect of CAP-DES on the thermogenic function

of BAT. The hypothesis was that since CAP-DES impairs both central and peripheral warmth receptors, the procedure would prevent the central sensation of warmth. Suppression of BAT thermogenesis by the hypothalamus in response to an increase in body temperature induced by the ingestion of food (Himms-Hagen 1989; Himms-Hagen et al., 1989) would thus be attenuated. In other words, it was expected that diet-induced thermogenesis in BAT would be enhanced in CAP-DES rats. When it was found, unexpectedly, that BAT was atrophied in CAP-DES rats and did not respond to feeding a cafeteria diet, a second experiment was done in which rats were exposed to cold to see whether their BAT would grow in response to this stimulus. In the second experiment, the effect of cold exposure on brown adipose tissue of CAP-DES rats was studied. It was known that CAP-DES rats can regulate body temperature normally at a cold temperature, thus cold-induced nonshivering thermogenesis in BAT should occur.

In addition, the thermogenic response of anaesthetized rats to intravenous infusion of noradrenaline was measured, known to be a good index of the amount of BAT present and of its response to noradrenergic stimulation (Foster and Frydman, 1978b; Foster et al., 1980).

Objective:

To find out whether CAP-DES can enhance thermogenesis in BAT induced by a cafeteria diet.

Methods:

Male Sprague-Dawley rats were obtained from Charles River Laboratories at 5 weeks of age. They were housed in individual wire mesh cages at 26°C with 12 hour light/dark cycle (lights on at 0700 hours). They had free access to food and water. At 6 weeks of age, rats were injected with capsaicin or vehicle as described in Chapter 3.5.

In the first experiment, 7 days after recovering from the last injection, control (vehicle-injected) and CAP-DES rats ate either chow or chow plus a cafeteria diet for 7 or 21 days. The cafeteria diet was fed on a rotating schedule of 3 menus (see Chapter 3.2). Food intake was measured twice a week in order to study the food intake of each menu twice. Digestible energy content and nutrient composition of the various foods was obtained from food tables (Pennington, 1989). Body weight gain was calculated from initial and final body weight.

In the second experiment, control and CAP-DES rats were either exposed to cold (4°C) or remained in the warm (26°C) for 1 or 7 days, starting 10 days after the last day of injections.

Rats were killed by decapitation between 08:00-09:00 hours, blood was collected and their rectal temperature was measured immediately. Both interscapular BAT and epididymal adipose tissue were removed, cleaned and weighed. BAT was then homogenized in isolation medium (0.25 M sucrose, 1mM N-2-hydroxyethylpiperazine-N-2ethanesulfonic acid (HEPES), 0.2 mM EDTA (potassium salt), adjusted to pH 7.2 with 1.0 M KOH) using a motor-driven teflon-glass homogenizer. Samples of homogenate were removed, frozen in liquid nitrogen, and stored at -80°C for later assay of protein, UCP, and T_4 5'-deiodinase activity. Mitochondria were isolated from the remainder of the homogenate and GDP-

binding was measured (see Chapter 3.8 and 3.10). The protocol of assays for protein, UCP, T_4 5-deiodinase, serum T_3 and T_4 was the same as described in Chapter 3.

Measurements of oxygen consumption and temperatures of BAT and colon of anaesthetized rat are the same as described in Chapter 3.19. For the infusion, a cannula was placed in a tail vein of anaesthetized rats. Infusion started with saline (0.0197 ml/min, Harvard pump, 2 ml glass Luer-Lok syringe, position 8). When BAT and body temperature and oxygen uptake were stable, noradrenaline D-bitartrate was infused at a rate of 0.25 μg base/100 cm^2.min and 0.0197 ml/min for 20 minutes. A stable and elevated rate of oxygen uptake was usually achieved within 8-10 minutes. Rectal and interscapular BAT temperatures were noted periodically before and during the infusion. The values shown are for two times, one immediately before the infusion, the other exactly 20 min after the start of the infusion of norepinephrine. The dose of norepinephrine used was sufficient to produce a maximum increase in oxygen uptake (Foster et al., 1980; Foster and Frydman, 1978).

Results are expressed as means±SEM for the numbers of animals indicated. Statistical analysis used two-way or three way ANOVA followed by Tukey's HSD (honestly significant difference) post-hoc test. The level of significance was $P < 0.05$.

Results:

Experiment 1: effect of diet

After CAP-DES, BAT was small and pale in rats eating chow in comparison with that of control rats eating the same diet. The weight of tissue was much less in both 1 week and

3 weeks experiments (Table 3). Total protein content in BAT was significantly decreased (Fig. 7). The most remarkable effect of CAP-DES was found in the reduction of total uncoupling protein content. Only 10 percent of the uncoupling protein remained at 1 week (2 week after capsaicin injection) and 45 percent at 3 weeks (4 weeks after capsaicin injection) (Fig. 8). Recovery of mitochondria from BAT of the CAP-DES rats was very low. At 1 week it was not enough to measure mitochondrial UCP concentration in these same samples. Mitochondrial GDP binding measured in CAP-DES rats was much lower than that of control rats at 1 week (Fig. 9). However, by three weeks, mitochondrial concentration of UCP (Fig. 11) and GDP binding (Fig. 9) were in the normal range (although total UCP remained low). Total thyroxine 5'-deiodinase activity in BAT of CAP-DES rats was relatively normal at 1 week, but it was decreased at 3 weeks (Fig. 10). Serum T3 concentration was also decreased at 3 weeks (Fig. 12).

Cafeteria diet increased BAT mass at both times studied (Table 3). There was no change in total protein content at 1 week, but there was a significant increase at 3 weeks (Fig.7) in control but not CAP-DES rats. Mitochondrial GDP binding was increased by cafeteria diet in control rats at 1 week and in both types of rats at 3 weeks (Fig. 9). The increase in GDP binding was independent of changes of mitochondrial concentration of UCP (Fig. 11) or in total tissue UCP (Fig. 8). As can be seen in Figs. 9 and 11, cafeteria diet increased mitochondrial GDP binding at 3 weeks in the CAP-DES rats although concentration of UCP was low. This suggested that BAT of the CAP-DES rats still has some thermogenic response to diet.

From Table 4, it can be seen that cafeteria diet contained more fat than the chow

TABLE 3
BODY, BAT AND WAT WEIGHT OF RATS EATING CHOW OR CHOW PLUS A CAFETERIA DIET

	CONTROL		CAP-DES	
1 WEEK FEEDING	CHOW (5)	CAFE (5)	CHOW (5)	CAFE (5)
Body Weight, g initial	282 ±3.3	282 ±3.5	268 ±5.4	269 ±3.5
final	341 ±4.4	353 ±4.7	335 ±7.9	343 ±14.3
BAT Weight, mg	439 ±44.3	566^{+} ±20.5	264* ±21.0	561^{+} ±57.3
WAT Weight, g	2.9 ±0.21	5.5^{+} ±0.31	3.0 ±0.10	5.5^{+} ±0.66
Body temperature °C	37.6 ±0.13	37.9 ±0.23	37.0 ±0.32	37.7 ±0.24
3 WEEKS FEEDING	(8)	(8)	(10)	(10)
Body Weight, g initial	277 ±5.9	293 ±6.2	260 ±3.3	272 ±3.7
final	438 ±10.6	541^{+} ±16.3	423 ±7.8	505^{+} ±15.4
BAT Weight, mg	664 ±66.9	1153^{+} ±46.1	468* ±26.1	820^{*+} ±76.9
WAT Weight, g	6.4 ±0.50	14.2^{+} ±0.96	5.7 ±0.27	13.6^{+} ±1.44
Body temperature °C	37.0 ±0.11	37.7^{+} ±0.11	36.9 ±0.17	37.6^{+} ±0.18

* Significant effect of CAP-DES, comparing animals fed the same diet

+ Significant effect of diet, comparing rats treated in the same way

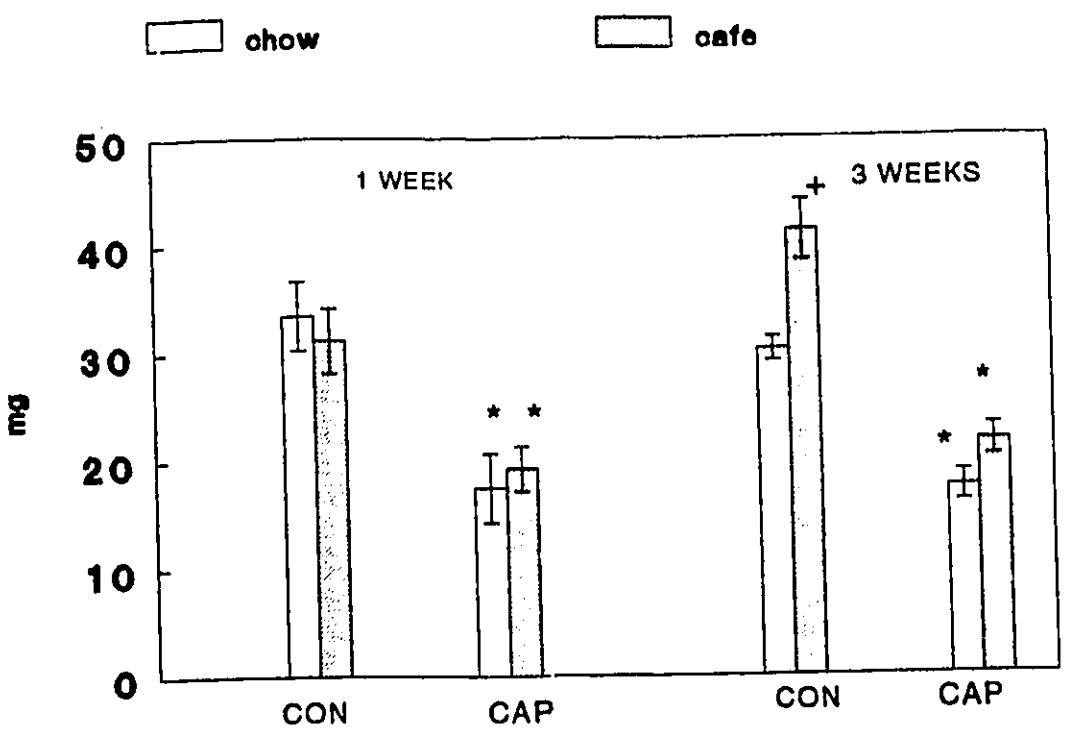


Fig. 7 Total protein content of interscapular brown adipose tissue (IBAT) in control (CON) and capsaicin-desensitized (CAP) rats eating chow or a cafeteria diet for 1 week or 3 weeks.

* Significant effect of CAP-DES. Total protein in BAT of CAP-DES rats is significantly less than that of control rats eating the same diet and studied at the same time.
+ Significant effect of diet. After 3 weeks cafeteria feeding, total protein content of BAT is increased in control rats.

Values are means ± SEM for the number (n) of rats per group.
A significant difference is indicated when $p < 0.05$.
In 1 week experiment, n = 5
In 3 weeks experiment, n = 8 in CON, n = 10 in CAP

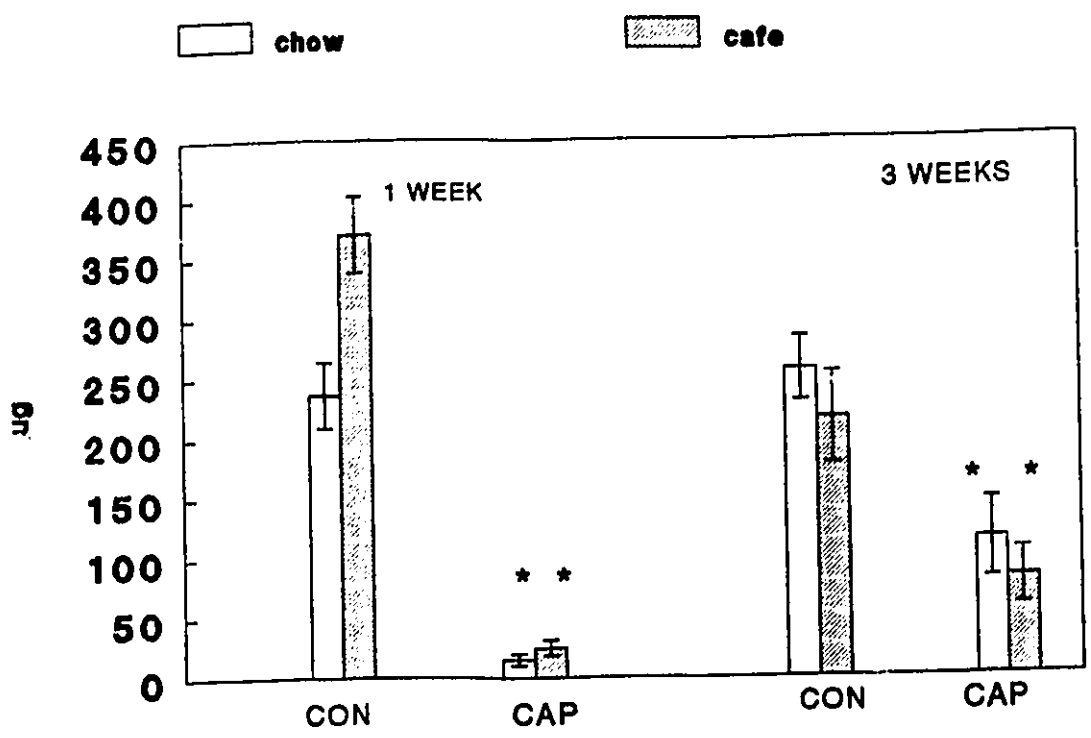


Fig. 8 Total uncoupling protein (UCP) of interscapular brown adipose tissue (IBAT) in control (CON) and capsaicin-desensitized (CAP) rats eating chow or a cafeteria diet for 1 week or 3 weeks.

* Significant effect of CAP-DES. UCP level in CAP-DES rats is significantly less than that of CON rats eating the same diet and studied at the same time.

Values are means±SEM for the number (n) of rats per group. A significant difference is indicated when $p<0.05$.
In 1 week experiment, n=5
In 3 weeks experiment, n=8 in CON, n=10 in CAP

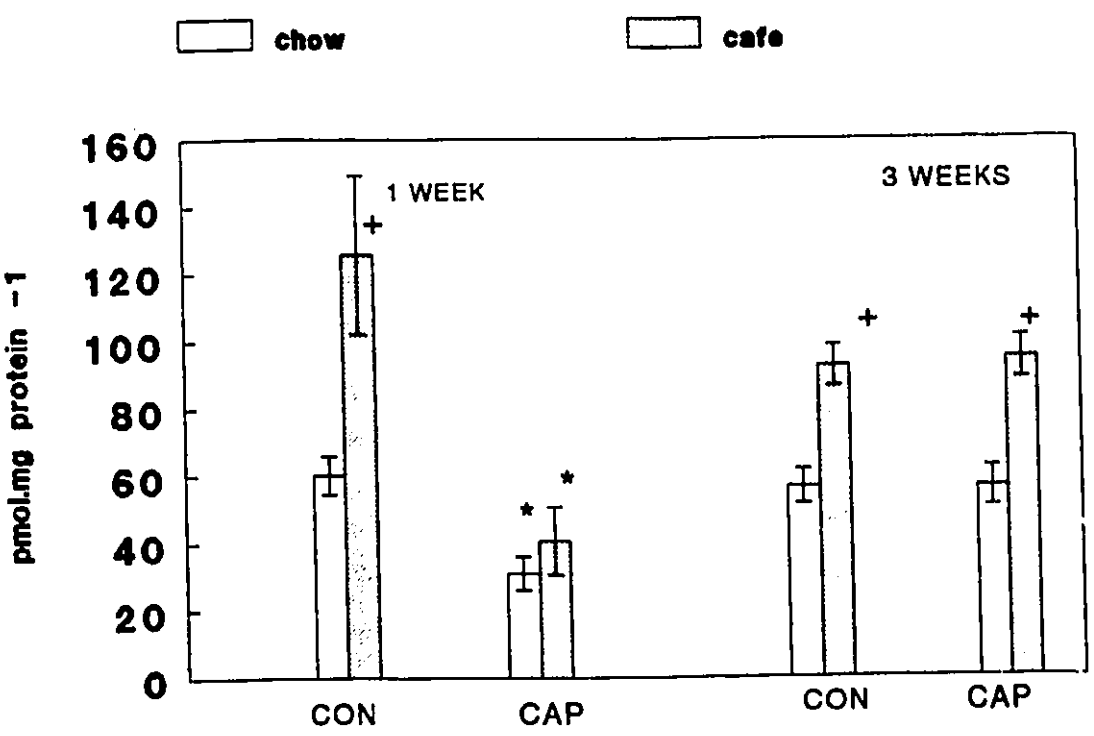


Fig. 9 GDP binding by mitochondria isolated from interscapular brown adipose tissue (IBAT) in control (CON) and capsaicin desensitized (CAP) rats eating chow or a cafeteria diet for 1 week or 3 weeks.

* Significant effect of CAP-DES. The level of GDP binding in CAP-DES rats is significantly less than that of CON rats at 1 week (both diets), but not at 3 weeks.
+ Significant effect of diet. GDP binding is significantly increased by cafeteria diet in controls at 1 week and in both types of rat at 3 weeks.

Values are means±SEM for the number (n) of rats per group. A significant difference is indicated when $p<0.05$.
In 1 week experiment, n=5
In 3 weeks experiment, n=8 in CON, n=10 in CAP.

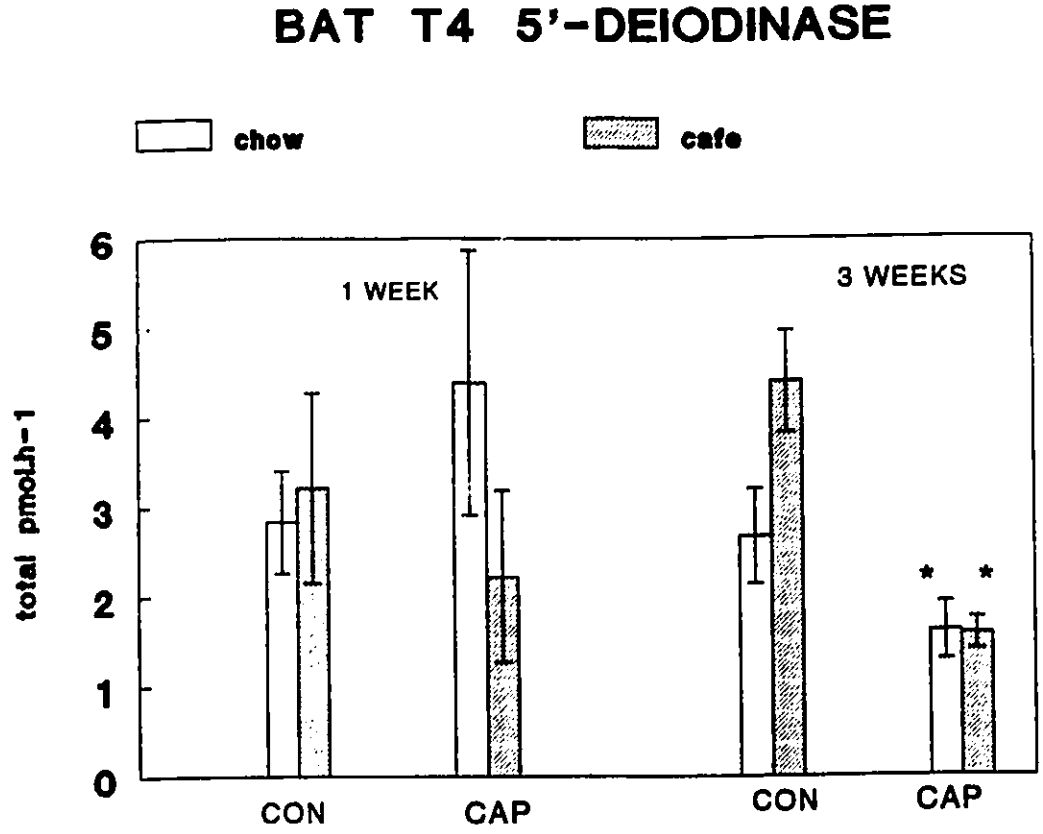


Fig. 10 Total T_4 5'-deiodinase activity in interscapular brown adipose tissue (IBAT) in control (CON) and capsaicin desensitized (CAP) rats eating chow or a cafeteria diet for 1 week or 3 weeks.

* Significant effect of CAP-DES. The level of T_4 5'-deiodinase activity in IBAT of CAP-DES rats is significantly reduced in both diet groups at 3 weeks, but not at 1 week.

Values are means ± SEM for the number (n) of rats per group.
A significant difference is indicated when $p < 0.05$.
In 1 week experiment, n=5
In 3 weeks experiment, n=8 in CON, n=10 in CAP.

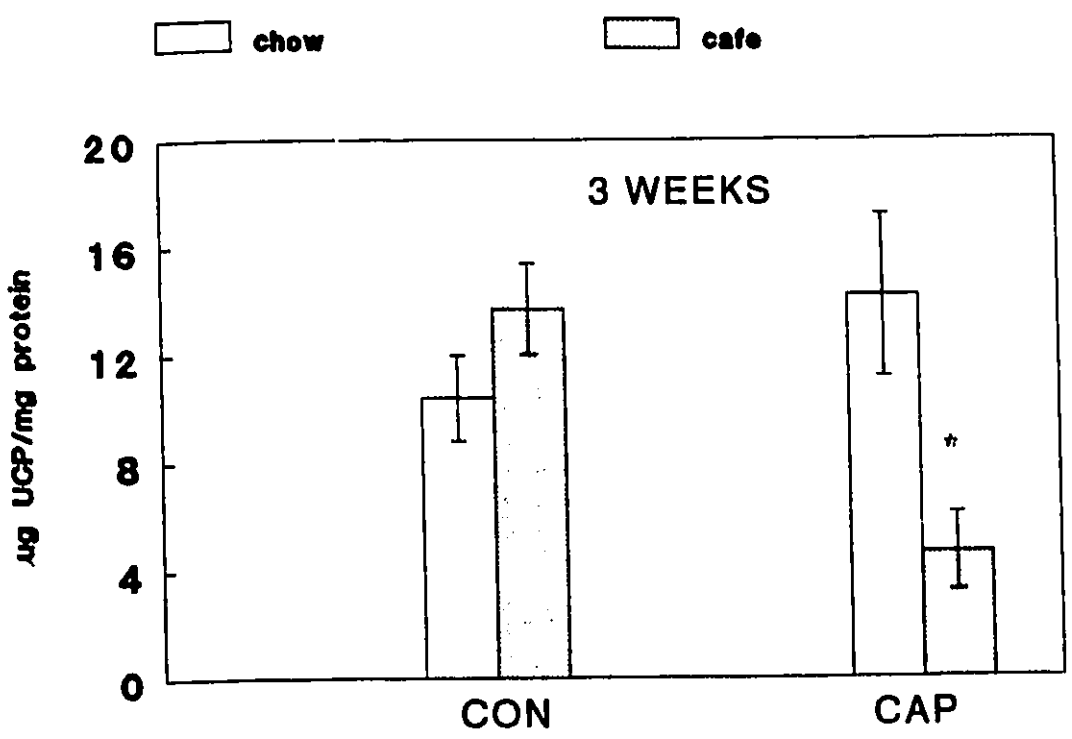


Fig. 11 Concentration of UCP in mitochondria isolated from interscapular brown adipose tissue (IBAT) in control (CON) and capsaicin-desensitized (CAP) rats eating chow or a cafeteria diet for 3 weeks.

* Significant effect of CAP-DES,comparing animals eating the same diet. There was too low a yield of mitochondria to measure concentration of UCP in CAP-DES rats at 1 week.

Values are means±SEM for the number (n) of rats per group. A significant difference is indicated when $p<0.05$.
In 3 weeks experiment, n=8 in CON, n=10 in CAP.

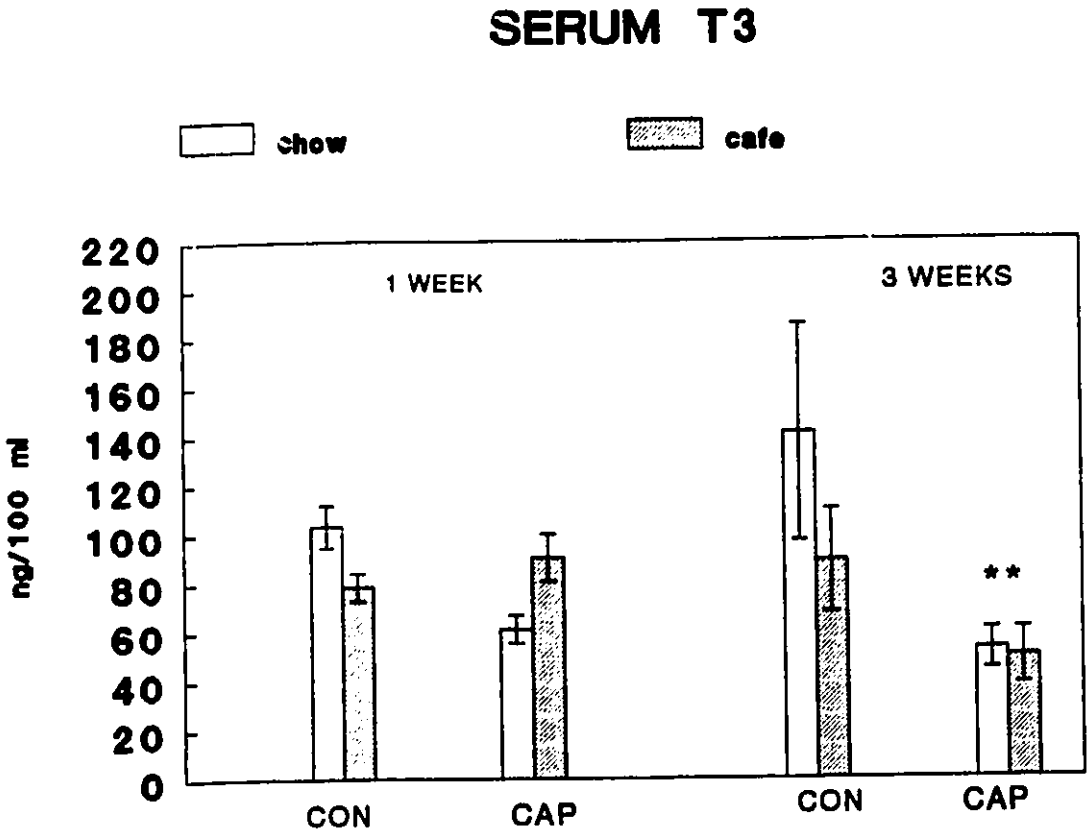


Fig. 12 The level of serum T_3 in control (CON) and capsaicin-desensitized (CAP) rats eating chow or a cafeteria diet for 1 week or 3 weeks.

* Significant effect of CAP-DES. T_3 level of in CAP-DES rats is significantly lower than that of CON rats eating the same diet and studied at three weeks.

Values are means±SEM for the number (n) of rats per group. A significant difference is indicated when $p<0.05$.
In 1 week experiment, n=5
In 3 weeks experiment, n=8 in CON, n=10 in CAP

TABLE 4
FOOD INTAKE AND COMPOSITION OF CHOW AND CAFETERIA-FED CONTROL AND CAP-DES RATS

	CONTROL		CAP-DES	
	CHOW	CAFE	CHOW	CAFE
Energy intake kcal/day	101.6 ± 3.15	151.0+ ± 4.39	97.6 ± 1.75	137.7+ ± 4.94
Composition (%) carbohydrate	59.8	38.5 ± 1.59	59.8	37.6 ± 1.62
fat	14.2	47.7+ ± 1.40	14.2	48.4+ ± 1.56
protein	26	13.8 ± 0.17	26	14.0 ± 0.29

There was no significant difference in total intake of carbohydrate and protein between chow and cafeteria diet. But, the total fat intake was significantly increased in cafeteria-fed rats.

Values are means± SEM.
+ denotes a significant difference between chow and cafeteria diet.

diet. Fat intake increased, but because rats were hyperphagic, total carbohydrate and protein intake remained unchanged. Energy intake in cafeteria-fed rats was increased by about 50% due to the palatable food. Food intake of CAP-DES rats was similar to that of control rats, whether they were eating chow or choosing from chow plus a cafeteria diet. CAP-DES rats exhibited the same food selection as control rats, both choosing a high-fat diet.

Body weight of both CAP-DES and control rats were significantly increased by cafeteria diet. They showed similar body weight gains (Table 3). Weight of epididymal white adipose tissue was increased by the high fat diet in both CAP-DES and control rats at both 1 week and 3 weeks. Body temperature in both types of cafeteria-fed rats was increased at 3 weeks (Table 3).

Experiment 2: effect of cold

Similar to the results obtained in the first experiment, CAP-DES reduced total BAT protein and UCP at 10-18 days after injections (Fig. 13 and 14). BAT wet weight was smaller than that of control (Table 5). However, mitochondrial GDP binding (Fig.15) and UCP concentration in mitochondria (Fig. 16) were normal.

After cold exposure, total protein (Fig. 13), mitochondrial GDP binding (Fig. 15) and T_4 5'-deiodinase activity (Fig. 17) were increased within 1 day, the increase persisted at 7 days in the control rats. BAT of CAP-DES rats had a similar response to the cold, except for a lack of increase in total protein at 1 day, but it was increased to the normal extent by 7 days (Fig. 13). There were no changes in UCP at 1 day (Figs. 14), but substantial increases in UCP by 7 days in both control and CAP-DES rats. Concentration of mitochondrial UCP

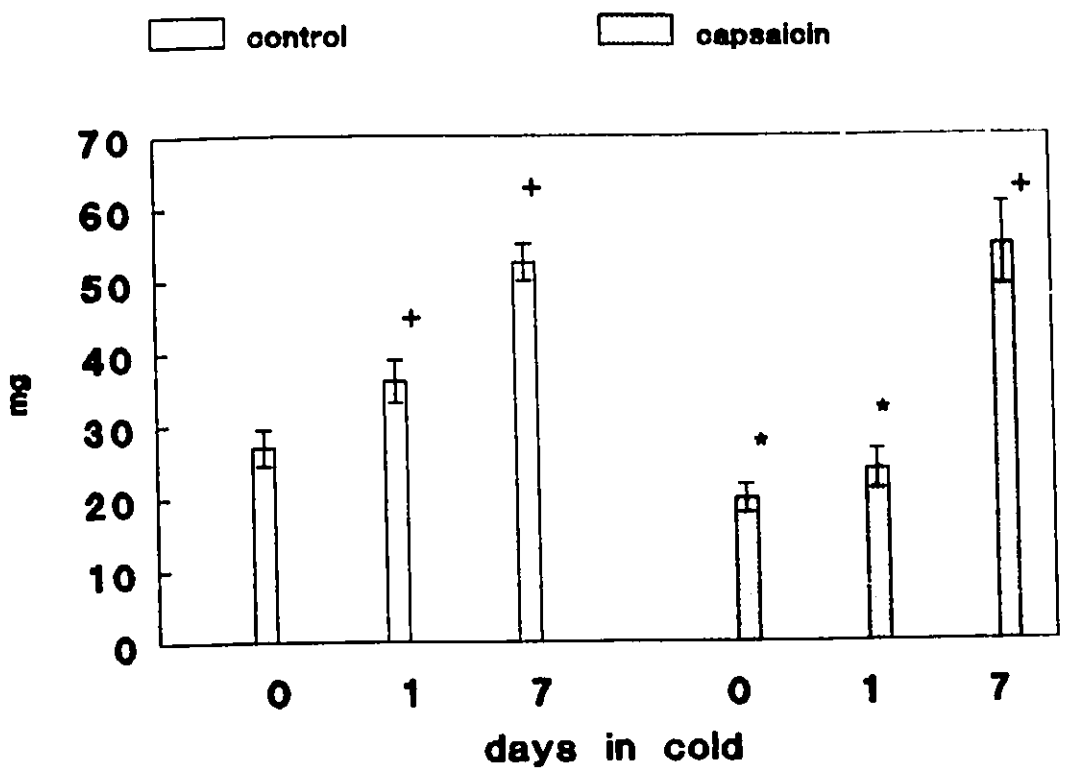


Fig. 13 Total protein in interscapular brown adipose tissue (IBAT) of control and capsaicin-desensitized rats exposed to cold (4 °C) for 0, 1 and 7 days.

* Significant effect of CAP-DES. After exposure to cold 0 and 1 day (not in 7 days), total protein content in BAT of CAP-DES rats is significantly lower, compared with control rats exposured to the same temperature.
+ Significant effect of cold. Total protein is significantly increased by cold exposure.

Values are means ± SEM for the number (n=6) of rats per group. A significant difference is indicated when $p<0.05$.

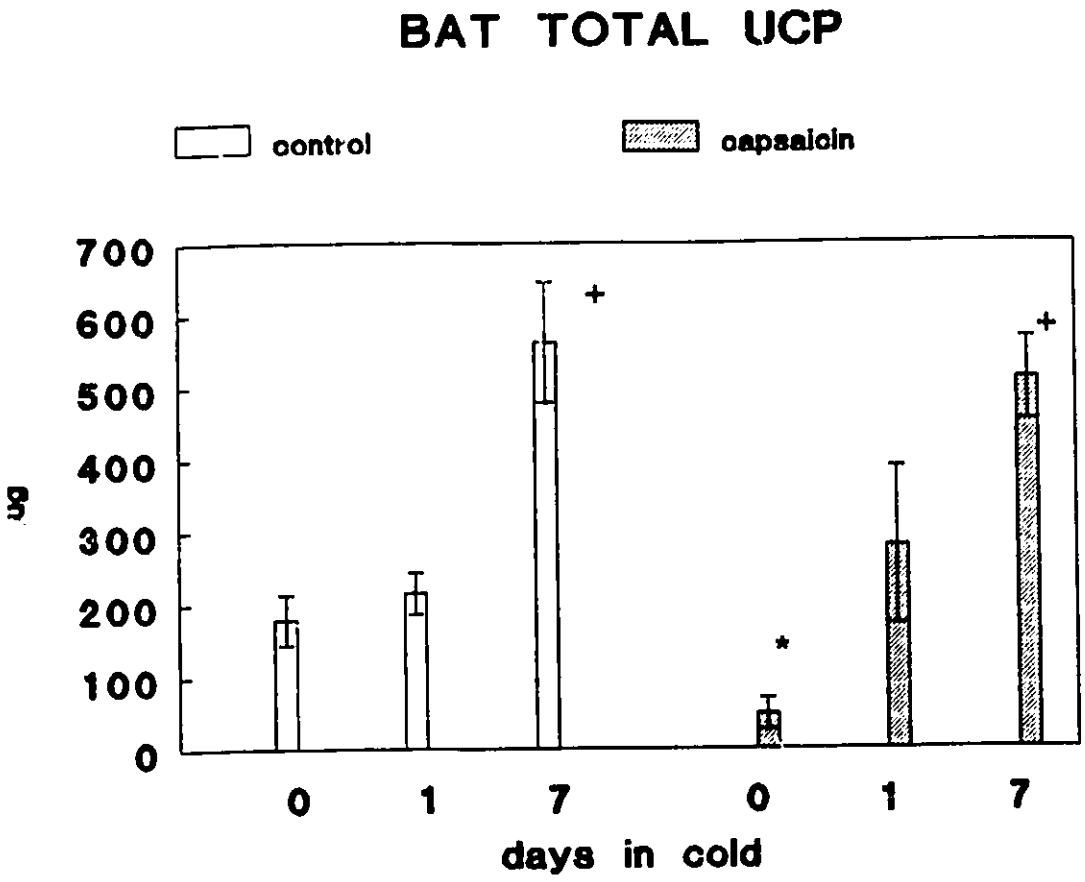


Fig. 14 Total uncoupling protein (UCP) in interscapular brown adipose tissue (IBAT) of control and capsaicin-desensitized rats exposed to cold (4°C) for 0, 1 and 7 days.

* Significant effect of CAP-DES, compared with control rats exposed to the same temperature.
+ Significant effect of cold. UCP level is increased after exposure to cold at 7 days in both groups of rats.

Values are means±SEM for the number (n=6) of rats per group. A significant difference is indicated when $p<0.05$.

TABLE 5
BODY AND TISSUE WEIGHTS, BODY TEMPERATURE, AND SERUM THYROID HORMONE LEVELS OF CONTROL AND CAP-DES RATS EXPOSED TO COLD

	CONTROL			CAPSAICIN		
Days at 4°C	o (6)	1 (6)	7 (6)	0 (6)	1 (6)	7 (6)
Body Weight, g (at start)	344.2 ±7.2	347.7 ±11.3	291.8 ±4.3	343.7 ±9.0	312.7 ±5.2	284.7 ±4.8
Body Weight Change in cold, g	-	-6.3 ±1.7	+23.7 ±3.3	-	-5.2 ±1.6	+21.0 ±4.5
BAT Weight, mg	535.7 ±82.1	394.8+ ±29.2	569.2 ±33.1	422.5* ±46.7	269.5+ ±28.2	554.5 ±45.6
WAT Weight, g	3.79 ±0.53	3.35 ±0.40	2.09+ ±0.22	3.47 ±0.24	2.56+ ±0.17	2.22+ ±0.12
Body Temperature °C	37.7 ±0.06	37.6 ±0.20	37.3 ±0.19	38.1 ±0.37	36.7* ±0.31	37.4 ±0.28
Serum T_3 ng/dl	43.0 ±5.87	60.7 ±6.80	46.5 ±4.23	33.2 ±3.53	57.5+ ±4.26	49.0 ±5.30
Serum T_4 μg/dl	9.11 ±0.94	10.07 ±1.03	7.78+ ±0.60	7.31 ±0.89	9.77 ±1.06	5.74+ ±0.43

* Significant effect of capsaicin-desensitization, comparing similarly-exposed rats

+ Significant effect of cold, comparing similarly-treated rats

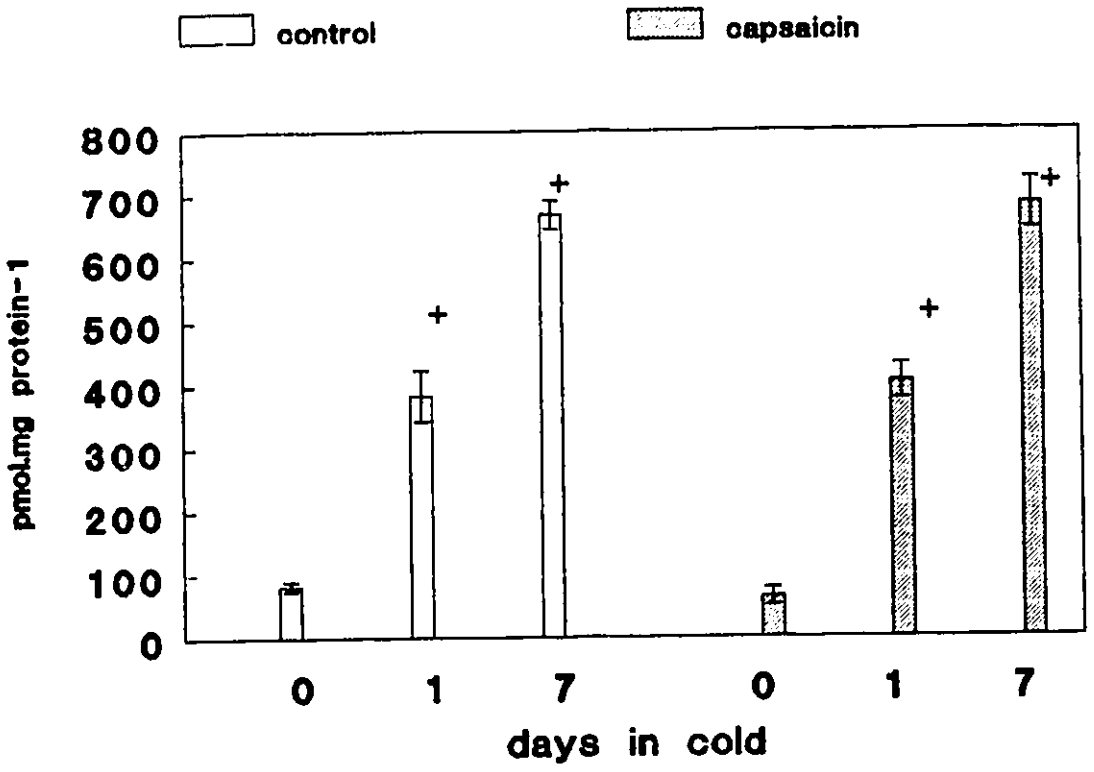


Fig. 15 GDP binding by mitochondria isolated from interscapular brown adipose tissue (IBAT) of control and capsaicin-desensitized rats exposed to cold (4 °C) for 0, 1 and 7 days.

+ Significant effect of cold. There are no significant effects of CAP-DES, comparing rats studied at the same time and exposure to same temperature.

Values are means ± SEM for the number (n=6) of rats per group. A significant difference is indicated when $p < 0.05$.

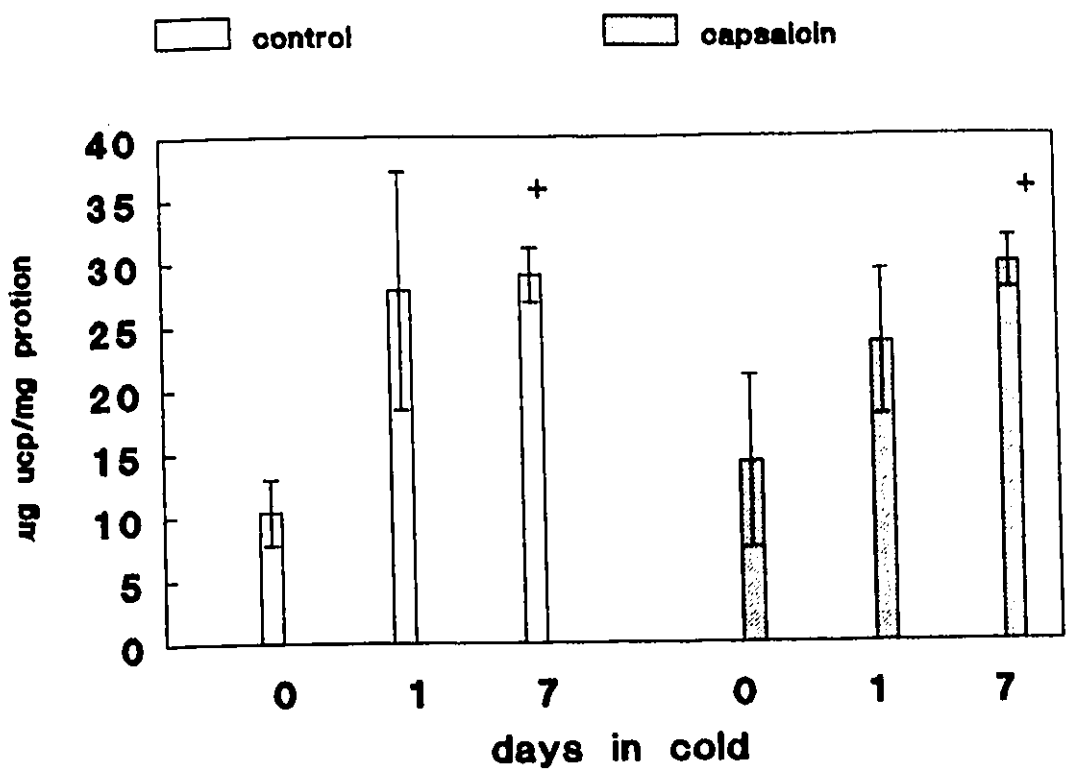


Fig. 16 Concentration of uncoupling protein (UCP) in mitochondria isolated from interscapular brown adipose tissue (IBAT) of control and capsaicin-desensitized rats exposed to cold (4 °C) for 0, 1 and 7 days.

+ Significant effect of cold. After cold exposure at 4 °C for 7 days, the concentration of UCP is significantly increased when compared with that of 0 day cold exposed rats.

Values are means ± SEM for the number (n = 6) of rats per group. A significant difference is indicated when $p < 0.05$.

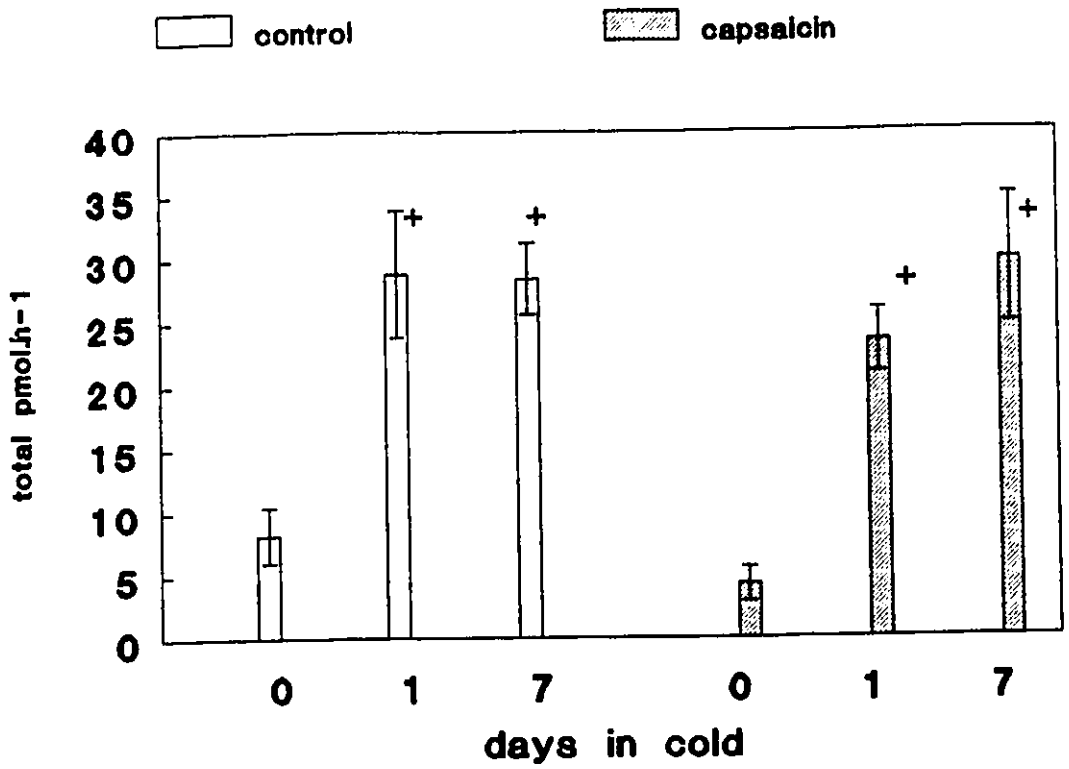


Fig. 17 Total T_4 5'-deiodinase activity in interscapular brown adipose tissue (IBAT) of control and capsaicin-desensitized rats exposed to cold (4°C) for 0, 1 and 7 days.

+ Significant effect of cold. UCP level is increased after exposure to cold at 1 and 7 days in both groups of rats.

Values are means±SEM for the number (n=6) of rats per group. A significant difference is indicated when $p<0.05$.

increased significantly only at 7 days (Fig. 16).

Both groups of rats lost body weight at 1 day and gained at 7 days after cold exposure. White adipose tissue became smaller after cold exposure for 7 days (Table 5). CAP-DES rats also were slightly hypothermic at 1 day cold exposure (Table 5), but returned to normal by 7 days (Table 5). There was a transient increase in T_3 level in both types of rat, reaching statistical significance only in CAP-DES rats (Table 5). The concentration of T_4 was decreased after 7 days in cold in both groups of rat (Table 5).

Experiment 3: thermogenic response to noradrenaline

Resting (anaesthetized) oxygen consumption of CAP-DES rats was 11-12% lower than that of control rats, whether expressed in terms of total body or of surface area (Fig. 18, Table 6). The increase induced by infusion of noradrenaline was also smaller, amounting to only 62% of that in control rats (Fig. 18). Colonic temperature of both types of rat increased during the infusion of noradrenaline, to an extent that was similar in the two groups of rats. However, interscapular BAT temperature rose to a much lesser extent in CAP-DES rats than in control rats (Fig. 19). Indeed, in the CAP-DES rats the increase in interscapular BAT temperature did not exceed the increase in colonic temperature whereas in the control rats it greatly exceeded the increase in colonic temperature (Fig. 19).

Discussion:

The first experiment showed that BAT was atrophied in CAP-DES rats and did not respond normally to diet. The atrophy of BAT was characterized by lower wet weight, a

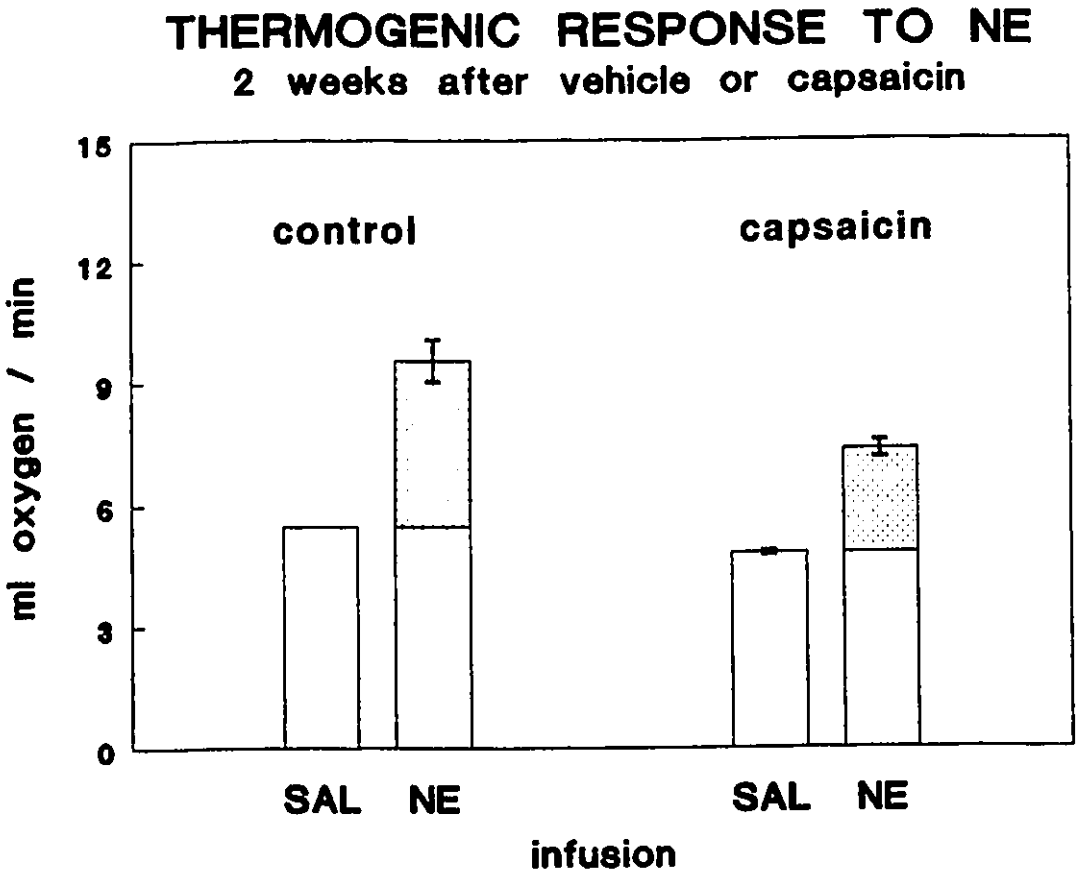


Fig. 18 Oxygen uptake of pentobarbital-anesthetized control and capsaicin-desensitized rats before and after 20 minute of infusion of norepinephrine.

The effect of norepinephrine (NE) is indicated by the stippled portions of the bars. The rate of oxygen consumption during saline (SAL) infusion was significantly less in CAP-DES rats, whether expressed as the total uptake (4.80 ± 0.06 versus 5.45 ± 0, $p < 0.001$) or in terms of body surface (1.32 ± 0.012 versus 1.48 ± 0.013 ml oxygen/100 cm^2.min, $p < 0.001$). Body surface was calculated from 7.5 x body weight (g) $^{0.67}$ = 100 cm^2 body surface as before (Himms-Hagen, 1969).

TABLE 6
BODY AND TISSUE WEIGHTS AND COLONIC TEMPERATURES OF ANAESTHETIZED CONTROL AND CAP-DES RATS

	CONTROL	CAP-DES	P
(n)	(4)	(8)	
Body weight, g	339 ±0.9	330 ±3.8	N.S
Interscapular BAT weight, mg*	567 ±79	280 ±41	<0.02
Colonic temperature °C (at start of infusion)†	36.2 ±0.34	35.7 ±0.12	N.S
Interscapular BAT temperature °C (at start of infusion)†	34.7 ±0.23	34.6 ±0.10	N.S

* Weighed after the infusion of noradrenaline

† These are the temperatures at the start of the infusion. The change during the infusion is shown in Fig. 19.

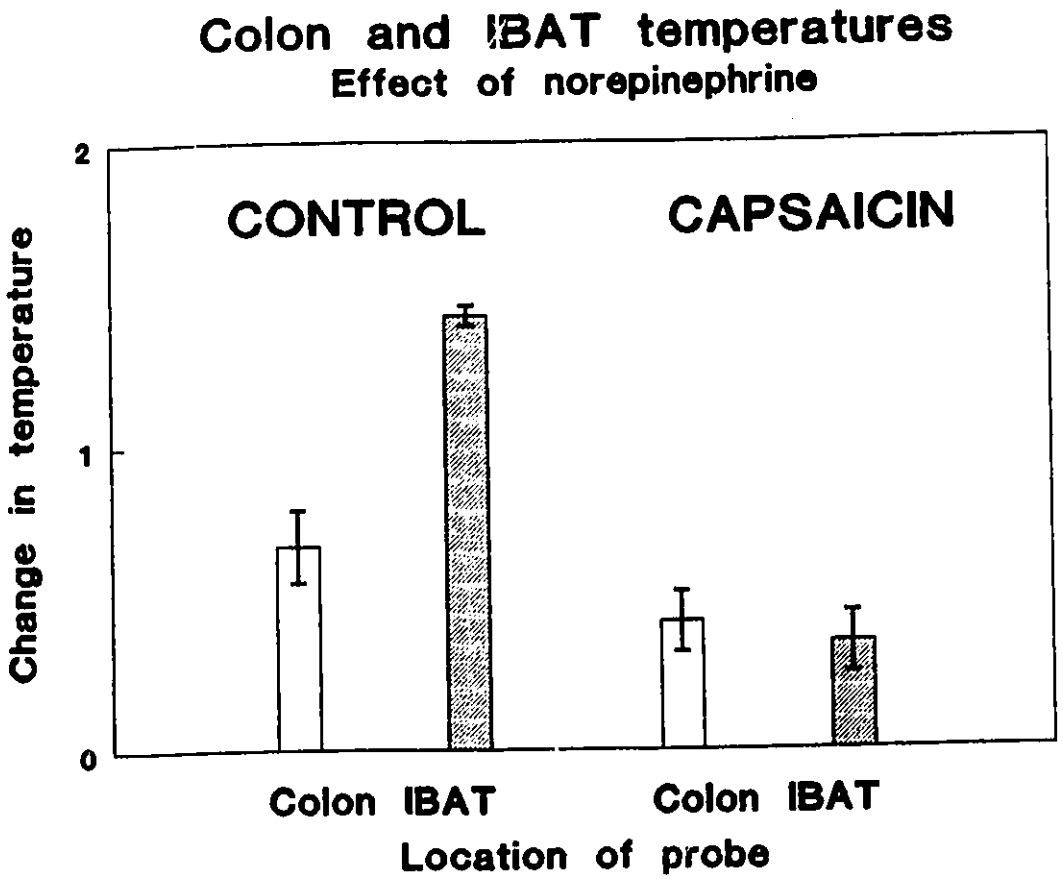


Fig. 19 Changes in colonic (COL) and interscapular brown adipose tissue (IBAT) temperature during 20 minutes infusion of norepinephrine in anesthetized control and capsaicin-desenstized rats.

Temperatures were recorded immediately before and after 20 min of infusion of norepinephrine. The actual starting temperatures are in Table 6. All changes are significantly different from zero. The increase in IBAT temperature in significantly greater than the increase in colonic temperature in control rats ($P < 0.05$) but not in CAP-DES rats. The increase in IBAT temperature in CAP-DES rats is significantly less than in control rats ($P < 0.001$) whereas the increase in colonic temperature is not different from that in control rats.

reduced total protein content and as little as 10% of the normal content of UCP. Since the mitochondrial concentration of UCP was normal and the amount of mitochondria recovered from BAT were very low, it seems that most mitochondria of BAT of CAP-DES have been lost. The finding was entirely different from what was expected, suggesting the working hypothesis was incorrect.

It is well known that capsaicin treatment can destroy a subpopulation of sensory nerves in which the neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) occur. Both SP and CGRP normally play a mediator or transmitter role in the function of the sensory nervous system. CGRP is also a potent vasodilator (Brain et al., 1985). Sensory innervation of white adipose tissue (Fishman and Dark, 1987) lead to prediction of sensory innervation of BAT. This prediction was confirmed by some researchers (Himms-Hagen et al., 1989; Norman et al., 1988; Lever et al., 1988). They found BAT indeed has nerves containing SP and CGRP, characteristic of sensory nerves. Thus, the results obtained in the present experiments could be explained by one of the following possible reasons. First, after systemic administration of capsaicin, the neuropeptides SP and CGRP are depleted from sensory nerves in BAT, which may result in loss of a trophic effect on synthesis of UCP and of mitochondria or in disappearance of an inhibitory influence of these neuropeptides on degradation of UCP and of mitochondria. This idea is supported by the finding that CGRP is lost from nerves of BAT of CAP-DES rats (Himms-Hagen et al., 1990). Second, the large amount of neuropeptides initially released after capsaicin treatment may activate degradation of UCP. Third, capsaicin itself may exert a direct and destructive effect on BAT that results in degradation of UCP. It has been reported that capsaicin does

enter and accumulate in a variety of tissues after its systemic injection (Saria et al., 1982). A fourth possibility is that the combined actions of norepinephrine and sensory neuropeptides bring about the initial atrophy. It is known that capsaicin activates the sympathetic nervous system (Watanabe et al., 1987), increases overall metabolic rate (Kawada et al., 1986) and increases thermogenesis in BAT (Yoshida et al., 1988). Moreover, noradrenaline increases the excitability of peripheral sensory nerves (Maggi and Meli, 1988). Thus, BAT is likely to be subjected to stimulation by both noradrenaline and sensory neuropeptides after administration of capsaicin. However, all these interpretations need further studies. The atrophy of BAT produced by CAP-DES can not be attributed to the depletion of noradrenaline from sympathetic nerves since noradrenaline content of BAT is normal in CAP-DES rats (Himms-Hagen et al., 1990).

The trophic response of BAT to a cafeteria diet in CAP-DES rats was retarded, as judged from reduction of total protein and total UCP (index of thermogenic capacity), although a small diet-induced thermogenesis still occurred (increased in GDP binding). However, there was no direct correlation between BAT function and body weight gain. There were similar body growth rates in control rats and CAP-DES rats eating chow or chow plus cafeteria diet. Food intake was normal in CAP-DES rats, which was consistent with the finding by Castonguay and Bellinger (1987). Body fat in CAP-DES rats (as assessed by the weight of white epididymal adipose tissue) did not accumulate more than in control rats, even when these animals were fed highly palatable cafeteria food. This suggests that the thermogenic function of BAT is not critical in energy balance of CAP-DES rats under these conditions. Many studies have demonstrated that the reduced amount of UCP in BAT

of some obese animals and reduced diet-induced thermogenesis in BAT are directly related to a deficit in energy expenditure that contributes to the development of obesity (Himms-Hagen, 1989). This was not the case for CAP-DES rats. Whether other categories of thermogenesis take place to increase energy expenditure in compensation for the lack of BAT thermogenesis under these conditions needs further investigation. For example, it is possible that CAP-DES impaired both central and peripheral warmth receptor which would lead to enhancement of obligatory thermogenic processes as well as some facultative thermogenesis in other tissues. This complementary mechanism would contribute to the normal energy balance in CAP-DES rats.

As expected, cold-induced nonshivering thermogenesis in BAT did occur in CAP-DES rats in the second experiment. CAP-DES rats and control rats showed very similar responses to cold. After cold exposure, a trophic response of BAT occurred, as assessed from increases in total protein, in total UCP and T_4 5'-deiodinase activity. It can be suggested that cold receptors are innervated by sensory nerves that are not sensitive to capsaicin treatment.

The reduction in magnitude of the thermogenic response of the intact anaesthetized CAP-DES rat to noradrenaline would be in keeping with a reduced capacity of BAT for thermogenesis due to its low level of UCP. In the present study, the reduction of thermogenic response to noradrenaline in CAP-DES rats was 38%. Comparing with the results (Foster and Frydman, 1978) that derived from quantitative measurement of blood flow through BAT of warm-acclimated rats (42% of the thermogenic response to noradrenaline occurred in total BAT), only about 10% thermogenic capacity in BAT had remained in CAP-DES rats. Since BAT thermogenesis contribute to only about 1% of the

total resting metabolic rate in the unstimulated state (Foster and Frydman, 1978), the significant reduction in resting metabolic rate of CAP-DES rats can not be attributed solely to loss of thermogenic capacity of BAT. Since concentrations of both T_3 and T_4 were normal, it can also not be attributed to altered thyroid status.

The physiological implication of BAT atrophy in CAP-DES should be considered. The lower capacity of BAT thermogenesis has been indicated by its lesser UCP content and by the small thermogenic response to infused noradrenaline of intact CAP-DES rats. However, these rats were able to maintain a normal body temperature during cold exposure. In order to do so, CAP-DES rat could use shivering thermogenesis in muscle initially to compensate for atrophied BAT. Then, BAT will be stimulated to grow by cold acclimation and eventually nonshivering thermogenesis will occur normally in BAT.

In summary, the results of these three experiments show that, contrary to the prediction based on the working hypothesis, BAT is atrophied in CAP-DES rats and does not respond normally to diet. Function of BAT was, however, normal in the CAP-DES rat after cold-exposure. Despite the atrophy of BAT and lack of response to diet, energy balance of the CAP-DES rat appeared to be normal during the 1-3 week period studied. Results suggest a role for neuropeptides of sensory nerves in control of BAT function.

CHAPTER 6

EFFECT OF SYMPATHETIC DENERVATION ON THE GROWTH AND FUNCTION OF BROWN ADIPOSE TISSUE OF CAPSAICIN-DESENSITIZED (CAP-DES) RATS

Background:

Thermogenesis in BAT in response to either cold exposure or overfeeding is evoked by noradrenaline secreted from the sympathetic nerves which innervate this tissue (Himms-Hagen, 1991). The interscapular BAT (IBAT) of the rat is the most studied BAT depot. IBAT consists of two symmetrical pads, each of which receives five intercostal nerves as well as a nerve which runs along the thoracodorsal blood vessels (Foster et al., 1982a, b; Néchad, 1986). They arise from the middle and inferior cervical ganglia and the first five thoracic ganglia (Girardier and Seydoux, 1986). Many studies have shown that denervation of the pad on one side does not alter the noradrenergic innervation of the contralateral pad, indicating that there is no cross innervation between the two symmetrical pads (Foster et al., 1982 a,b; Park and Himms-Hagen, 1988). This morphological separateness of the two symmetrical pads allows the study in a single animal of denervated and innervated pads that are exposed to identical circulating hormones.

The sympathetic nerves in BAT are generally thought to play the major role in control of acute and chronic changes in thermogenesis. Activation of thermogenesis is mediated by both ß and α-adrenergic receptors located on brown adipocytes (Géloën et al., 1988; Himms-Hagen, 1989). Surgical denervation of BAT produces an atrophy which is associated with reduced total protein and UCP content (Desautels et al., 1986; Park and

Himms-Hagen, 1988). However, denervated BAT still retains some capacity to grow in response to chronic stimulation by cold (Park and Himms-Hagen, 1988). This means that sympathetic innervation may not be the sole mediator of the trophic response of BAT. Furthermore, the growth that occurs in response to cafeteria feeding of BAT is not associated with an increase in T_4 5'-deiodinase activity, which is totally dependent on the sympathetic innervation (Park and Himms-Hagen, 1988). Also, it has been shown that trophic changes in BAT induced by chronic administration of noradrenaline are usually smaller than those due to cold-acclimation (Géloën et al., 1988). Thus, factors other than noradrenaline could be involved in the control of the trophic response of BAT, particularly in a cold environment.

In preliminary experiments, as described in the previous chapter, I demonstrated that CAP-DES induces a marked atrophy of BAT in rats and prevents or retards the growth of BAT that occurs in response to diet or to cold. It seems that neuropeptides of sensory nerves of BAT play a trophic role in maintenance of BAT in a functional state. However, whether the atrophy produced by capsaicin treatment is similar to that of surgically denervated BAT is not known.

Objective:

The principal objectives of the present experiment were to find out whether sensory neuropeptides play any role in response to cold after surgical denervation of sympathetic nerves of BAT, namely, to find out whether BAT can grow in response to cold in the absence of both its sympathetic neurotransmitter, noradrenaline, and its sensory

neurotransmitters, CGRP and SP.

Methods:

Male Sprague-Dawley rats arrived at 5 weeks of age and were housed in individual plastic cages with 12 hours light cycle for 7 days. All rats had free access to water and chow (Agway, RMH 4020).

After 1 week adaptation to this environment, rats were anaesthetized with halothane and injected with either capsaicin or vehicle as described in chapter 3.5. 10 days later, surgical operations were performed. All rats were anaesthetized with a mixture of halothane and oxygen and interscapular BAT was denervated unilaterally as described in chapter 3.17.

Rats recovered to normal within 1 day after the operation. Half of the vehicle injected and half of the capsaicin injected rats were then maintained at 4°C. The other half of the rats remained at 26°C. After exposure to either 4°C or 26°C for 12 days, rats were killed by decapitation and their colonic temperature measured immediately. Interscapular BAT was removed and cleaned. Then, interscapular BAT was separated into right and left halves. Each half was processed separately and homogenized in 4ml of ice-cold isolation medium. Samples of homogenate were removed, frozen in liquid nitrogen and stored at -80°C for later assay of protein, UCP and T_4 5'D. Mitochondria were isolated as before and likewise frozen for later assay of protein and UCP. Due to only half BAT being used and very little mitochondria recovered from BAT of CAP-DES rats, GDP binding could not be performed in this experiment. Assays of protein, UCP and T_4 5'-deiodinase were done as described before.

Results are expressed as means ± SEM for the number of animals indicated. Statistical analysis used was two-way ANOVA. A significant difference is indicated when $p < 0.05$.

Results:

At 26°C, body weight and temperature of CAP-DES rats were normal (Table 7). Cold acclimation to 4°C for 12 days retarded body weight gain similarly in both CAP-DES and control rats. Like control rats, CAP-DES rats were able to regulate their body temperature at this time (Table 7).

Consistent with the previous experiment, wet weight of the intact side of BAT of CAP-DES rats was much less when compared with control rats in the warm environment (Table 7). Total protein content (Fig. 20) and UCP (Fig. 21) of BAT were significantly reduced by capsaicin treatment. In particular, the UCP, a key protein for BAT thermogenesis, was dramatically decreased (Fig. 21). However, concentration of BAT mitochondrial UCP (Fig. 22) was relatively normal, indicating that mitochondria were lost as a whole after CAP-DES. As before, the total activity of T_4 5'-deiodinase was normal (Fig. 23). After cold exposure for 12 days, wet weight of BAT in both CAP-DES and controls was significantly increased two fold (Table 7). Total protein content, UCP and T_4 5'-deiodinase activity were likewise increased, as well as concentration of mitochondrial UCP. The increases in CAP-DES rats were less than normal (Fig. 20-23).

In control rats at 26°C denervation did not alter wet weight of BAT (Table 7), protein content of BAT (Fig. 20), UCP level in BAT (Fig. 21) or in BAT mitochondria (Fig. 22), or T_4 5'-deiodinase activity in BAT (Fig. 23). As in the previous experiments (Chapter

TABLE 7
BODY AND TISSUE WEIGHTS AND BODY TEMPERATURES IN CONTROL AND CAP-DES RATS

	CONTROL		CAP-DES	
	WARM (26°C)	COLD (4°C)	WARM (26°C)	COLD (4°C)
(N)	8	8	9	8
Body Weight (g)	381.8 ±9.6	350.1‡ ±7.4	369.8 ±4.3	337.3‡ ±7.9
Body Temperature °C	37.3 ±0.2	37.6 ±0.3	37.6 ±0.2	38.2 ±0.1
BAT Weight (mg) Intact side	265.2 ±15.4	410.6‡ ±15.4	181.5* ±9.3	342.5‡* ±23.4
Denervated side	266.9 ±20.2	202.9† ±18.1	173.1* ±17.1	162.6† ±1.8

* Significant effect of capsaicin treatment, comparing tissue treated in the same way.
‡ Significant effect of cold, comparing similarly-treated rats.
† Significant effect of denervation, comparing tissues in the same animals.

(N) denotes the number of animals.
Note: There are two weights for BAT for each animal, one for the innervated side and one for the denervated side.

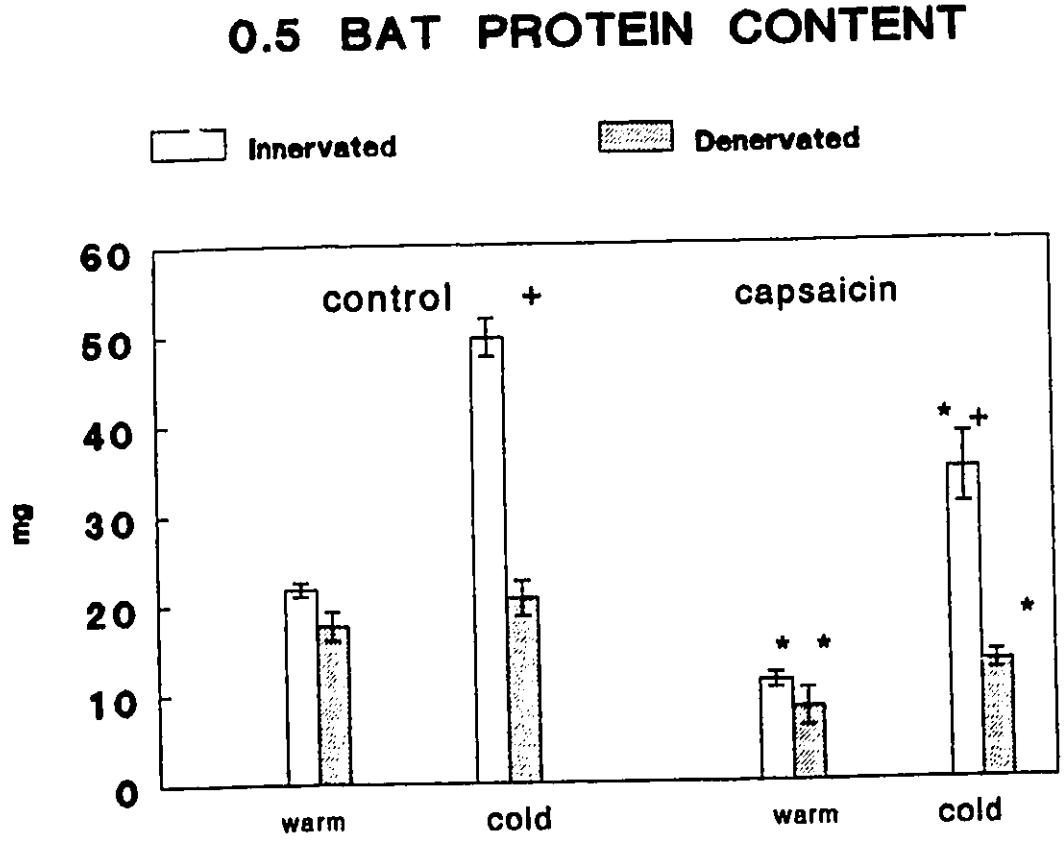


Fig. 20 Protein content in half interscapular brown adipose tisssue (IBAT), either innervated or denervated, of control vehicle-injected rats (control) and of capsaicin-desensitized rats (capsaicin). Rats were either at 26°C (warm) or had been at 4°C for 12 days (cold).

* Significant effect of capsaicin treatment. CAP induced a significant decrease in protein content in innervated BAT in rats in the warm that was not further altered by denervation (DEN). The level of protein in DEN BAT of CAP-DES rats was significantly lower than that in DEN BAT of control rats. In control rats, DEN did not signficantly alter protein content.
+ Significant effect of cold. Cold induced a significant increase in protein in innervated BAT in both control rats and CAP rats; the level in CAP rats in the cold is siginificantly lower than that in control rats in the cold. Cold did not significantly alter protein content of denervated BAT in either control or CAP rats.

Values are means±SEM for the number (see Table 7) of rats per group. A significant difference is indicated when $p < 0.05$.

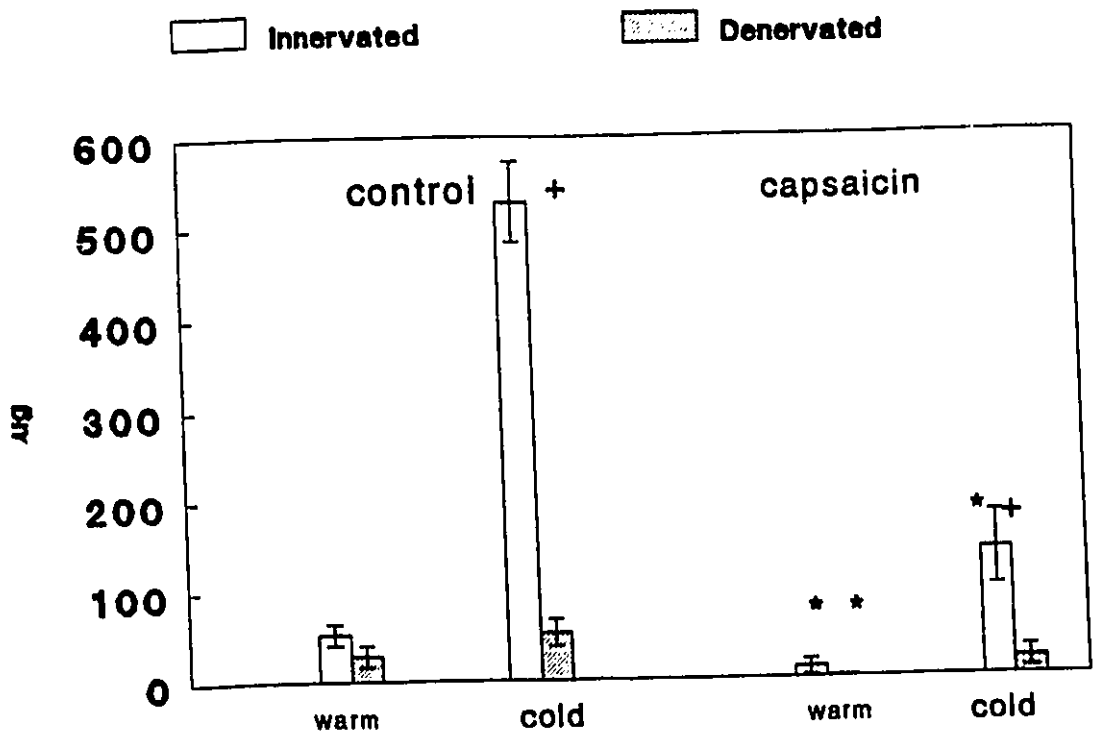


Fig. 21 Total uncoupling protein (UCP) content in half interscapular brown adipose tisssue (IBAT), either innervated or denervated, of control vehicle-injected rats (control) and of capsaicin-desensitized rats (capsaicin). Rats were either at 26°C (warm) or had been at 4 °C for 12 days (Cold).

* Significant effect of capsaicin treatment. CAP induced a significant decrease in UCP content of innervated and denervated BAT; the level was not further decreased by denervation (DEN). UCP in DEN BAT of CAP rats was significantly less than that in DEN BAT of control rats. DEN alone did not significantly alter UCP in BAT of control rats.
+ Significant effect of cold. Cold induced significant increases in UCP in both control and CAP rats; the level in CAP rats in the cold was significantly less than that in control rats in the cold. Cold did not significantly alter UCP level in DEN BAT in both control and CAP rats.

Values are means±SEM for the number (see Table 7) of rats per group. A significant difference is indicated when $p < 0.05$.

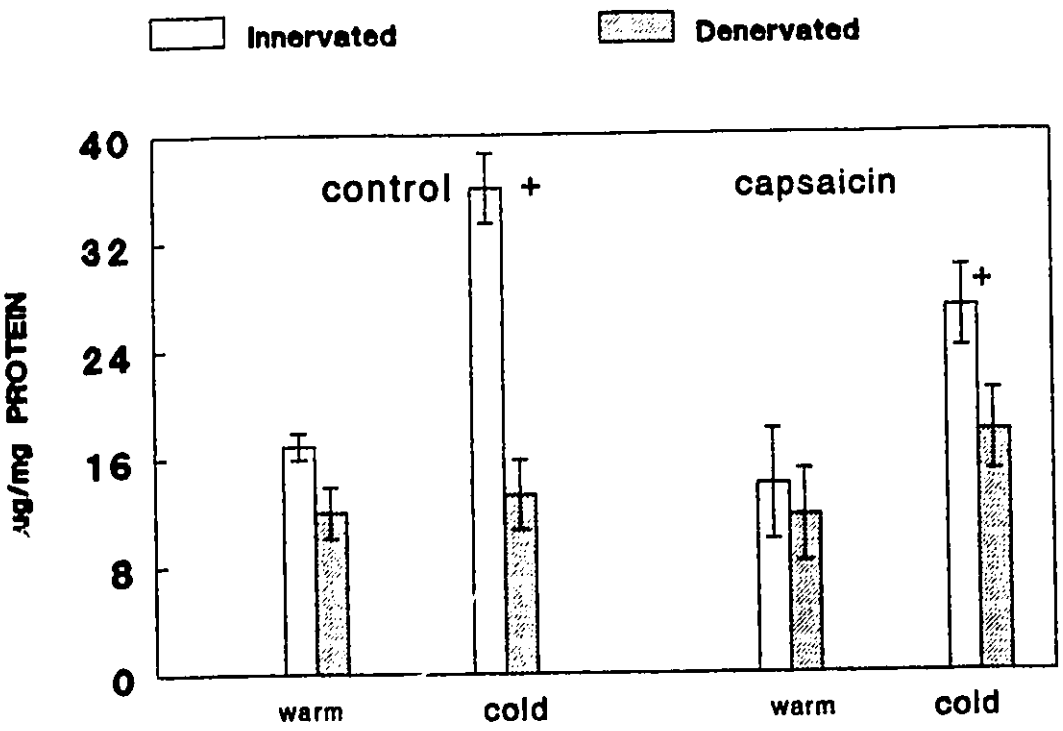


Fig. 22 Concentration of uncoupling protein (UCP) in mitochondria isolated from half interscapular brown adipose tisssue (IBAT), either innervated or denervated, of control vehicle-injected rats (control) and of capsaicin-desensitized rats (capsaicin). Rats were either at 26°C (warm) or had been at 4°C for 12 days (Cold).

There is no significant effect of CAP on either innervated BAT or on denervated BAT in rats in the warm.
+ Significant effect of cold. Cold induced a significant increase in innervated BAT, but not in denervated BAT in both control and CAP rats.

Values are means±SEM for the number (see Table 6) of rats per group. A significant difference is indicated when $p < 0.05$.

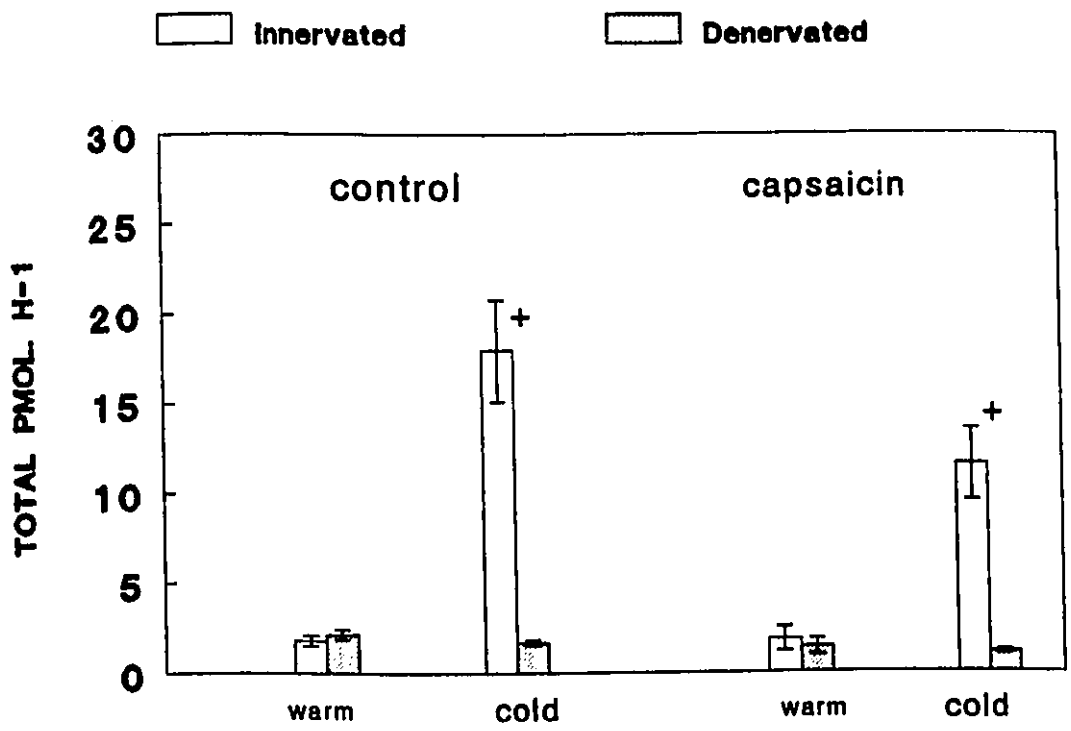


Fig. 23 Total content of thyroxine (T_4) 5'-deiodinase in half interscapular brown adipose tisssue (IBAT), either innervated or denervated, of control vehicle-injected rats (control) and of capsaicin-desensitized rats (capsaicin). Rats were either at 26°C (warm) or had been at 4°C for 12 days (Cold).

+ Significant effect of cold. Cold induced a significant increase in T_4 5'-deiodinase in innervated BAT in both control and CAP rats; the level in cold-acclimated CAP rats is not significantly different from that in cold-acclimated control rats.

Values are means±SEM for the number (see Table 6) of rats per group. A significant difference is indicated when $p < 0.05$.

5), the most dramatic effect of CAP-DES on BAT was the very marked reduction in total UCP content, almost reaching to zero level (Fig. 21). However, denervation caused only 40% reduction in UCP of BAT of control rats, to a level which was not significantly different from that of innervated BAT. No UCP was detectable in denervated BAT in CAP-DES rats (Fig. 21).

In cold-exposed control rats, denervation markedly reduced the increases in BAT total protein and UCP content, in concentration of mitochondrial UCP and in T_4 5'-deiodinase activity (Fig. 20-23). Similar to control rats, denervation resulted in reduction of increased protein and UCP content of BAT in CAP-DES rats after cold exposure. The concentration of UCP was not significantly altered by denervation in CAP-DES cold-acclimated rats and the concentration was not significantly different from that in denervated BAT of control cold-acclimated rats (Fig. 22). Denervation fully prevented the increase in activity T_4 5'-deiodinase in both control and CAP-DES rats (Fig. 23).

Discussion:

The previous studies (Chapter 5) demonstrated that CAP-DES caused atrophy of BAT which was mainly characterized by a lower total protein and total UCP. The same results were obtained in this experiment for the side of interscapular BAT with intact innervation. It has been known for many years that atrophy of BAT usually occurs when sympathetic tone in this tissue is absent or very low. The best studied example is the atrophy that occurs after surgical denervation. The present experimental design provided conditions that allowed atrophy produced by CAP-DES to be compared with the atrophy produced by

surgical denervation. Comparison of the effect of denervation in control rats with the effect of CAP-DES on intact BAT showed that the pattern of atrophy of BAT produced by CAP-DES differed from the atrophy induced by surgical denervation in that the decrease in total protein content was much greater and the depletion of UCP content much more profound. This pattern was not further modified by surgical denervation of BAT in the CAP-DES rats. Thus, the atrophying effect of CAP-DES differs from that produced by surgical denervation.

An immunohistochemical study done at the same time demonstrated that CGRP was absent in BAT after CAP-DES whereas noradrenaline content, measured by HPLC, was normal (Himms-Hagen et al., 1990). It seems likely, therefore, that the effect of CAP-DES can be attributed to loss of sensory neuropeptides, such as CGRP or SP whereas the effect of surgical denervation is more likely to be due to removal of noradrenaline, because immunoreactive CGRP still persists in denervated tissue (Himms-Hagen et al., 1990).

CHAPTER 7

TIME-COURSE OF ATROPHY OF BROWN ADIPOSE TISSUE IN CAPSAICIN-DESENSITIZED (CAP-DES) RATS

Background:

The previous studies (Chapters 5 and 6) have shown that CAP-DES produces atrophy of BAT and reduction in responses to diet or cold (Cui et al., 1990; Himms-Hagen et al., 1990). The atrophy of BAT is associated with a reduced content of total protein and total mitochondrial UCP. However, the concentration of UCP in isolated mitochondria of BAT in CAP-DES rats is the same as in control rats, indicating mitochondria were lost as a whole during atrophy. The atrophy is not due to the lack of the sympathetic innervation to the tissue since the noradrenaline content of BAT in CAP-DES is normal (Himms-Hagen et al., 1990) and the pattern of atrophy differs from that produced by surgical denervation (Chapter 6). As described in Chapters 5 and 6, CAP-DES rats are able to regulate thermogenesis in a cold environment. After exposure (1 day) or acclimation (7 or 12 days) to cold, thermogenesis of BAT in CAP-DES rat was increased and a trophic response occurred. The trophic response was characterized by increased content of total protein and UCP, mitochondrial concentration of UCP and activity of T_4 5'-deiodinase. But the extent of the increase in BAT in CAP-DES was smaller compared with control rats. In response to infusion of noradrenaline intravenously, CAP-DES rats also had a smaller capacity to increase metabolic rate consistent with the smaller size of their BAT (Chapter 5).

In the experiments described in Chapters 5 and 6, the function of BAT in CAP-DES

rats was assessed from 11 to 28 days after the last injection. However, the rapidity of atrophy that occurs in CAP-DES rats at shorter times after the injections is unknown.

Objectives:

The objective of this study was to assess the time-course of atrophy of BAT in CAP-DES rats at shorter times after the desensitization procedure, and to characterize more fully the nature of the atrophy induced by the capsaicin desensitization. Also, to examine whether the effect of capsaicin is specific for BAT or whether atrophy of other tissues also occurs.

Methods:

Male Sprague-Dawley rats were obtained from Charles River Laboratories at 5 weeks of age. As described before, all rats were housed in individual plastic cages at 26°C with 12 hours light cycle. They had free access to food (Agway, RMH 4020) and water.

One week later, rats were anaesthetized with halothane and given 3 consecutive days of capsaicin injections as described in chapter 3.5.

Two groups of rats were obtained and injected in June 1989 and studied 1, 3 or 14 days later. Two more groups of rats were injected in October 1989 and studied at 28 or 52 days later. Thus, function of BAT in CAP-DES rats was studied at 5 different time points. Food intake and body weight were recorded once a week. The difference between initial and final body weight is described as body weight gain.

Rats were killed by decapitation at 08:30-09:00 hours. Colonic temperature was measured immediately. Interscapular BAT was removed and placed in ice-cold isolation

medium as before. White epididymal adipose tissue was removed and weighed. Organs (liver, heart and kidneys) were also removed, weighed and frozen for later analysis. BAT was cleaned of white adipose tissue and muscle, weighed and homogenized using a motor-driven Teflon-glass homogenizer. Samples were frozen in liquid nitrogen and stored at -80°C for later assay of protein, DNA, UCP and cytochrome oxidase (COX). These assays were performed as described as Chapter 3.

Statistical analyses used t-tests, comparing control and CAP-DES rats studied at the same time. A significant difference is indicated when $P < 0.05$. The effect of time was not analyzed because the different time points were obtained at different times of year using different batches of rats.

Results:

The food intake of CAP-DES was decreased during the 3 days of injections and body weight was lower when compared with that of control rats (data not shown) . Final body weight (Fig. 24) and body weight gain (Fig. 25) of CAP-DES rats were significantly less than vehicle-injected rats at 1 day, 3 days and 14 days. Gradual recovery of body weight and body weight gain of CAP-DES started at 28 days and reached the normal range by 52 days. The lower body weight was accompanied by a lesser epididymal adipose tissue weight (Fig. 26). When the white fat weight was expressed per unit body weight, the white fat was significant lower only at 3 days (Fig. 27).

As in previous studies, appearance of BAT of CAP-DES rats was pale and small and weight of BAT was significantly lower at 1, 3 and 14 days, and normal by 52 days (Fig. 28).

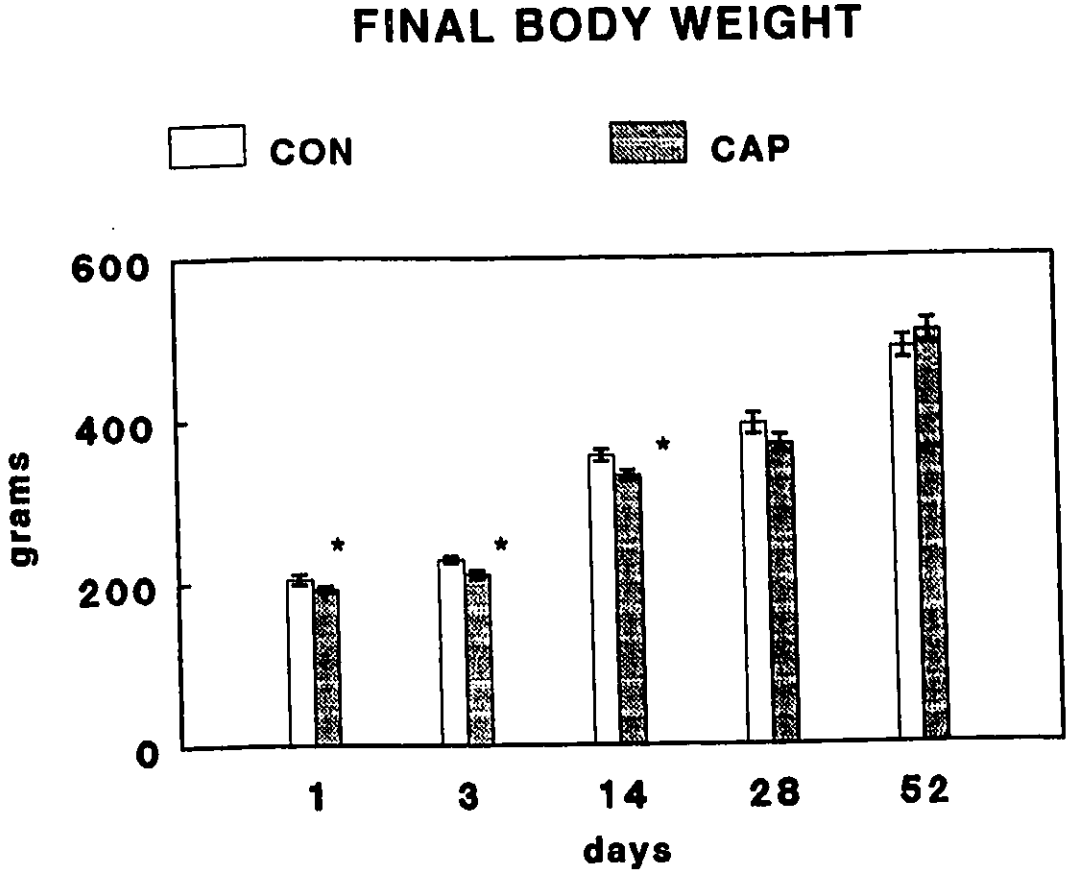


Fig. 24 Body weights of control (CON) and capsaicin-desensitized (CAP) rats at various times after the last day of injection.

* Significant effect of CAP-DES. CAP-DES significantly decreases body weight at 1, 3 and 14 days.

Values are means ± SEM for the number (n) of rats per group. A significant difference is indicated when $p < 0.05$.

n = 6 at 1, 14, 28 and 52 days.
n = 7 at 3 days.

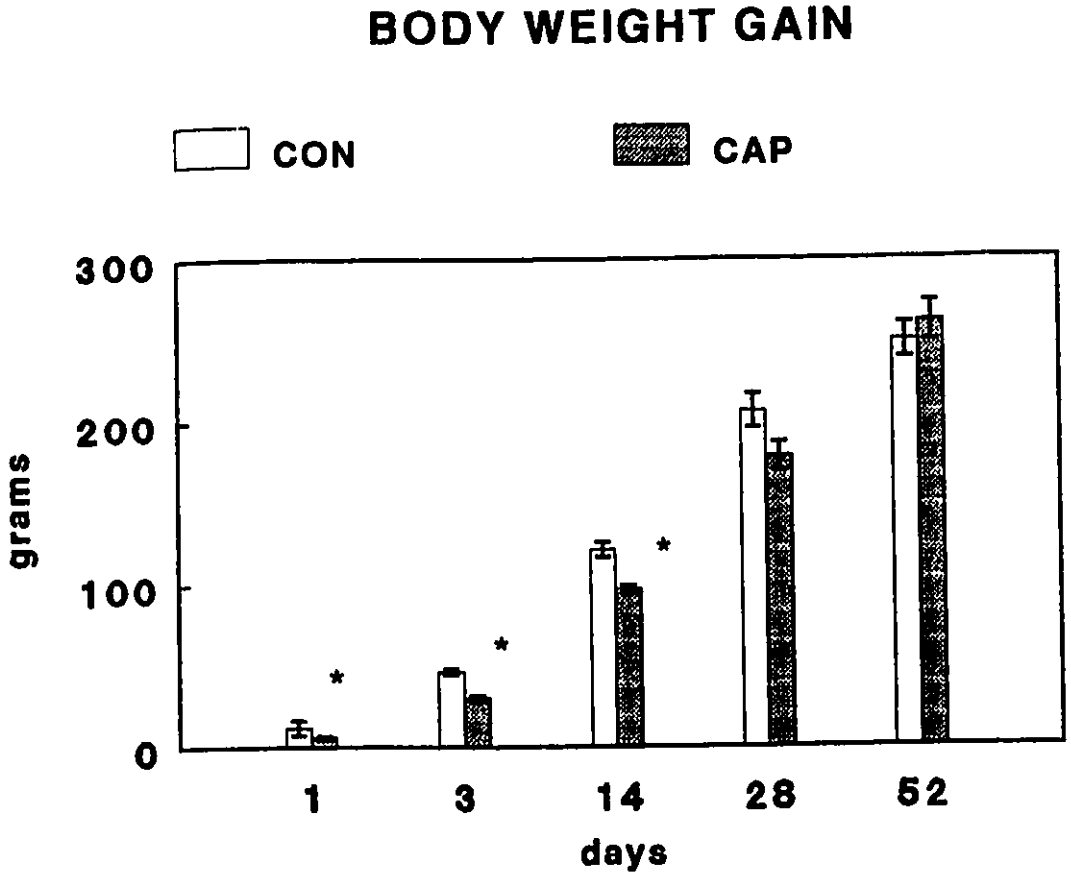


Fig. 25 Body weight gain of control (CON) and capsaicin-desensitized (CAP) rats at various times after the last day of injection. Gain is calculated from the weight before the first injection and the weight on the day after the last day of injection indicated.

* Significant effect of CAP-DES. CAP-DES significantly decreases body weight gain at 1, 3 and 14 days.

Values are means ± SEM for the number (n) of rats per group. A significant difference is indicated when $p < 0.05$.

n = 6 at 1, 14, 28 and 52 days.
n = 7 at 3 days.

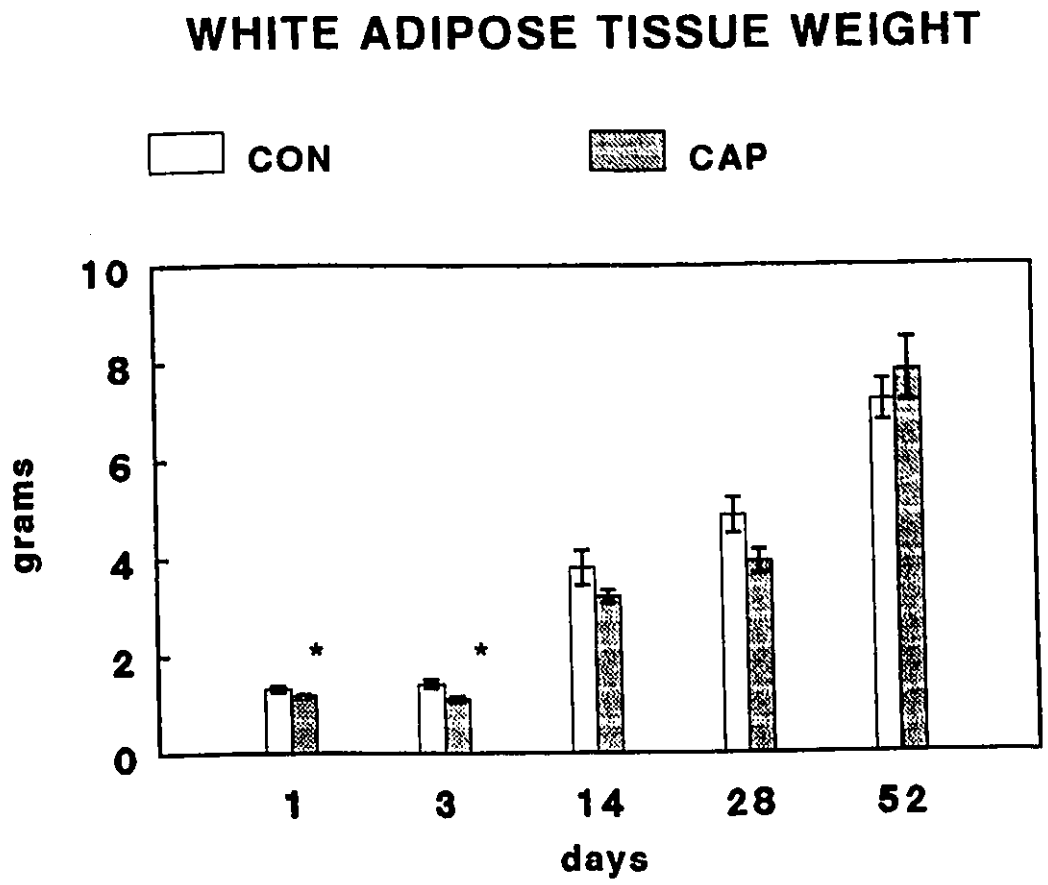


Fig. 26 Weight of white epididymal adipose tissue of control (CON) and capsaicin-desensitized (CAP) rats at various times after the last day 6 injection.

* Significant effect of CAP-DES. CAP-DES significantly decreases white fat weight at 1 and 3 days.

Values are means±SEM for the number (n) of rats per group. A significant difference is indicated when $p < 0.05$.

n = 6 at 1, 14, 28 and 52 days.
n = 7 at 3 days.

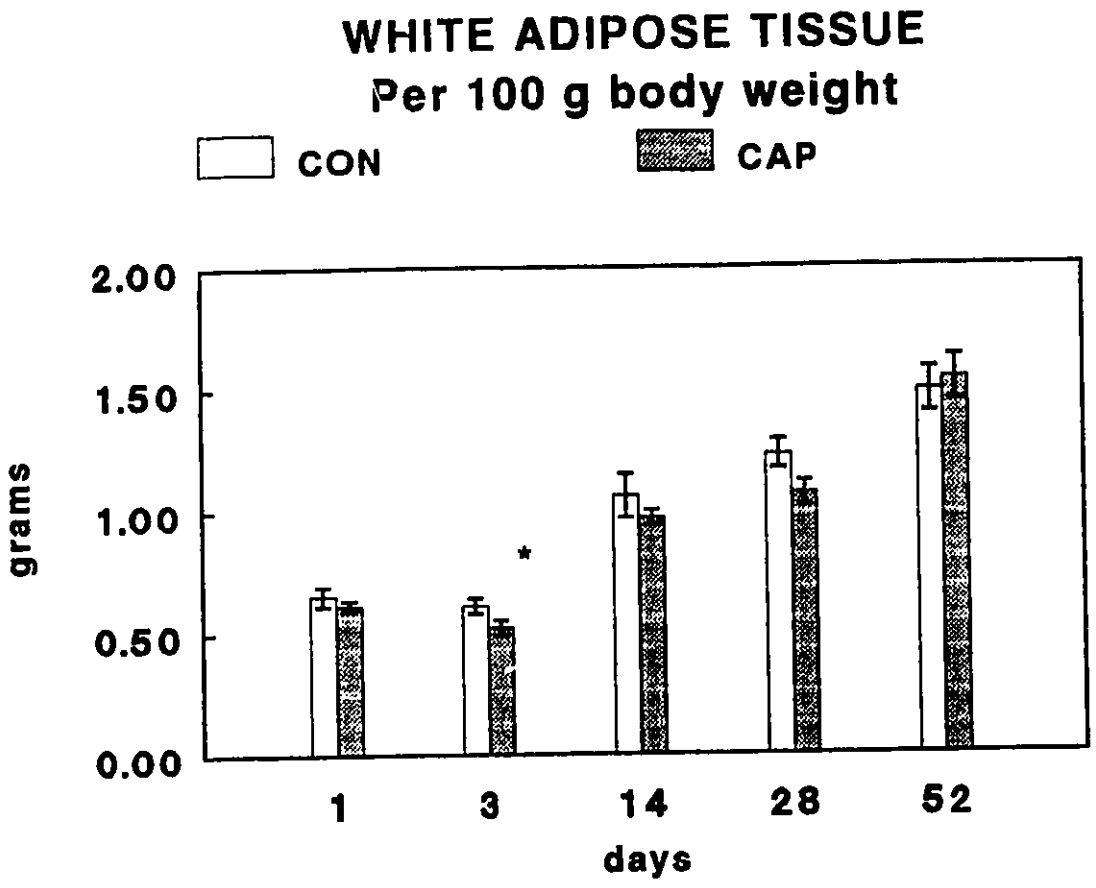


Fig. 27 Weight of white epididymal adipose tissue expressed in terms of body weight of control (CON) and capsaicin-desensitized (CAP) rats at various times after the last day of injection.

* Significant effect of CAP-DES. CAP-DES significantly decreases white fat weight only at 3 days.

Values are means±SEM for the number (n) of rats per group. A significant difference is indicated when $p < 0.05$.

n = 6 at 1, 14, 28 and 52 days.
n = 7 at 3 days.

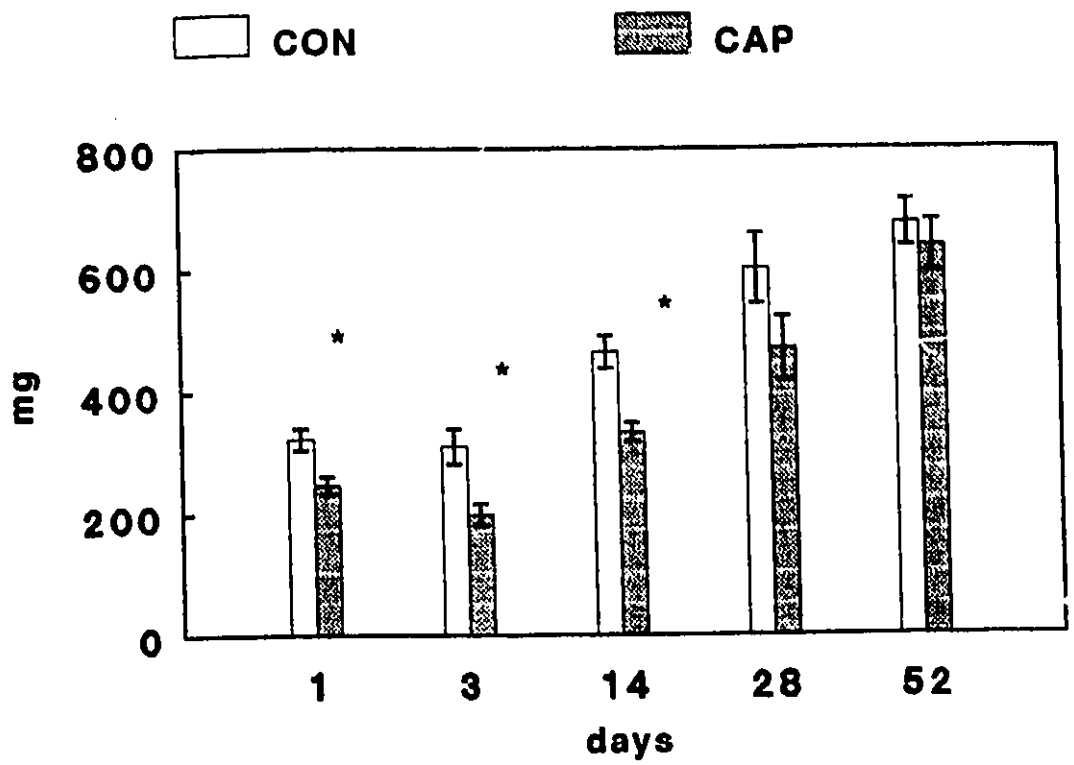


Fig. 28 Weight of interscapular BAT of control (CON) and capsaicin-desensitized (CAP) rats at various times after the last day of injection.

* Significant effect of CAP-DES. CAP-DES significantly decreases weight of brown adipose tissue at 1, 3 and 14 days.

Values are means±SEM for the number (n) of rats per group. A significant difference is indicated when $p < 0.05$.

n = 6 at 1, 14, 28 and 52 days.
n = 7 at 3 days.

In CAP-DES rats, there was very rapid loss from BAT of protein (Fig. 29), UCP (Fig. 30), COX (Fig. 31) and DNA (Fig. 32). At 1 day, the total protein, UCP, COX and DNA were reduced by about 30%, 90%, 85% and 17% respectively (Fig. 29-30). At 3 days, similar patterns were obtained, except that large variability did not show a significant reduction in UCP and COX content. However, at 14 days significant reductions in protein, UCP, COX and DNA still persisted. By 28 days, all these reductions had recovered to normal. At 52 days, except for a decrease in total protein, all other functional indexes of BAT were similar to those of control rats.

A small and transient loss of COX occurred in liver, heart and kidney at 3 days only (Table 8). There was a temporary decrease in protein and DNA content in liver of CAP-DES only at 3 days. All organs could grow with age, as judged from the increasing DNA, protein content and organs weight. By 28 days, all measurements of three organs in CAP-DES rats showed the same results as control rats.

Discussion:

In the previous studies (Chapter 5), atrophy of BAT had occurred by 11-14 days after the three consecutive days of capsaicin injections and appeared to persist, to a variable extent, for 23-28 days (Chapter 5 and 6). The present study shows that the most extensive destruction of BAT mitochondria content occurred at 1 day as judged from the parallel reductions in UCP and COX. As seen in Fig. 29 and 32 protein and DNA content were also significantly decreased at this time. The reduction in DNA content indicated atrophy of BAT is accompanied by cell loss and retarded growth of BAT. The lowest content of DNA was

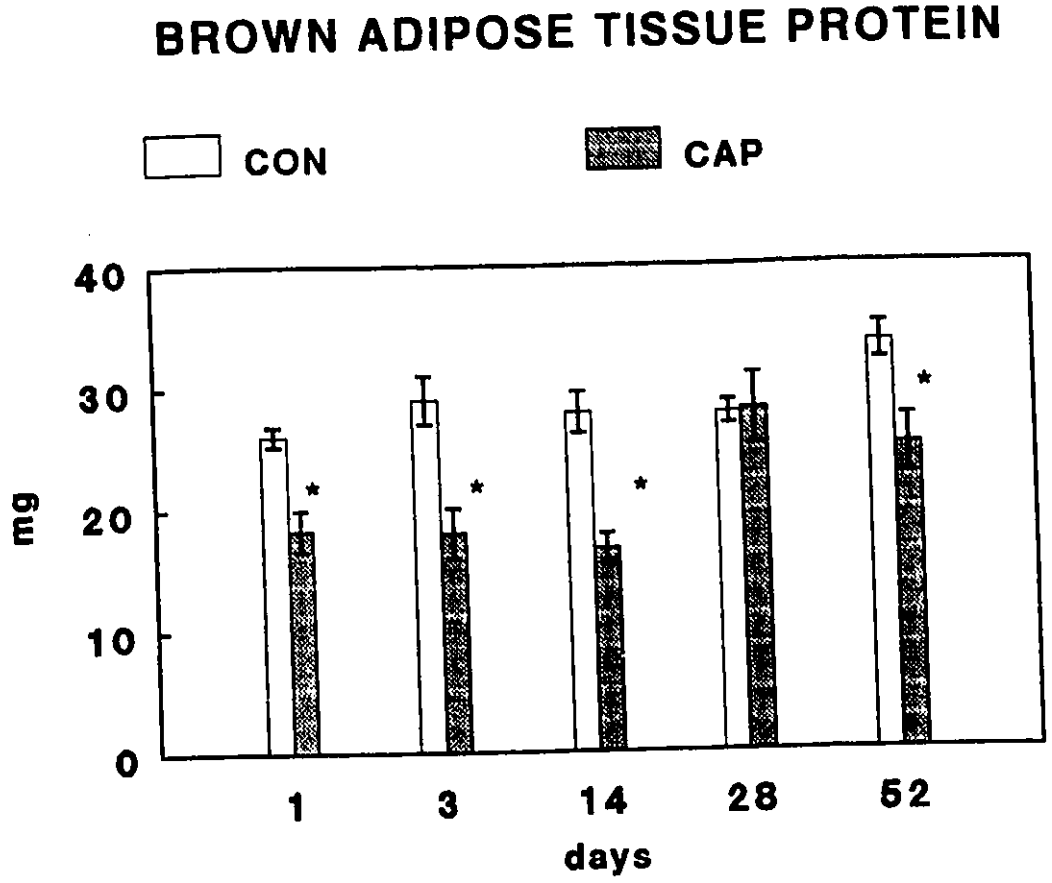


Fig. 29 Total protein in interscapular BAT of control (CON) and capsaicin-desensitized (CAP) rats at various times after the last day of injection.

* Significant effect of CAP-DES. CAP-DES significantly decreases BAT protein only at 1, 3, 14 and 52 days.

Values are means ± SEM for the number (n) of rats per group. A significant difference is indicated when $p < 0.05$.

n = 6 at 1, 14, 28 and 52 days.
n = 7 at 3 days.

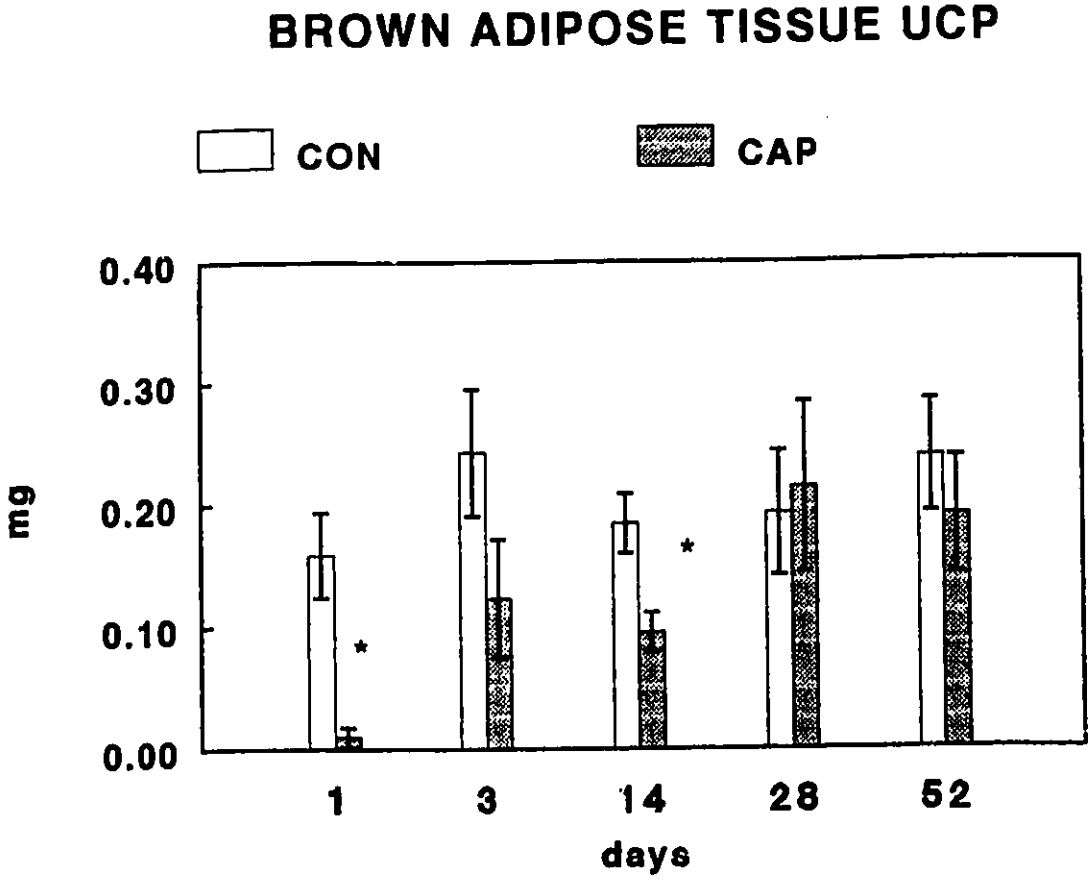


Fig. 30 Total uncoupling protein (UCP) content of interscapular BAT of control (CON) and capsaicin-desensitized (CAP) rats at various times after the last day of injection.

* Significant effect of CAP-DES. CAP-DES significantly decreases UCP in BAT only at 1 and 14 days.

Values are means±SEM for the number (n) of rats per group. A significant difference is indicated when $p < 0.05$.

n = 6 at 1, 14, 28 and 52 days.
n = 7 at 3 days.

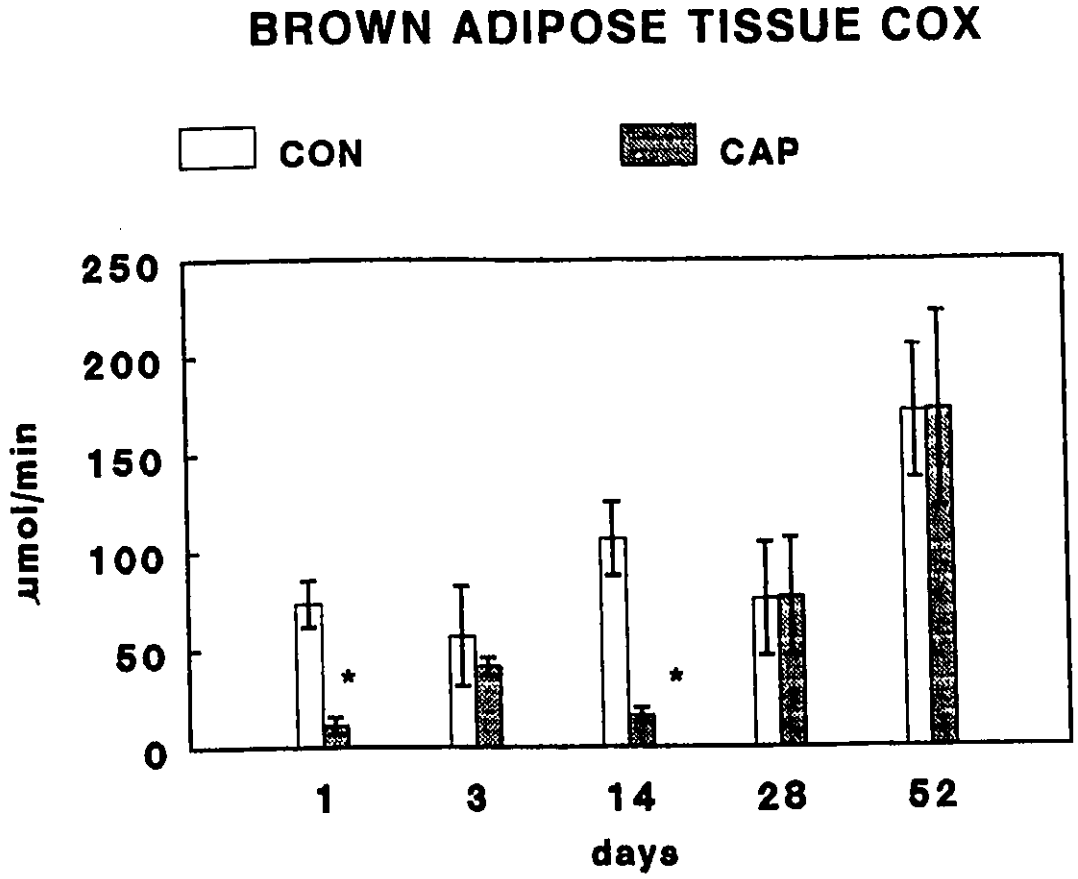


Fig. 31 Total cytochrome oxidase (COX) content of interscapular BAT of control (CON) and capsaicin-desensitized (CAP) rats at various times after the last day of injection.

* Significant effect of CAP-DES. CAP-DES significantly decreases COX content in BAT only at 1 and 14 days.

Values are means ± SEM for the number (n) of rats per group. A significant difference is indicated when $p < 0.05$.

n = 6 at 1, 14, 28 and 52 days.
n = 7 at 3 days.

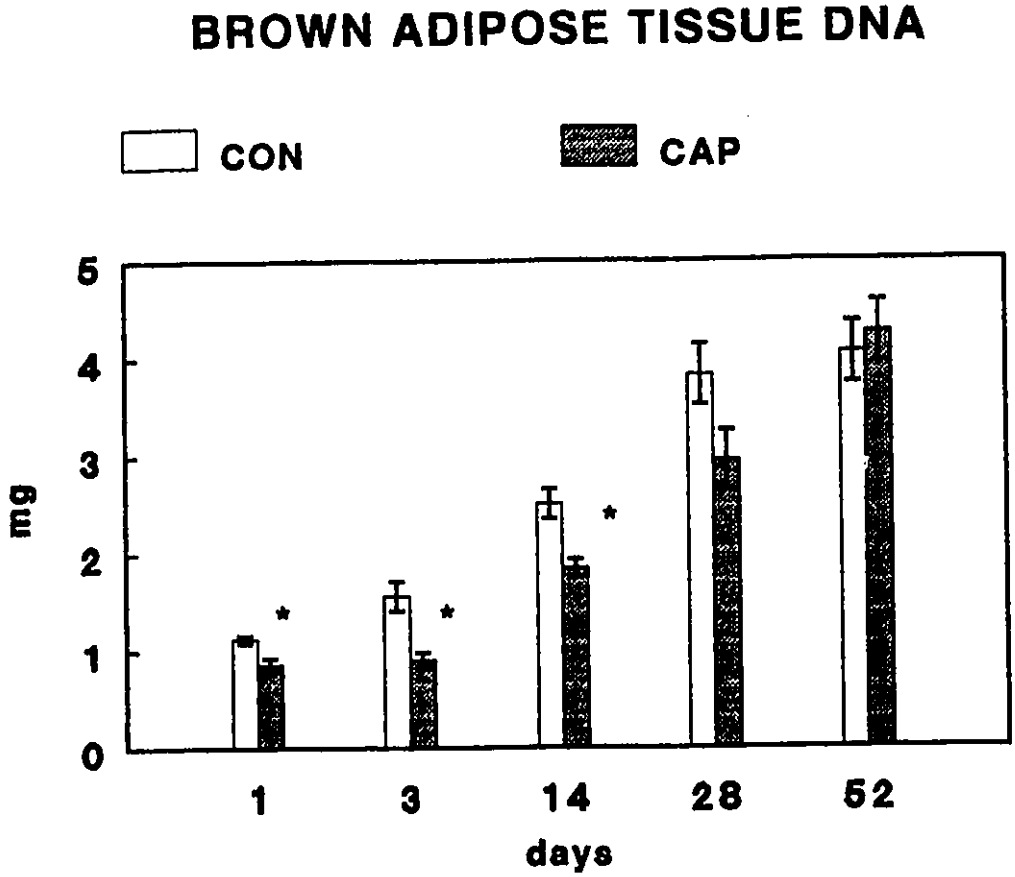


Fig. 32 DNA content of interscapular BAT of control (CON) and capsaicin-desensitized (CAP) rats at various times after the last day of injection.

* Significant effect of CAP-DES. CAP-DES significantly decreases DNA content in BAT only at 1, 3 and 14 days.

Values are means±SEM for the number (n) of rats per group. A significant difference is indicated when $p < 0.05$.

n = 6 at 1, 14, 28 and 52 days.
n = 7 at 3 days.

TABLE 8
WEIGHT AND PROTEIN, DNA AND CYTOCHROME OXIDASE (COX) CONTENTS OF LIVER, HEART AND KIDNEY OF CONTROL (CON) AND CAPSAICIN-DESENSITIZED (CAP) RATS AT VARIOUS TIMES AFTER THE LAST INJECTION

	1 day		3 days		14 days		28 days		52 days	
	CON (6)	CAP (6)	CON (7)	CAP (7)	CON (6)	CAP (6)	CON (6)	CAP (6)	CON (6)	CAP (6)
LIVER										
Weight, g	9.5 ±0.49	8.7 ±0.27	11.5 ±0.33	10.6 ±0.47	15.4 ±0.74	13.9 ±0.12	16.9 ±0.82	15.8 ±0.70	18.3 ±0.56	20.4 ±0.62
Protein, g	2.4 ±0.09	2.2 ±0.07	2.6 ±0.07	2.3 * ±0.06	3.2 ±0.14	3.1 ±0.12	4.3 ±0.30	3.8 ±0.17	5.0 ±0.18	4.8 ±0.10
DNA, mg	63.3 ±3.31	60.6 ±2.76	74.4 ±2.46	65.8 * ±2.35	96.3 ±7.83	87.5 ±3.24	115.4 ±4.90	108.3 ±4.87	199.5 ±4.84	205.9 ±6.66
COX, mmol/min	6.0 ±0.79	6.3 ±0.79	7.8 ±1.20	3.9* ±0.37	7.8 ±1.48	10.4 ±1.90	10.4 ±1.86	7.1 ±1.36	16.9 ±1.21	14.7 ±3.20

TABLE 8 (cont'd)

	1 day		3 days		14 days		28 days		52 days	
	CON (6)	CAP (6)	CON (7)	CAP (7)	CON (6)	CAP (6)	CON (6)	CAP (6)	CON (6)	CAP (6)
HEART										
Weight, g	0.67 ±0.02	0.62 ±0.02	0.72 ±0.03	0.70 ±0.03	0.97 ±0.02	0.87 * ±0.02	1.03 ±0.04	1.03 ±0.03	1.15 ±0.06	1.20 ±0.03
Protein, mg	141 ±4.2	144 ±4.9	196 ±6.9	196 ±6.2	202 ±3.1	188 * ±4.0	222 ±6.9	235 ±6.2	294 ±10.5	286 ±13.6
DNA, mg	4.51 ±0.11	4.18 ±0.17	5.33 ±0.32	4.87 ±0.22	6.37 ±0.20	6.52 ±0.18	8.15 ±0.34	7.89 ±0.38	5.98 ±0.42	5.70 ±0.20
COX, mmol/min	0.29 ±0.06	0.31 ±0.05	1.67 ±0.25	0.87 * ±0.08	0.59 ±0.05	0.56 ±0.07	0.93 ±0.18	0.67 ±0.06	2.07 ±0.54	2.07 ±0.35
KIDNEY										
Weight, g	1.84 ±0.04	1.72 ±0.06	2.08 ±0.08	1.98 ±0.07	2.68 ±0.05	2.58 ±0.05	2.80 ±0.10	2.91 ±0.12	3.25 ±0.10	3.40 ±0.09
Protein, mg	362 ±7.3	344 ±7.5	389 ±10.5	372 ±9.3	521 ±25.3	493 ±13.8	532 ±13.4	545 ±20.2	736 ±24.0	834 ±29.0
DNA, mg	11.0 ±0.35	11.6 ±0.35	13.3 ±0.97	12.3 ±0.51	6.7 ±0.20	7.7 ±0.83	8.1 ±0.36	8.2 ±0.43	21.6 ±0.94	22.3 ±0.74
COX, mmol/min	1.00 ±0.03	0.87 ±0.14	0.81 ±0.09	0.59 * ±0.09	1.39 ±0.42	0.96 ±0.11	0.78 ±0.11	0.66 ±0.08	1.82 ±0.22	2.08 ±0.49

* Significant difference between control and capsaicin-desensitized rats, comparing animals studied at the same time.

seen at 3 days. The functional capacity of BAT gradually recovers to normal by 28-52 days. CAP-DES also affected mitochondria of the three major organs, liver, heart and kidney. But the effect was much smaller and more transient, being apparent only at 3 days, compared with the more profound and longer-lasting effects on BAT.

As explained in the previous chapter , dramatic loss of mitochondria from BAT of the CAP-DES rat might be due to the removal of a trophic influence of sensory neuropeptides on BAT, such as CGRP and SP. They might normally stimulate synthesis of mitochondria in BAT or have an inhibitory influence on mitochondriolysis. The other possibility is that the capsaicin itself can directly act on mitochondria or cell membrane of BAT and destroy mitochondria and cells (Szolcsanyi et al., 1971; Marsh et al., 1987; Holzer, 1991). This can be supported by loss of DNA content and mitochondria mass.

The recovery of BAT was slow, taking about 28-52 days. This might be due to the slow regeneration of the sensory nerves and consequent restoration of the normal influence of sensory neuropeptides. The slow recovery of BAT might also be due to compensation by the trophic influence of the sympathetic nervous system for the missing influence of the sensory nerves. BAT of CAP-DES rat has a normal noradrenaline content (Himms-Hagen et al., 1990) and a normal sympathetic nervous system activity which can be stimulated by feeding of sucrose or exposure to cold and suppressed by fasting (J.B. Young, personal communication). In fact, BAT of CAP-DES rats can indeed grow in response to the increased sympathetic nervous system stimulation that occurs during cold-acclimation (Chapter 5 and 6).

Atrophy of BAT has been associated with altered energy balance in many kinds of

obese animal. Atrophy of BAT could cause a deficit in energy expenditure and contribute to the development of obesity in these animals (Chapter 1). Thus, it can be speculated that atrophy of BAT produced by CAP-DES should make the rats have a tendency to obesity. However, results obtained from chapter 5 did not show any agreement with this hypothesis. Even when a cafeteria diet was eaten by CAP-DES rats for 1 or 3 weeks, the increased weight of white adipose tissue was similar to that of control rats. In the present experiments, obesity did not appear by 52 days after injection. Instead, CAP-DES rats had normal body weight and body weight gain. Their food intakes were also similar to those control rats. But, we do not know whether longer term effect of CAP-DES or long term high fat diet feeding would produce obesity in the CAP-DES animal. Further studies are needed.

In summary, rapid and extensive destruction of BAT mitochondria occurs in CAP-DES rats, the most marked state being seen at 1 day. The atrophy of BAT by CAP-DES was accompanied by loss of cells. Therefore, it can be suggested that sensory nerves play a role in control of metabolic capacity of BAT, especially in control of the thermogenic capacity of its mitochondria and its growth. CAP-DES has relatively less effect on liver, heart and kidney.

CHAPTER 8

ACUTE EFFECT OF CAPSAICIN INJECTION ON TEMPERATURE OF BAT

Background:

Capsaicin, the pungent principle in red pepper, induces a "hot" sensation and, in high concentration, burning pain when applied to the skin or mucous membranes. The consumption of certain spiced foods is well known to lead to a sensation of warmth.

Recently, in man, Henry and Emery (1985) have demonstrated that the consumption of a spiced meal containing both chili and mustard sauces increased diet-induced thermogenesis by 25% over a 3 hour period above the same meal without spice addition. Dietary supplementation of capsaicin in high fat diets lowers the perirenal adipose tissue weight and serum triglyceride concentration in rats (Kawada et al., 1986a) due to the enhancement of energy metabolism (Kawada et al., 1986b). Intraperitoneal injection of capsaicin stimulates oxygen consumption (Kawada et al., 1986b), probably by stimulating release of adrenaline from the adrenal glands (Watanabe et al., 1987), since it was blocked by ß-adrenergic blocker (propranolol) (Kawada et al., 1986b).

As mentioned before, BAT is the most important site of thermogenesis in cold-adapted rats stimulated by noradrenaline (Foster and Frydman, 1978) or by cold exposure (Foster and Frydman, 1979). BAT is also the site of diet-induced thermogenesis in the rat (Rothwell and Stock, 1979) although this view has recently been challenged by Ma et al. (1988). Administration of capsaicin activates BAT function in rats (Yoshida et al., 1988). It has been demonstrated that intramuscular injection of capsaicin increases significantly

interscapular BAT temperature without affecting the rectal temperature and increases significantly GDP binding and mitochondrial oxygen consumption in interscapular BAT (Yoshida et al., 1988). Whether capsaicin-induced increase in temperature of BAT could cause overheating and destruction of BAT is unknown.

Objective:

The objective of this experiment was to measure the temperature of BAT after subcutaneous injection of capsaicin at the same initial dose as used in the capsaicin desensitization procedure.

Methods:

Male Sprague-Dawley rats were obtained from Charles River Laboratories at 7 weeks of age. They were housed in individual plastic cages with free access to chow and water. Rats were divided into two groups and maintained at either 26°C or 4°C for 5 weeks. These rats were not injected with capsaicin until the day of experiment. Rats at 4°C were transferred to 26°C for one day before the measurement of their oxygen consumption. The measurement of oxygen consumption after injection of capsaicin in anaesthetized rats was the same as described as in Chapter 3.

In addition, intravenous infusion of CGRP was also performed to see whether BAT and colonic temperatures could be changed. The CGRP dose used in this experiment was 10 pmol/100g. min which has shown to have a vasodilatation effect in the rat (Marshall et al., 1987). After 40 minutes infusion with CGRP, noradrenaline was infused subsequently

(0.5 μg/100 cm^2. min for 20 min.) for comparison.

Results:

There was a small but reproducible and delayed increase in oxygen consumption after capsaicin injection in pentobarbital-anaesthetized rats (Fig. 33). The response to the drug started at 50-80 minutes after administration of capsaicin and terminated by 100 to 120 minutes. The increase in oxygen consumption was only 16.5±2.29% ($n=6$, $P<0.001$) and preceded an increase in temperature of BAT. In 3 warm-acclimated rats the increase in BAT temperature was 0.27±0.133°C and in 3 cold-acclimated rats the increase was 0.68±0.088°C. However, these same animals, subsequently in response to intravenous infusion of noradrenaline, had a large increase in BAT temperature which was followed by large increase in oxygen consumption. The increase in rectal temperature was much smaller.

Intravenous infusion of CGRP did not alter temperatures of BAT and colon (Fig. 34) but, infusion of noradrenaline in the same rat significantly increased both BAT and colonic temperature (Fig. 34).

Discussion:

It has been shown that capsaicin produces atrophy of BAT in the rat (Chapters 5, 6 and 7). With respect to the mechanism of BAT atrophy, several hypotheses were proposed. One of them is that capsaicin itself could destroy BAT. Capsaicin might cause overheating and consequent selective activation of proteolysis since it can activate sympathetic nerves

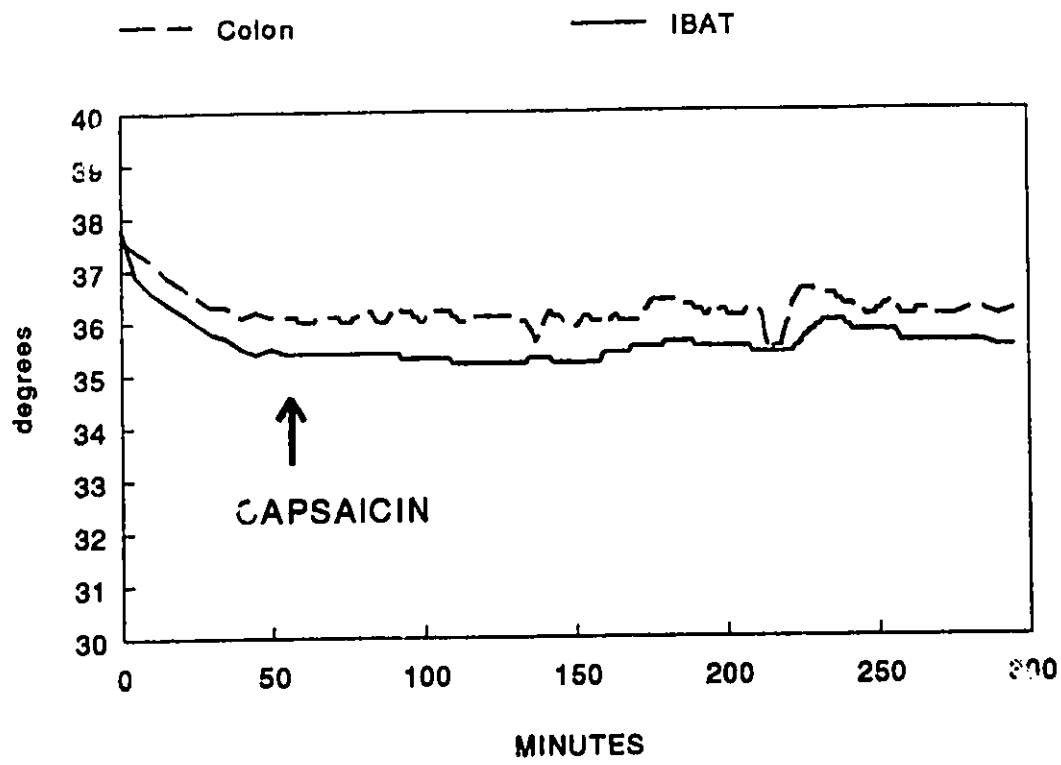


Fig. 33 Change in colonic and interscapular BAT (IBAT) temperatures in anaesthetized cold-acclimated rat after capsaicin injection (12.5 mg/kg s.c.).

A typical experiment is shown. In six experiments, the response to drug started at 50-80 min after capsaicin injection and terminated by 100 to 120 min. There was no significant effect on colonic temperature and only a very small effect on BAT temperature.

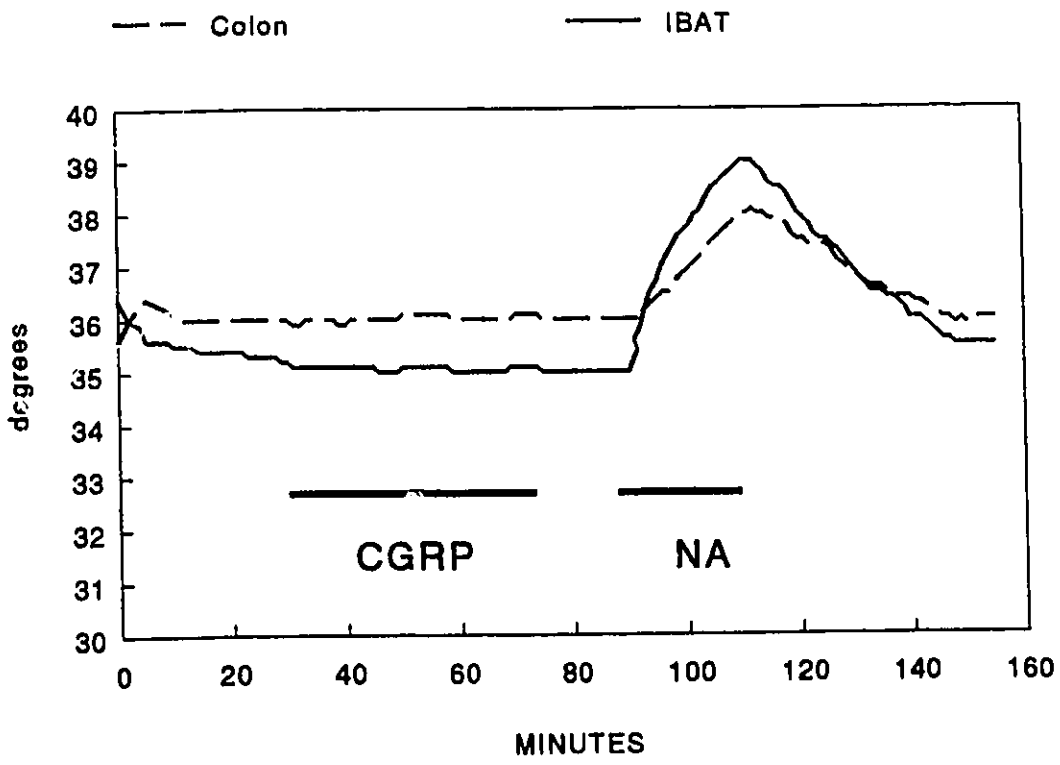


Fig. 34 Change in colonic and interscapular BAT (IBAT) temperatures during 40 minutes intravenous infusion of CGRP and 20 minutes infusion of noradenaline (NA) in anaesthetized cold-acclimated rat.

Infused CGRP: 10 pmol/100g.min.
NA: 0.5μg/100cm^2.min.

There was no significant change in colonic and IBAT temperatures during intravenous infusion of CGRP for 40 min, but infusion of NA resulted in large increase in both colonic and IBAT temperatures.

and induce an acute effect on BAT thermogenesis (Yoshida et al., 1988; Kawada et al., 1986). However, this hypothesis was not supported by the present results. There was a small increase in oxygen consumption. This result agrees with findings of Kawada et al. (1986). It can been seen that only a small increase in BAT temperature occurs after administration of capsaicin. The dose used was the same as the dose used for the first injection in the desensitization procedure. Thus, elevated BAT temperature by CAP-DES could not account for the atrophy of BAT. Also, other studies have demonstrated that lateral hypothalamic lesions induce a very high temperature of BAT but do not have any acute atrophying effect on BAT (Park et al., 1988; Corbett et al., 1988).

Infusion with CGRP did not have any acute thermogenic effect on BAT in the anaesthetized rats. If CGRP play a role in control of BAT thermogenesis, as suggested in previous chapters, its mechanism of action on BAT must differ from that of noradenaline. The activation of BAT thermogenesis by noradrenaline is largely mediated by activation of adenylate cyclase (Bukowiecki, 1984). However, stimulation of adenylate cyclase by CGRP in BAT has not been reported, although such an effect occurs in other cells (Haegerstrand et al., 1990; Mile et al., 1989). CGRP may promote other metabolic systems to stimulate BAT function, not detectable by measurement of BAT temperature. CGRP increases phosphoinositide turnover in skeletal muscle cell (Laufer and Changeux, 1989). And, the activation of this transmembrane pathway can contribute to thermogenesis of BAT (Mohell et al., 1984; Nanberg and Putney, 1986). Thus, it is possible that CGRP has some effect on BAT by modulation of the phosphatidylinositol pathway.

Chapter 9

Effect of chronic administration of calcitonin gene-related (CGRP) on brown adipose tissue in CAP-DES rats.

Background:

The previous chapters have clearly demonstrated that BAT atrophied after capsaicin desensitization. Atrophied BAT was not responsive to stimulation by cafeteria feeding (Chapter 5). The growth of BAT in response to cold was significantly decreased (Chapter 5 and 6). The time course studies (Chapter 7) have shown that functional impairment of BAT in CAP-DES rats is rapid and lasts for a long time. These observations suggest that neuropeptides of sensory nerves in BAT could be involved in maintenance of BAT in a functional state and play a trophic role in control of BAT growth.

However, the mechanism of atrophy caused by capsaicin is unknown. Two proposals should be considered in terms of the release of neuropeptides from sensory nerves. As discussed in chapter 5, one is that the atrophy of BAT is induced by releasing large amounts of neuropeptides that damage the tissue after capsaicin treatment. The other possibility is that atrophy is induced by the absence of sensory neuropeptides after they have been depleted by capsaicin. This experiment was designed to directly investigate the role of one neuropeptide, CGRP, characteristic of sensory nerves, in BAT growth.

Objective:

To find out whether CGRP exerts a direct trophic influence on BAT in normal rats

or improves the atrophy of BAT in CAP-DES rats.

Methods:

Male Sprague-Dawley rats were obtained from the same sources as previous studies. They arrived at 5 weeks of age and were housed in individual plastic cages at 26°C with 12 hours light cycle. Laboratory chow and water were provided ad libitum.

After one week, rats received capsaicin or vehicle injections, as described in chapter 3.5. Three days later, all rats were implanted with a mini-osmotic pump with cannula containing either CGRP or saline as control. The details of preparation were as described in Chapter 3.

After exactly two weeks infusion with CGRP or saline, all rats were killed by decapitation. The skin around the scapular area was opened and interscapular BAT was carefully removed. Some mini pumps with cannula were displaced, with the cannula about 3-4 mm away from BAT. Both epididymal and retroperitoneal white adipose tissue were cleaned and weighed. The preparation of BAT was the same as described before. Samples of BAT homogenate and mitochondria were stored for the assays of protein, UCP, COX, and DNA. These assays were performed as described as before. Food intake and body weight were measured weekly.

Results are expressed as means ± SEM for the number of animals per group (n=10). Statistical analysis was used two-way ANOVA. A significant difference is indicated when $p < 0.05$.

Results:

As in previous experiments, body weights of CAP-DES rats were significantly lower than those of control rats (Table 9). There was no difference in weight of white adipose tissue between CAP-DES rats and control rats at this time (Table 9). The wet weight of BAT was significantly reduced after capsaicin treatment (Table 9). Total protein content (Fig. 35), concentration of mitochondrial UCP (Fig. 36) and COX (Fig. 37), total COX content (Fig. 38) as well as DNA content (Fig. 40) were significantly decreased in CAP-DES rats. Total UCP content was also decreased, but it did not reached significant level due to large variation between control animals (Fig. 39)

Infusion of CGRP did not significantly alter BAT wet weight in either control or CAP-DES rats (Table 9). The concentration of mitochondrial UCP of BAT in CAP-DES rats was significantly increased by CGRP treatment (Fig.36). DNA content was also significantly increased in both types of animals (Fig. 40). Total UCP content and COX content as well as COX concentration in mitochondria were not altered by infusion of CGRP (Fig. 37-39).

Body temperature of CAP-DES was normal. Food intake in CAP-DES rats was similar to that of control rats. Infusion of CGRP did not change either body temperature or food intake in both CAP-DES and control rats (Table 9).

Discussion:

As expected, CAP-DES produced atrophy of BAT which was associated with reduction of total protein, UCP and COX although the low UCP was not significant in this

TABLE 9
EFFECTS OF INFUSION OF CGRP IN VEH-TREATED AND CAP-DES RATS

	Control		Capsaicin	
Infusion	Saline (n=10)	CGRP (n=10)	Saline (n=10)	CGRP (n=10)
Body Temperature °C	38.2 ±0.13	38.1 ±0.13	37.8 ±0.21	38.2 ±0.27
Body Weight (g)	346.7 ±5.86	348.2 ±3.63	333.2* ±4.21	333.3* ±4.69
BAT Weight (mg)	479.0 ±13.45	521.9 ±24.56	345.8* ±40.85	441.7* ±29.52
WAT Weight (g) epididymal	3.92 ±0.21	3.85 ±0.15	3.86 ±0.51	4.00 ±0.31
retroperitoneal	2.62 ±0.20	2.96 ±0.31	3.00 ±0.77	2.46 ±0.35
Food intake (g)	28.2 ±0.57	28.4 ±0.58	27.3 ±0.67	27.2 ±0.80

* Significant effect of capsaicin-desensitization
+ Significant effect of infusion with CGRP

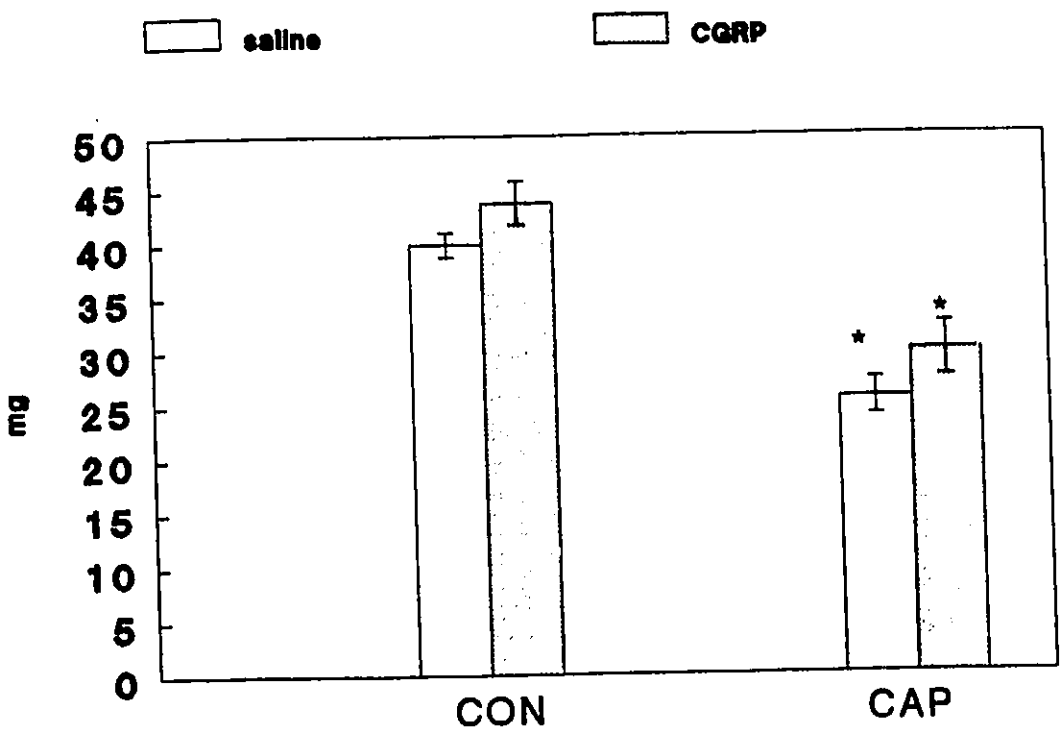


Fig. 35 BAT protein content in vehicle-treated (CON) and capsaicin-desensitized (CAP) rats infusion with either CGRP or saline.

* Significant effect of capsaicin treatment. CAP-DES induced a significant decrease in protein content of BAT.

Values are mean ± SEM for the number (n = 10) of rats per group. A significant difference is indicated when $p < 0.05$.

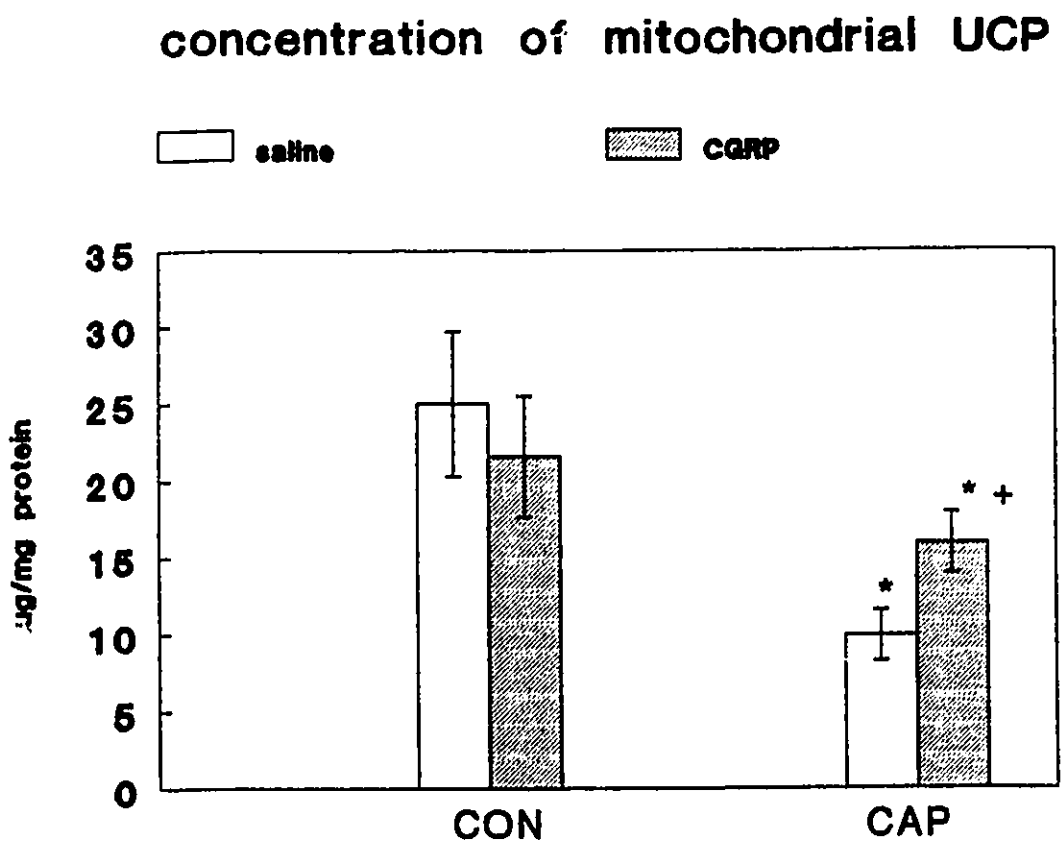


Fig. 36 Concentration of mitochondrial UCP of BAT in vehicle-treated (CON) and capsaicin-desensitized (CAP) rats infusion with either CGRP or saline.

* Significant effect of capsaicin treatment. CAP-DES induced a significant decrease in concentration of mitochondrial UCP.

+ Significant effect of infusion with CGRP. Concentration of mitochondria UCP was significantly increased after CGRP infusion when comparing with rat infused with saline in CDP-DES rats.

Values are mean ± SEM for the number (n = 10) of rats per group. A significant difference is indicated when $p < 0.05$.

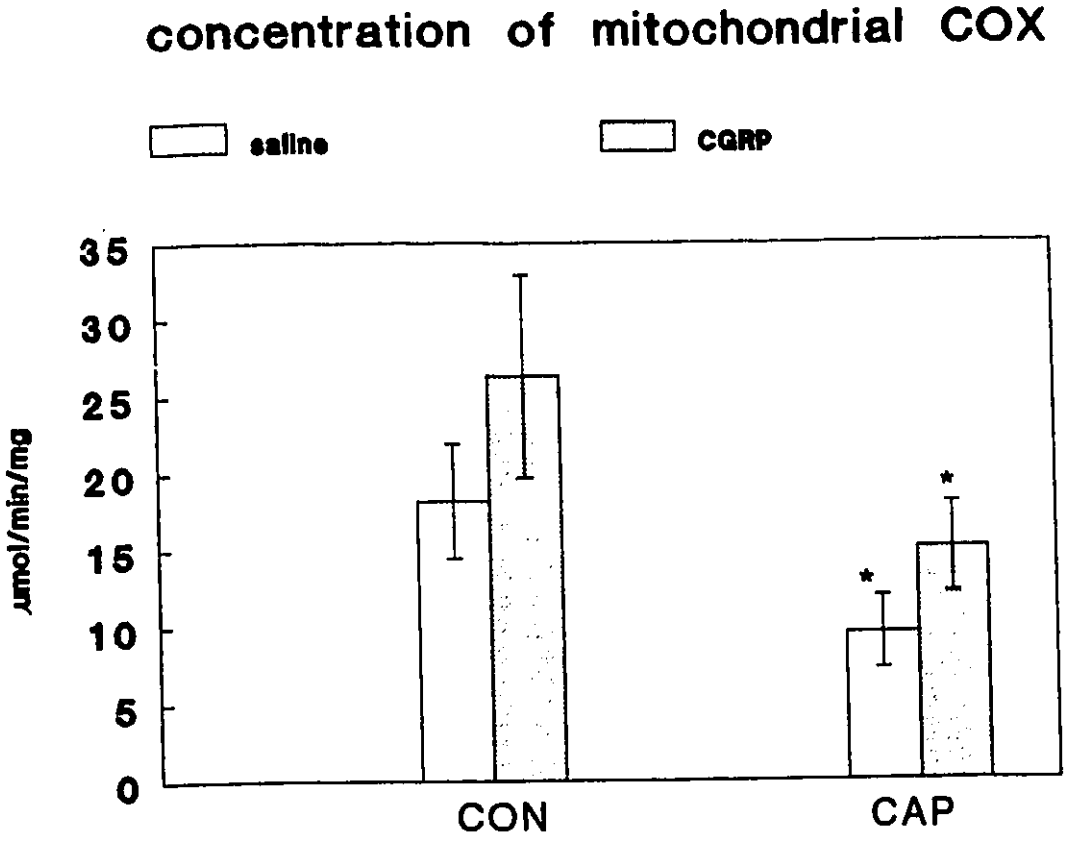


Fig. 37 Concentration of mitochondrial COX of BAT in vehicle-treated (CON) and capsaicin-desensitized (CAP) rats infusion with either CGRP or saline.

* Significant effect of capsaicin treatment. Capsaicin induced a significant decrease in concentration of mitochondrial COX.

There is no significant effect of CGRP on either control or CAP-DES rats.

Values are mean ± SEM for the number (n = 10) of rats per group. A significant difference is indicated when $p < 0.05$.

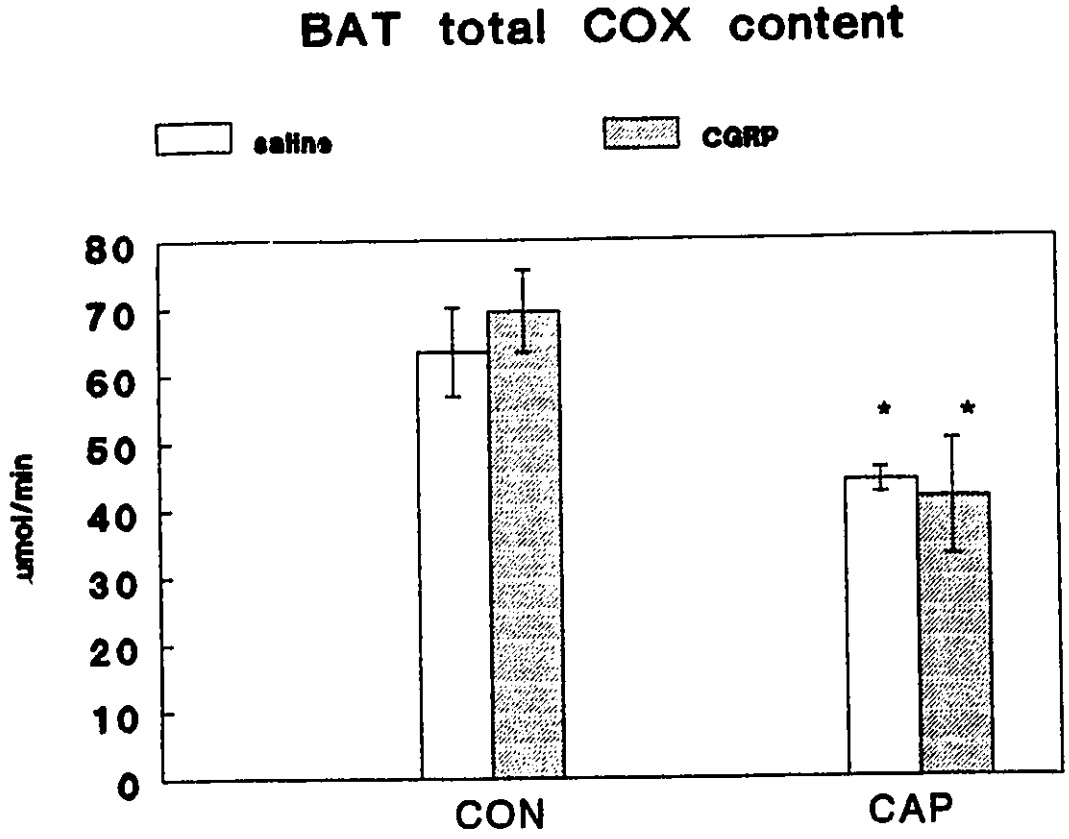


Fig. 38 BAT total COX content in vehicle treated (CON) and capsaicin-desensitized (CAP) rats infusion with either CGRP or saline.

* Significant effect of capsaicin treatment. Capsaicin induced a significant decrease in BAT total COX content.

There is no significant effect of CGRP on either control or CAP-DES rats.

Values are mean±SEM for the number (n=10) of rats per group. A significant difference is indicated when $p < 0.05$.

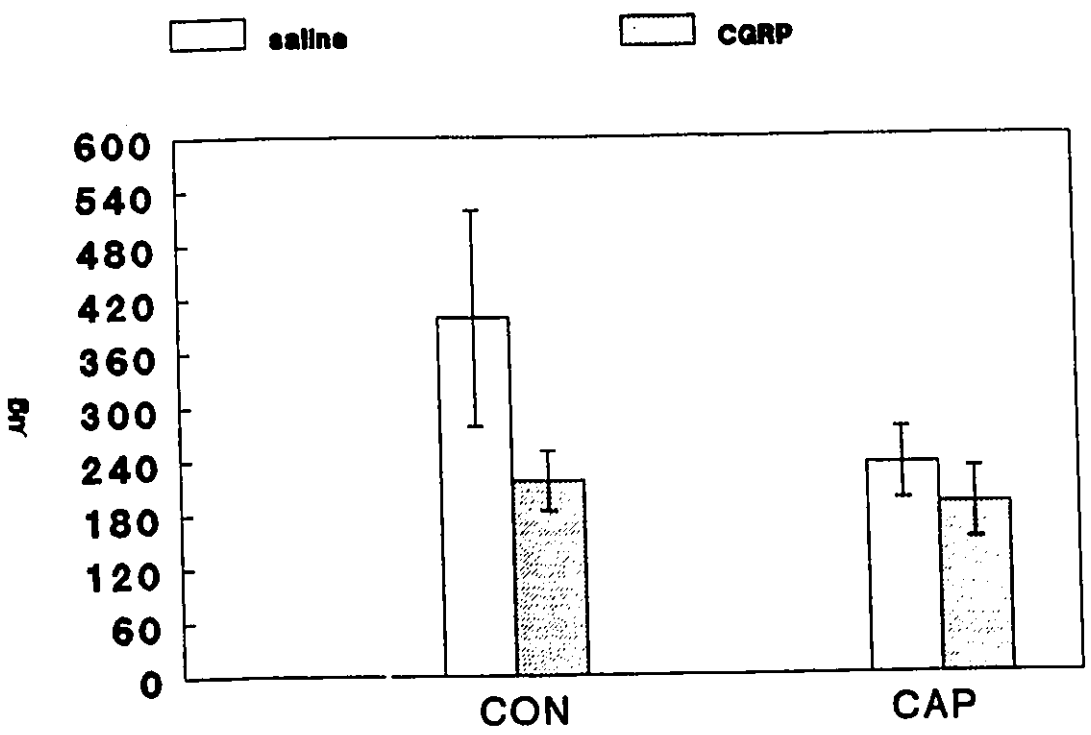


Fig. 39 BAT total UCP content in vehicle-treated (CON) and capsaicin-desensitized (CAP) rats infusion with either CGRP or saline. Capsaicin induced a decrease in BAT UCP content althrough it is not reached to significant level.

There is no effect of CGRP on BAT UCP content in either control or CAP-DES rats.

Values are mean±SEM for the number (n=10) of rats per group. A significant difference is indicated when $p < 0.05$.

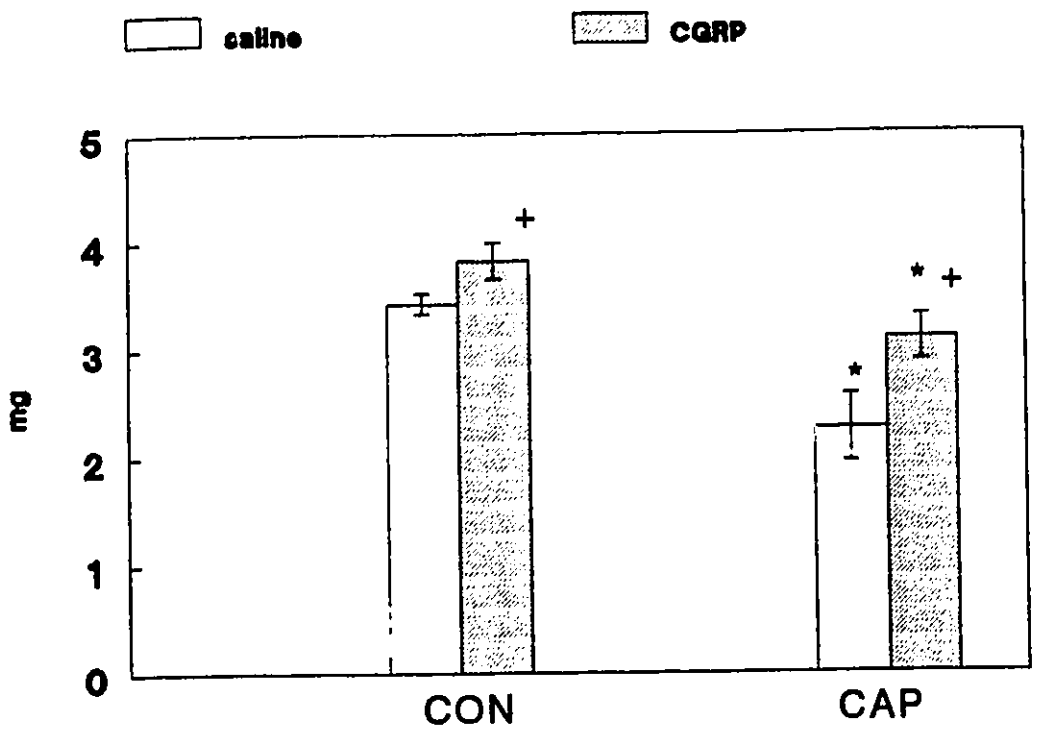


Fig. 40 DNA content of BAT in vehicle-treated (CON) and capsaicin-desensitized (CAP) rats infusion with either CGRP or saline.

* Significant effect of capsaicin treatment. Capsaicin induced a significant decrease in DNA content of BAT in CAP-DES rats.

+ Significant effect of infusion with CGRP. DNA content was significantly increased after CGRP infusion when comparing with the rat of infused with saline in both control and CAP-DES rats.

Values are mean±SEM for the number (n=10) of rats per group. A significant difference is indicated when $p < 0.05$.

experiment due to large variation among control rats. The concentrations of mitochondrial UCP and COX were significantly decreased. This was different from previous findings. The loss of certain component of mitochondria may be attributed to the different experimental manipulations. Rats used for the present study were subjected to surgery after CAP-DES. The stress of surgery and load of the filled pump on the body could further decrease thermogenesis of BAT in animals that have been CAP-DES.

Infusion of CGRP on BAT significantly increased the concentration of mitochondrial UCP in CAP-DES rats and the DNA content in both CAP-DES and control rats, suggesting that CGRP does have a trophic influence on BAT. This trophic effect of CGRP could involve DNA synthesis and cell proliferation. The finding agrees with observations of other researchers that CGRP can stimulate proliferation of endothelial cells in culture (Haegerstrand et al., 1990). Since whole BAT consists of many types of cells including mature brown adipocyte, interstitial cells and preadipocytes as well as endothelial cells, the type of cell stimulated to grow by CGRP in the present study is not clear. Quantitative histological studies would be needed. Based on distribution and function of CGRP, endothelial cells could be the ones to grow, which could contribute to proliferation of BAT vasculature during thermogenesis. It is known that CGRP is distributed in nerve endings associated with the smooth muscle of blood vessels and that it can regulate local blood flow (Rosenfeld et al., 1983; Brain et al., 1985). The other finding that also supports that other types of cells in BAT, like mature brown adipocytes, did not proliferate was that total content of protein, UCP and COX were not changed by the CGRP infusion. Whether an extended period of CGRP infusion could increase these components in BAT is unknown.

The CGRP-induced increase in concentration of UCP in mitochondria of CAP-DES rats, but not control rats, suggests a role for this neuropeptide in control of mitochondrial capacity in mature brown adipocytes.

It should be noted that results obtained from present study are preliminary. The experiment involved a new technique to insert mini-osmotic pump with cannula directed towards the interscapular BAT. The pump can deliver endogenous substrate continuously and maintain the presence of CGRP in BAT at high levels. However, as mentioned earlier, some pumps with cannula were displaced from interscapular BAT after two weeks infusion. In this case, some rats actually received less direct infusion with CGRP, which could affect the results. Thus, the effect of chronic infusion with CGRP should be more clear after the experiment is repeated.

Nevertheless, it can be concluded that CGRP can stimulate growth of BAT in both normal and CAP-DES rats.

CHAPTER 10

EFFECT OF ATROPHY OF BAT ON ENERGY BALANCE OF CAPSAICIN-DESENSITIZED (CAP-DES) RATS.

Background:

The studies described in Chapters 5-7 showed that the major effect of CAP-DES on BAT was atrophy. The atrophy occurred rapidly and persisted for up to 28 days. Another interesting observation from these studies is that the atrophy of BAT was not associated with obesity. CAP-DES rats had normal or less than normal body weight gain and body fat. When fed a palatable high fat "cafeteria" diet for 1 to 3 weeks, starting one week after injections, CAP-DES rats did not become any more obese than did control rats (Chapter 5). In the time course study, CAP-DES did not show greater tendency to obesity by 52 days (Chapter 7). These finding were not expected, since atrophy of BAT usually is associated with reduced energy expenditure and a high metabolic efficiency that contributes to obesity (see Chapter 1, Introduction).

It is possible, however, that CAP-DES rats might be more susceptible to diet-induced obesity, such as that seen in rats fed a high-fat diet for long period, or to aging-induced obesity. Further studies, therefore, were done to study the long-term susceptibility of CAP-DES rats to obesity. The diet chosen to induce obesity was a high-fat lard-based diet which we knew already would promote growth of BAT as well as induce obesity in normal rats (Harper et al., 1991).

Objectives:

The objectives of these experiments were to study the long-term effect of a high fat diet on BAT thermogenesis and body fat in CAP-DES rats and the long-term effects of capsaicin-desensitization on body weight and composition in rats eating chow and developing aging-induced obesity.

In part I, rats were fed a high fat diet for 10 weeks, and then BAT was studied. In part II, rats were studied at 14 and 32 weeks after the injections.

Methods:

Male Sprague-Dawley rats were obtained from Charles River laboratories at 5 weeks of age. They were maintained in wire-mesh cages individually at 26°C with 12 hours light cycle. All rats had free access to food (Agway Chow RMH 4020) and water.

At 6 weeks of age, rats were anaesthetized with halothane and injected with capsaicin or vehicle as described in Chapter 3. All rats were injected within a one-week period in 1990.

In part I, groups of CAP-injected and VEH-injected rats were divided into two sub-groups. One sub-group of each type was fed high fat diet and chow. The other sub-group was fed laboratory chow only. Food intake and body weight were measured weekly. Body composition was measured frequently by total body electrical conductivity (TOBEC) with a model SA-1 body composition analyzer (EM-SCAN™) as described in Chapter 3. After 10 weeks, all rats were killed and their body temperatures were measured. Epididymal plus retroperitoneal white adipose tissue (WAT) pads were weighed, then returned to the

carcass. Interscapular BAT was cleaned of adhering muscle and white adipose tissue and weighed. BAT was homogenized and samples were kept for later protein, UCP, COX and DNA measurement. Methods used were as described in Chapter 3. For the carcass analysis, whole body, excluding BAT, tail, paws and skin, were homogenized and lipid extracted as described in Chapter 3.

In part II, groups of rats were studied at 14 and 32 weeks following the injections. Rats were killed and samples were prepared as before. For body composition analysis procedure both TOBEC and carcass analysis were used. Carcass analysis did not include three organs (liver, heart and kidney) which were used for later assays.

For measurement of naso-anal length (done only for the rats studied at 32 weeks), rats were anaesthetized with sodium pentobarbital (35 mg/kg sc) and naso-anal lengths were measured. The results were used to calculate the Lee index of obesity using the body weight of the rat at this same time.

Measurement of resting oxygen consumption was done at 30 weeks for the group of rats studied at 32 weeks. Rats were placed in a cylindrical insulated metabolic chamber and their oxygen uptake at 28 °C measured as described in Chapter 3.19. All measurements were made between 0900 and 1300h. Rats had access to food until the time of the measurement. Usually, 2 rats were studied per day. Each rat took about 2 hours to become accustomed to the measurement conditions (45-60 min) and have a steady state of oxygen consumption (60 min). Results are expressed in terms of total body weight, per 100g of body weight, per 100 cm^2 of body surface (12.5 x body weight $^{0.60}$ (Lee, 1929)), in terms of metabolic mass (kg $^{0.75}$) and in terms of fat-free mass. Fat free mass was calculated for these rats studied at 30

weeks from the % body fat found by carcass analysis in the same rats at 32 weeks.

It should be noted that some results are described in both part I and II of these experiments in order to describe the effect of CAP-DES over a longer period, namely from 10 weeks to 32 weeks after injections.

Data are presented as means±SEM. Statistical analysis used t-tests and differences were considered to be statistically significant when $p < 0.05$.

Results:

Part I: Effect of high fat diet

Chow-fed CAP-DES rats showed a similar growth rate to chow-fed control rats for most of the time (Fig. 41), while the CAP-DES rats eating a high fat diet gained less body weight when compared with control rats eating the same diet. The difference became larger with time (Fig. 41). Energy intakes of high fat fed rats in either CAP-DES or control groups were the same as in similar groups of rats eating chow (Table 10). Body weights were greater in the high fat fed rats compared with chow-fed rats of the same type (Table 10). Body weight gain in CAP-DES rats eating the high fat diet was significantly lower, compared with control rats eating the same diet (Table 10). The high-fat diet increased body fat accumulation (Table 11) which was assessed in three different ways: by carcass analysis, by TOBEC, and by weighing the two major white adipose tissue (WAT) depots (epididymal and retroperitoneal). As expected, all rats eating high fat diet had much more body fat than rats eating chow. Consistent with body weight gain, CAP-DES rats eating high fat diet had less body fat (measured by WAT weight) than that of control rats. The percentage of body

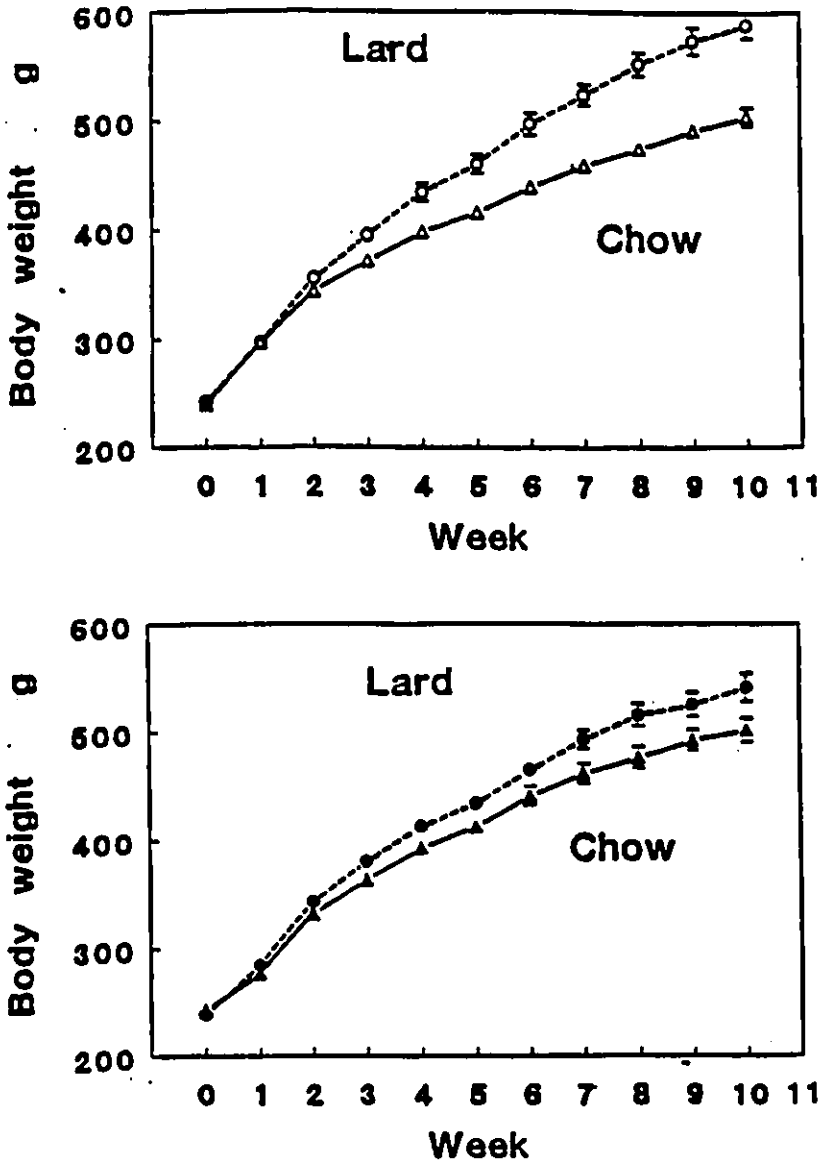


Fig. 41 Growth of control rats eating chow or a high-fat (lard-based) diet (top).

Growth of CAP-DES rats eating chow or a high-fat (lard-based) diet (bottom).

Numbers of rats and other data are in Tables 10-11.

Table 10. Energy intakes and body weights of control and CAP-DES rats eating chow or a high-fat diet for 10 weeks

Treatment	Control		CAP-DES	
Diet	Chow	Fat	Chow	Fat
Number	(10)	(12)	(14)	(11)
Energy intake kcal day	85.5 ±1.80	89.2 ±2.92	86.0 ±1.83	85.1 ±1.97
Body weights, g				
Initial	240.2 ±2.4	242.7 ±3.6	242.9 ±2.8	238.8 ±2.1
Final	510.9 ±7.1	599.0^{+} ±10.7	515.6 ±8.1	552.6^{+*} ±11.0
Change	270.7 ±6.4	356.3^{+} ±10.3	277.3 ±9.7	313.4^{+*} ±10.2
Body temperature °C	38.1 ±0.23	38.0 ±0.19	38.0 ±0.21	38.1 ±0.22

+ Significant effect of fat diet, comparing similarly-treated rats
* Significant effect of CAP-DES, comparing rats eating the same diet

Table 11. Body composition of control and CAP-DES rats eating chow or a high-fat diet for 10 weeks

Treatment	Control		CAP-DES	
Diet	Chow	Fat	Chow	Fat
Number	(10)	(12)	(14)	(11)
Carcass analysis				
Fat, g	41.1 ±2.89	120.5+ ±12.6	49.0 ±3.00	103.8+ ±5.96
Fat, %	8.1 ±0.56	21.9+ ±0.86	9.5 ±0.52	18.7+* ±0.77
WAT weight, g	18.9 ±0.83	50.9+ ±2.34	20.3 ±1.45	42.8+* ±2.98
TOBEC				
Fat,g	53.9 ±6.61	117.9+ ±7,50	62.9 ±5.15	107.6+ ±3.31
Fat, %	10.5 ±1.28	19.5+ ±1.10	12.3 ±1.04	19.4+ ±0.55
LEM, g	457.9 ±8.77	481.5 ±8.20	449.9 ±9.91	446.4* ±9.69
LEM, %	89.5 ±1.28	80.5+ ±1.09	87.6 ±1.04	80.6+ ±0.55

+ Significant effect of fat diet, comparing similarly-treated rats
* Significant effect of CAP-DES, comparing rats eating the same diet

fat in CAP-DES rats eating the high fat diet as assessed by carcass analysis, was less than that of control rats (Table 11). Chow-fed CAP-DES rats weighed the same as chow-fed control rats (Table 10) and also had a normal proportion of body fat (Table 11).

BAT weight in CAP-DES rats eating chow was normal, but the protein content was lower than in control rats. UCP and COX contents were not significantly lower than in control rats (Table 12). The high-fat diet induced significant increases in BAT weight, protein and UCP content in control rats but had no effect on BAT of CAP-DES rats, except for an increase in wet weight (Table 12). The UCP content in BAT of CAP-DES rats eating the high-fat diet was much less than in control rats; it was only 50% of UCP in BAT of control rats eating the same diet (Table 12).

Three methods for assessment of body fat were compared in present experiment. TOBEC is a recently developed non-invasive technique to estimate lean and fat content of small rodents. The principle of the technique is based on the widely differing electrical conductivities of lean tissue and body fat. The advantage of the method is its non-invasive assessment so that it can be used to follow changes in body composition in the course of long-term feeding experiments. However, this technique requires very strict conditions of performance such as position of animal and time of measurement. Thus, there is a limitation in the number of animals studied per day. Also, accuracy of measurement was not high. TOBEC gave a value 30% higher than that obtained by carcass analysis for chow-fed rats but the same mean value for rats eating the high-fat diet (Table 11). Fig. 42 shows correlation of body fat assessed by carcass analysis with body fat assessed by TOBEC. The correlation coefficient was $r=0.8786$. It was better when only control rats were used for the

Table 12. BAT of control and CAP-DES rats

Treatment Diet Number	Control Chow (10)	Control Fat (12)	CAP-DES Chow (14)	CAP-DES Fat (11)
BAT weight, g	0.83 ±0.037	1.52+ ±0.108	0.94 ±0.111	1.29+ ±0.135
Protein, mg	41.1 ±2.1	54.3+ ±2.7	33.0* ±2.9	43.2 ±5.9
UCP, μg	205 ±27.2	316+ ±29.3	140 ±32.7	152* ±19.7
COX, μmol min	53.8 ±8.1	74.1 ±13.8	36.1 ±5.2	59.4 ±24.8

+ Significant effect of fat diet, comparing similarly-treated rats
* Significant effect of CAP-DES, comparing rats eating the same diet

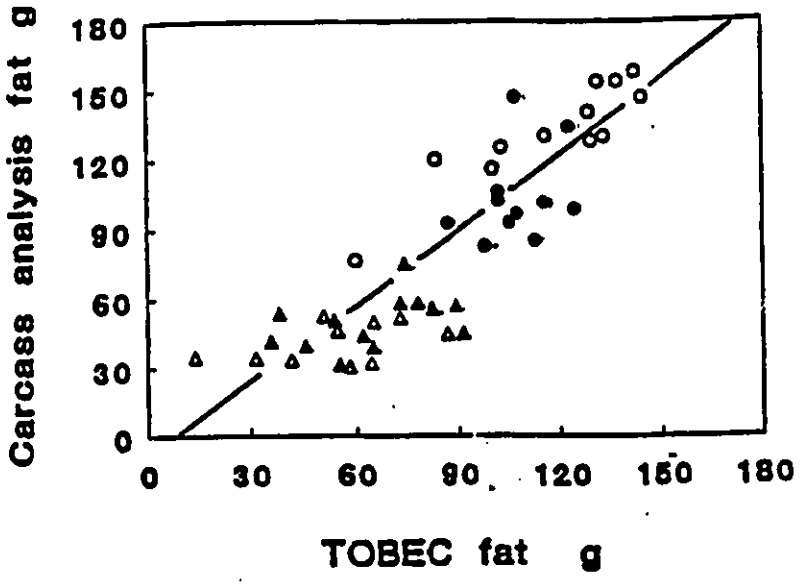


Fig. 42 Correlation of body fat as assessed by carcass analysis with body fat, as assessed by TOBEC. Symbols used are the same as in Fig. 41.

calculation ($r=0.9192$) than when all rats were included ($r=0.879$). Carcass analysis, of course, provides the highest accuracy. However, this method involves many laborious procedures and is very time consuming. The best way to estimate body fat is to weigh WAT depots. As shown in Fig. 43, there is very good correlation of body fat as assessed by carcass analysis with body fat as assessed by weighing two major WAT depots ($r=0.9509$). This method is much less expensive, easy to do, and provides a useful index of obesity.

Part II: Long-term effect of CAP-DES

Body weights of CAP-DES rats eating chow were the same as those of control rats at 10 weeks but significantly less by 14 weeks, and more obviously decreased by 32 weeks (Table 13). The lower body weight resulted from lower body fat and small body size (lower naso-anal length). Weights of both epididymal and retroperitoneal white adipose tissue depots were significantly decreased at 14 and 32 weeks (Fig. 44 and 45). Body fat content estimated by carcass analysis was similarly reduced at 32 weeks, both in absolute amount (Fig. 46) and when expressed as % body weight (Fig. 47). Body fat content was not changed at the earlier time of 10 weeks (Fig. 46 and 47). Lee index of obesity was, however, not significantly different from that of control rats (Table 13), possibly because this measure is not sensitive enough to detect the small difference of obesity in these animals. The food intake was normal in CAP-DES rats over 10 weeks, but significantly less over 14 and 32 weeks (Table 13), which was consistent with the lower body weights. CAP-DES can regulate body temperature normally, since no difference of body temperature between CAP-DES and control rat were detected (Table 13).

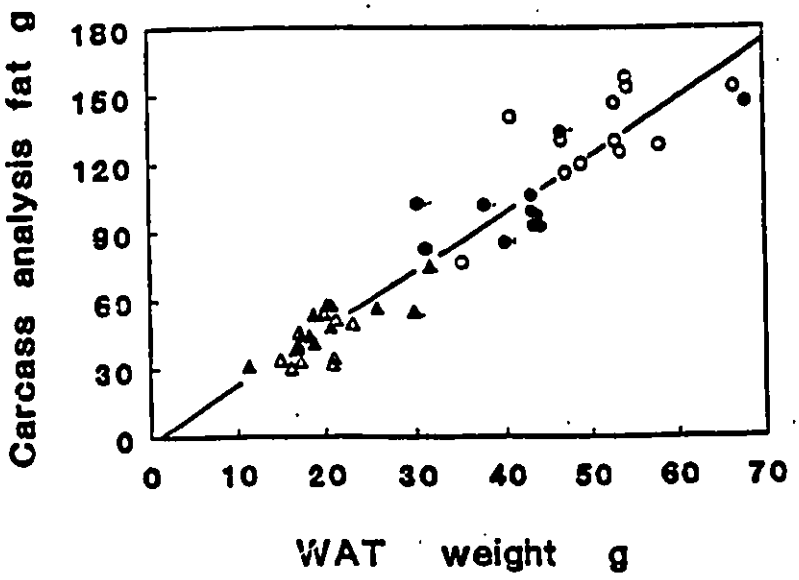


Fig. 43 Correlation of body fat, as assessed by carcass analysis, with body fat as assessed by weight of two major WAT depots. Symbols used are the same as in Fig. 41.

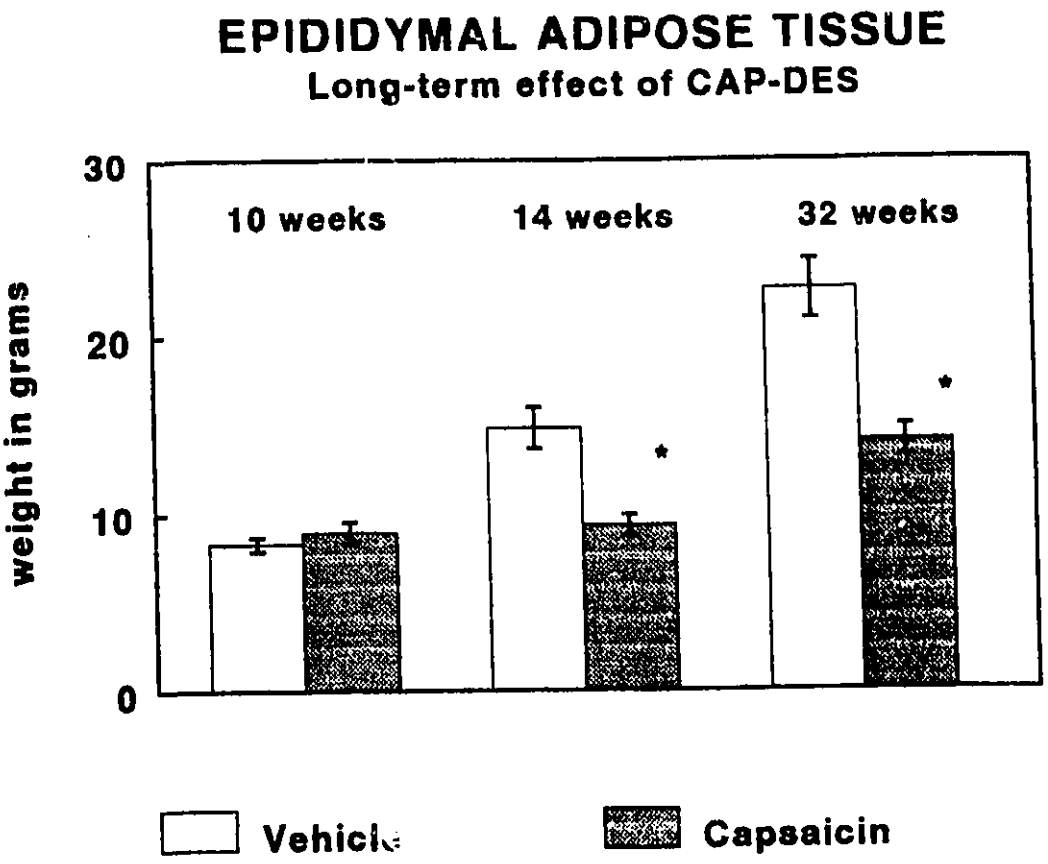


Fig. 44 Weight of epididymal white adipose tissue depots in vehicle-treated and capsaicin-desensitized rats studied at 10, 14 or 32 weeks after injections.

* denotes a significant difference between values for vehicle-treated and capsaicin-desensitized rats studied at the same time. Numbers of rats per group are in Table 10 and 11.

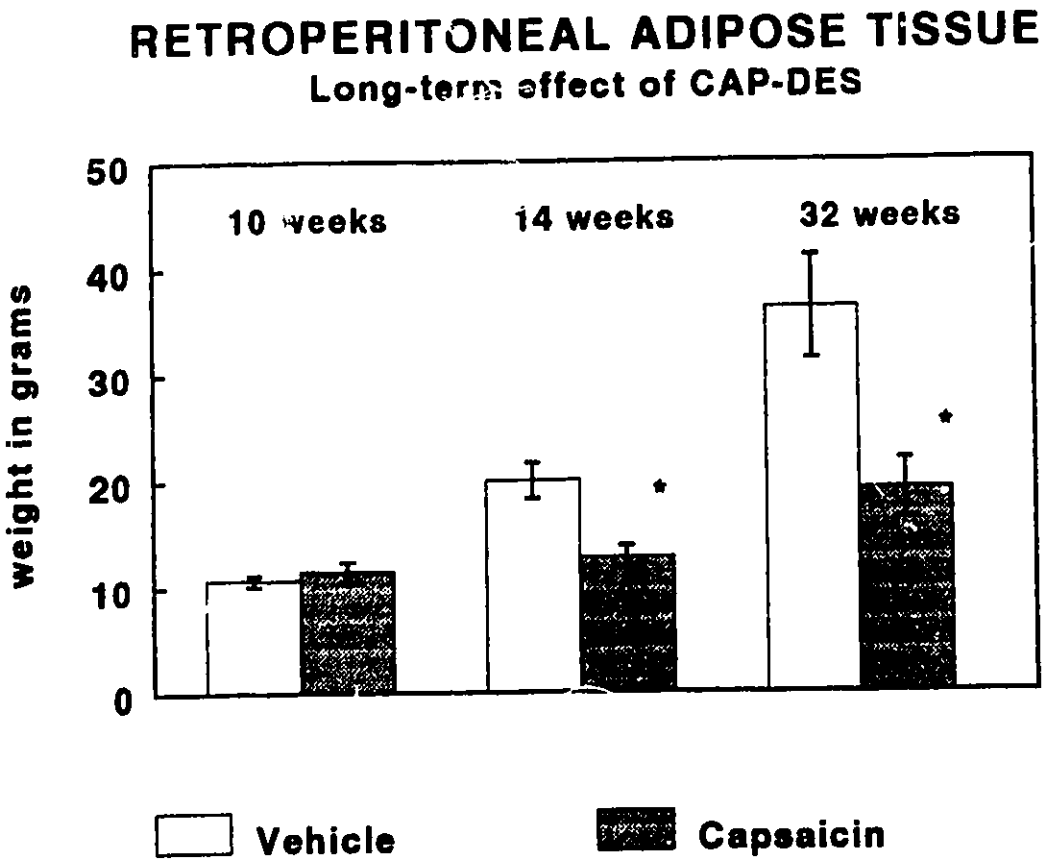


Fig. 45 Weight of retroperitoneal white adipose tissue depots in vehicle-treated and capsaicin-desensitized rats.

* denotes a significant difference between values for vehicl-treated and capsaicin-desensitized rats studied at the same time. Numbers of rats per group are in Table 10 and 11.

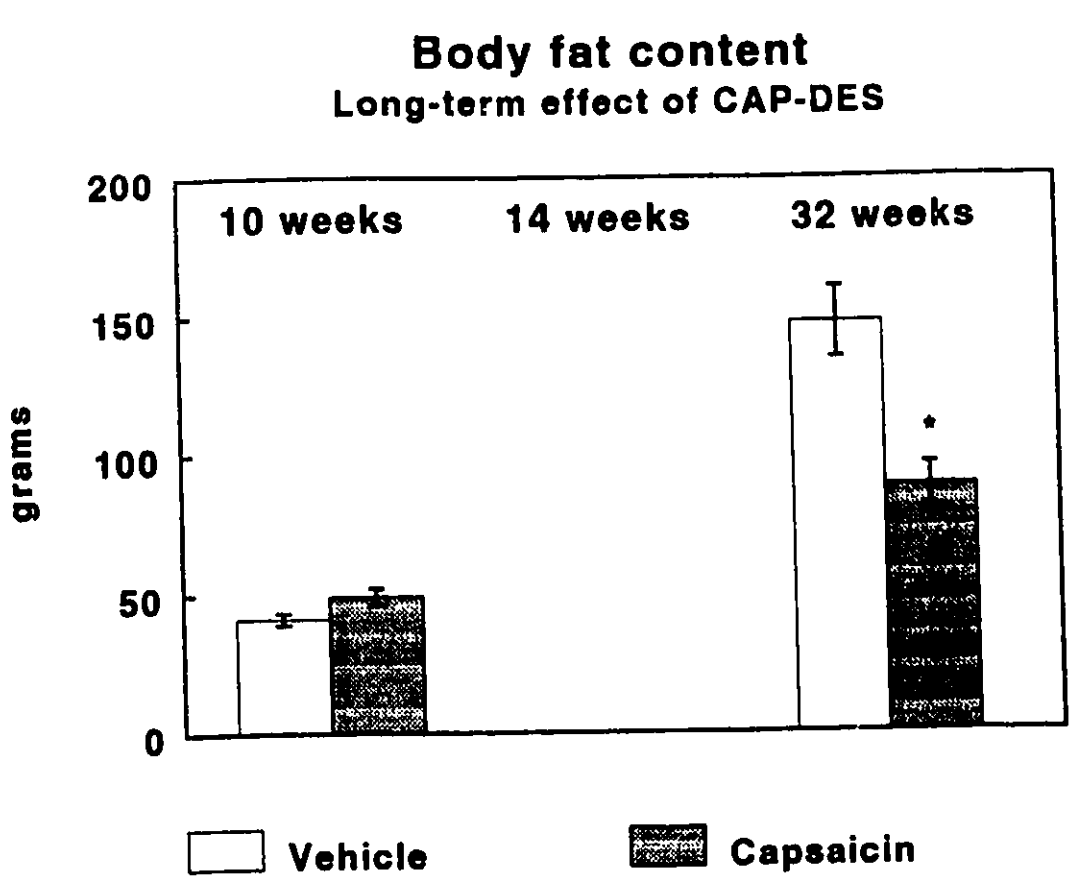


Fig. 46 Body fat content of vehicle-treated and capsaicin-desensitized rats. Body fat was estimated by carcass analysis. Measurement was not made for rats studied at 14 weeks.

* denotes a significant difference between values for vehicle-treated and capsaicin-desensitized rats studied at the same time. Numbers of rats per group are in Table 10 and 11.

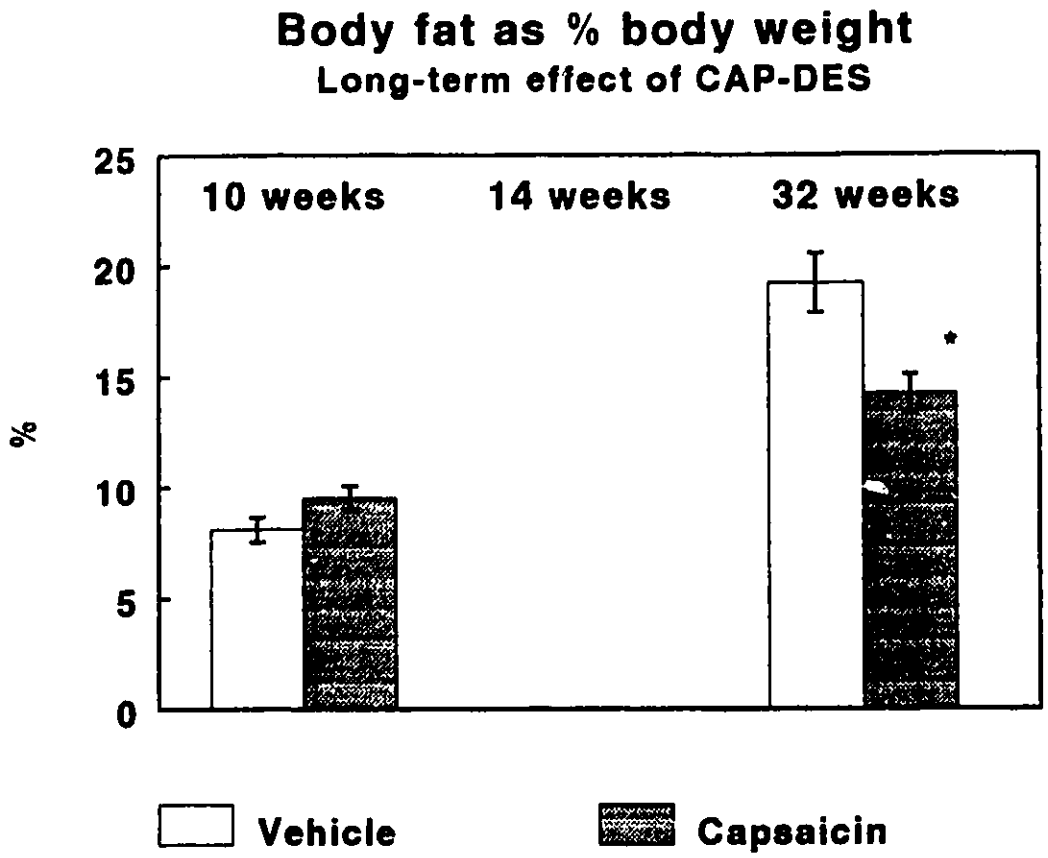


Fig. 47 Body fat content as % of body weight in vehicle-treated and capsaicin-desensitized rats. Body fat was estimated by carcass analysis. Measurement was not made for rats studied at 14 weeks.

* denotes a significant difference between values for vehicle-treated and capsaicin-desensitized rats studied at the same time. Numbers of rats per group are in Table 10 and 11.

Table 13: Characteristics of body size and temperature and food intake of vehicle-treated and CAP-DES rats at 10, 14 or 32 weeks after the last injection.

		10 weeks		14 weeks		32 weeks	
		Vehicle	CAP-DES	Vehicle	CAP-DES	Vehicle	CAP-DES
		(10)	(14)	(12)	(12)	(6)	(6)
Body weight	grams	511 ±7.1	515 ±8.1	599 ±17.4	536 * ±9.9	765 ±19.4	622 * ±21.9
Naso-anal length	cm					27.1 ±0.17	25.9 * ±0.38
Lee index						338.6 ±2.80	329.8 ±6.78
Colonic T°	°C	38.1 ±0.23	38.0 ±0.21	37.8 ±0.12	38.0 ±0.14	37.6 ±0.23	37.6 ±0.41
Food intake	g/day†	25.0 ±0.53	25.1 ±0.54	30.0 ±0.87	26.6 * ±0.39	30.9 ±0.87	25.9 * ±0.66

* Significant effect of capsaicin-desensitization, comparing rats studied at the same time

† Mean of averages of weekly one-day measurements, corrected for spillage, calculated for each rat for the number of weeks indicated and for the number of rats in each group indicated by the numbers in parentheses.

Weight of BAT in CAP-DES rats was normal at 10 week but low at 14 weeks, and significantly decreased at 32 weeks (Fig. 48). Total protein content of BAT of CAP-DES rats was lower than normal at all times studied (Fig. 49). Total UCP and COX content were significantly decreased about 70% at both 14 and 32 weeks (Fig. 50 and 51). DNA content was likewise reduced significantly at 32 weeks (Fig. 52). Thus, CAP-DES either produced progressive atrophy or prevented growth of BAT during the period between 10 weeks and 32 weeks.

As in the time course study of BAT atrophy (Chapter 7), three major organs were analyzed. Their weight and protein, DNA and COX content were estimated. The results from these measurement provided more information to further characterize the altered body composition of the CAP-DES animals at the later time. Liver weight and DNA content were reduced significantly in CAP-DES rats at 32 weeks but were normal when calculated in terms of the smaller body size (Table 14). Heart and kidney weight were normal but greater when expressed in terms of the reduced body size, as was heart protein content. Other measures did not show any differences in the CAP-DES rats, regardless of the way in which they were expressed.

In order to examine whether the lower body weight of CAP-DES was related to increased energy expenditure, resting metabolic rates of unrestrained, conscious rats were measured 30 weeks after injections. The total oxygen consumption of CAP-DES rats was significantly decreased by 18.7% when compared with that of control rats (Table 15). However, when expressed in terms of body weight, of body surface (in cm^2), of metabolic mass ($kg^{0.75}$), or of fat-free mass, oxygen consumption in the CAP-DES rats was not

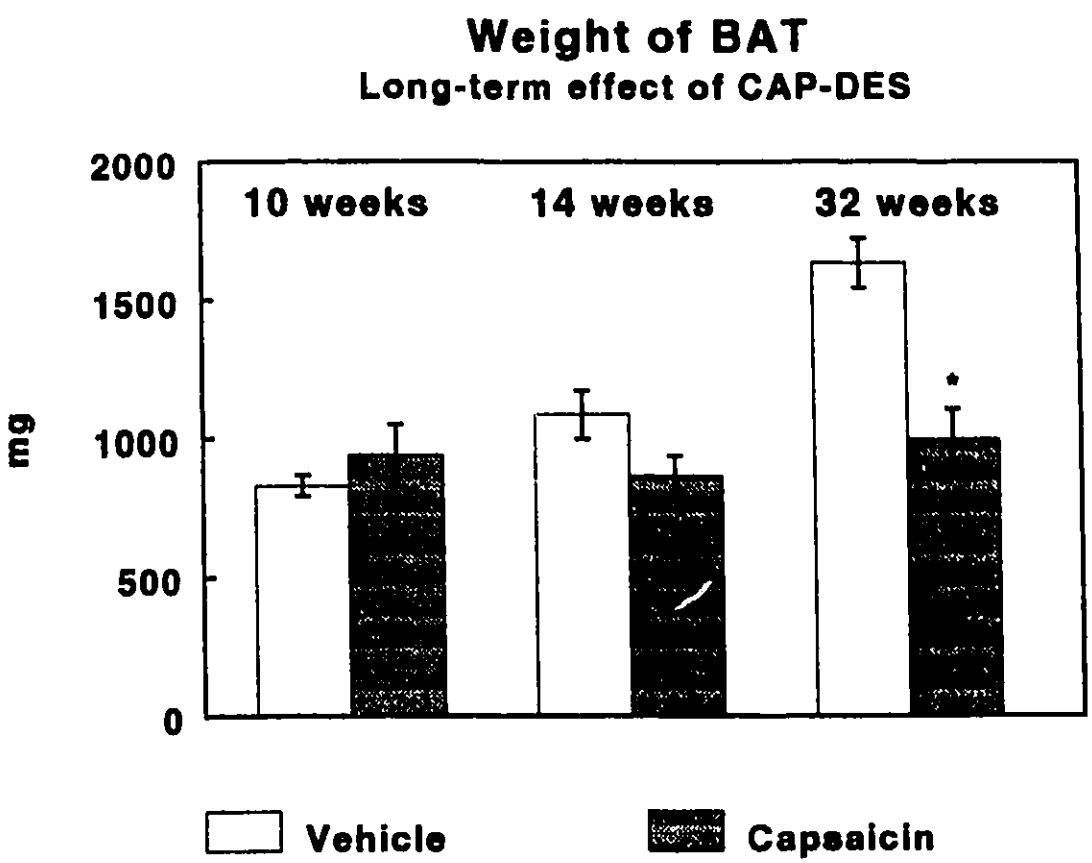


Fig. 48 Weight of interscapular BAT in vehicle-treated and capsaicin-desensitized rats.

* denotes a significant difference between values for vehicle-treated and capsaicin-desensitized rats studied at the same time. Numbers of rats per group are in Table 10 and 11.

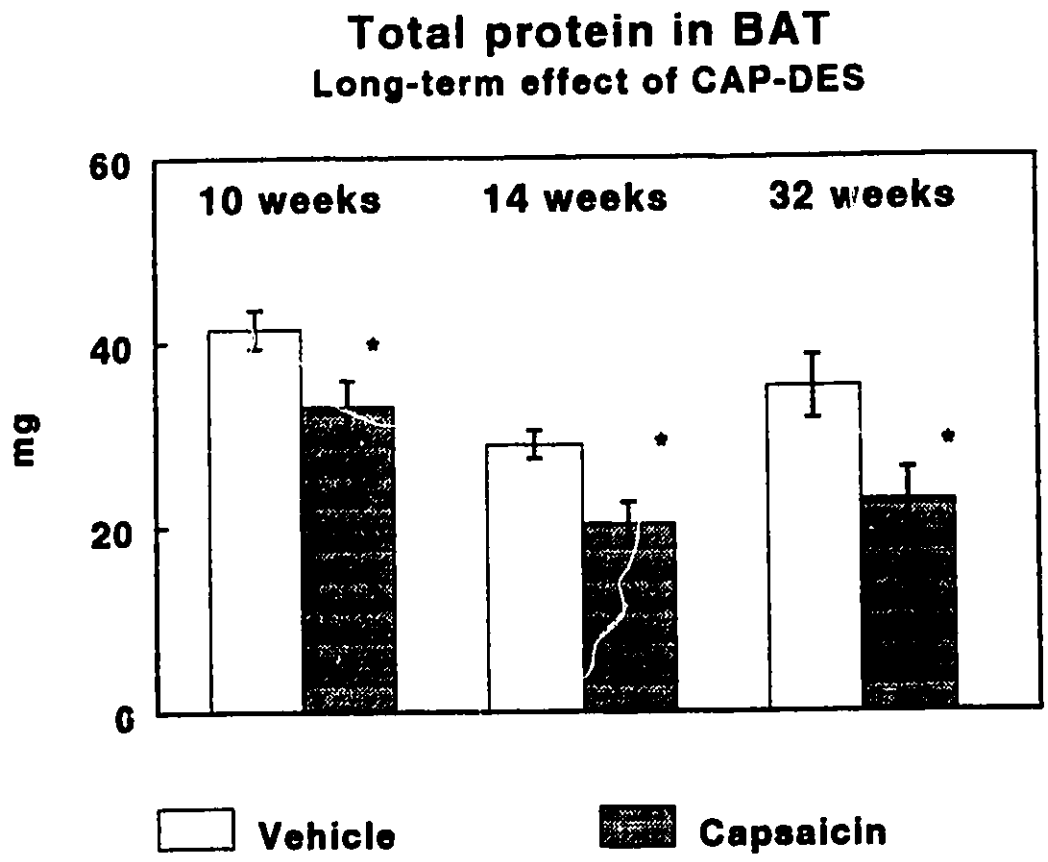


Fig. 49 Total protein content of interscapular BAT in vehicle-treated and capsaicin-desensitized rats. Protein was measured by a modified Lowry method.

* denotes a significant difference between values for vehicle-treated and capsaicin-desensitized rats studied at the same time. Numbers of rats per group are in Table 10 and 11.

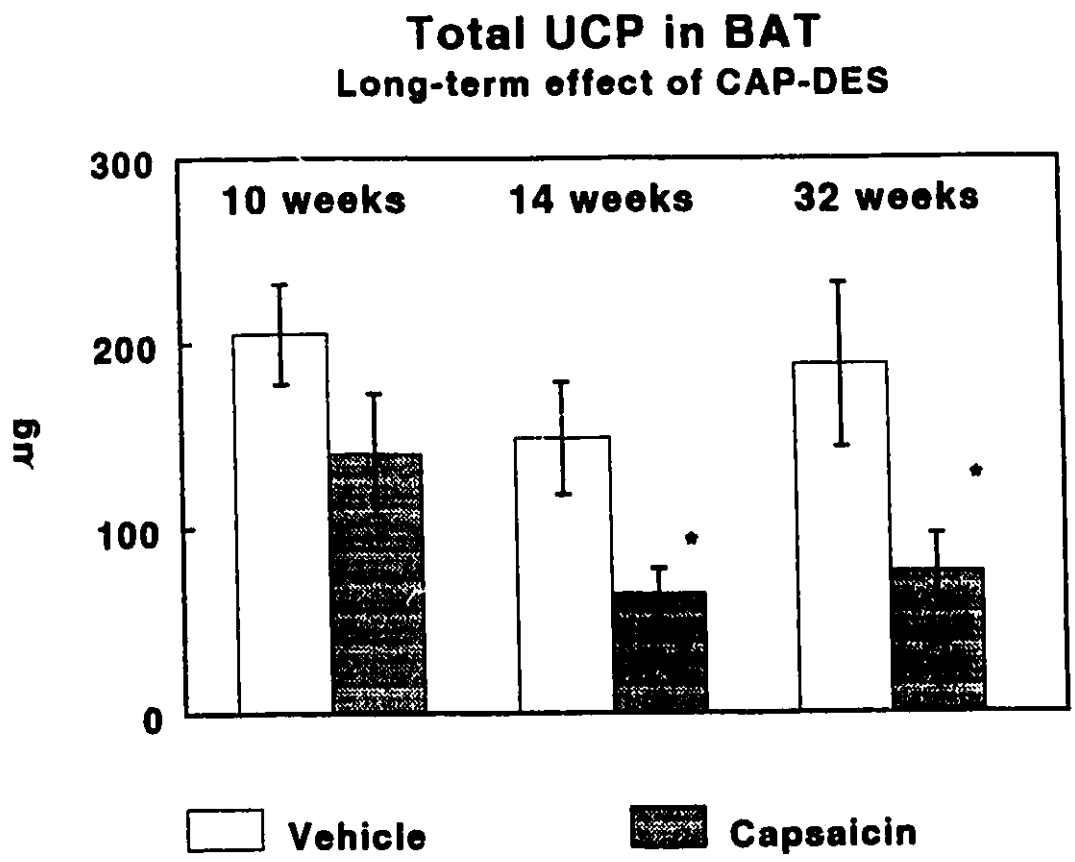


Fig. 50 Total content of uncoupling protein (UCP) in interscapular BAT of vehicle-treated and capsaicin-desensitized rats. UCP was measured in homogenates by a solid-phase radioimmunoassay using rabbit anti-hamster UCP antiserum and pure rat UCP as a standard.

* denotes a significant difference between values for vehicle-treated and capsaicin-desensitized rats studied at the same time. Numbers of rats per group are in Table 10 and 11.

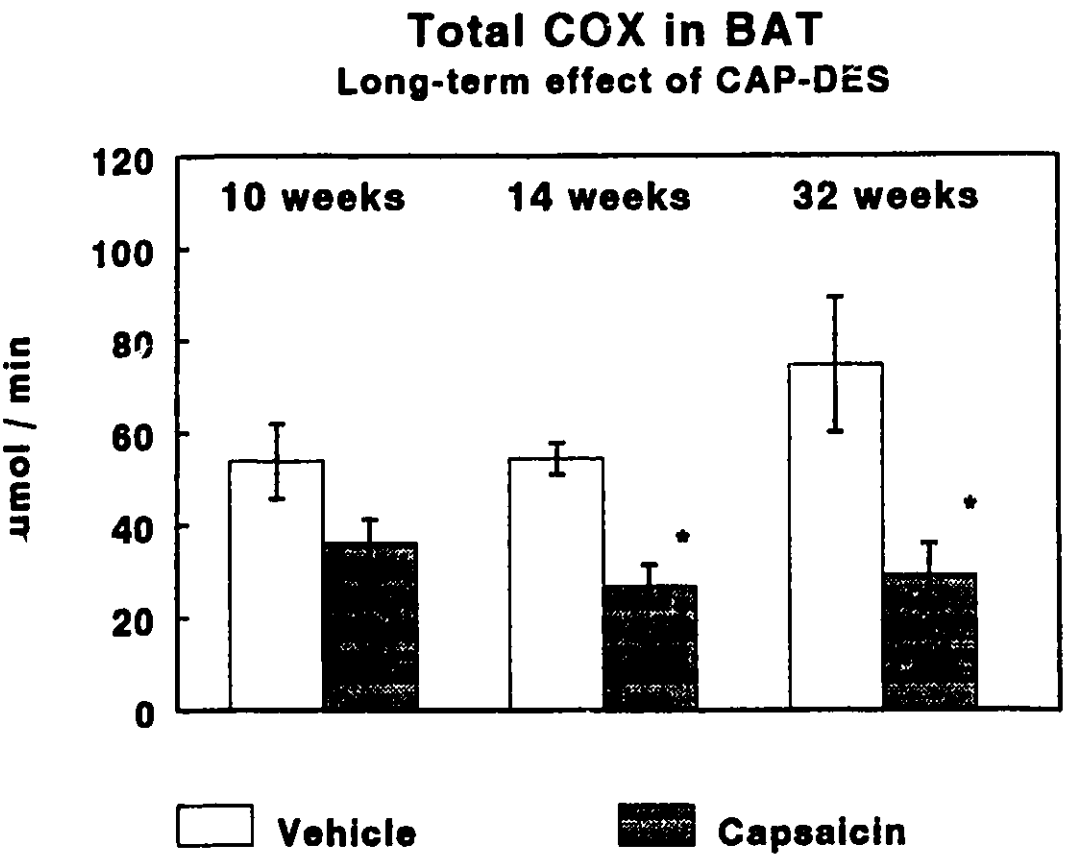


Fig. 51 Total content of cytochrome oxidase (COX) in interscapular BAT of vehicle-treated and capsaicin-desensitized rats. Cytochrome oxidase was measured in homogenates by a spectrophotometric method.

* denotes a significant difference between values for vehicle-treated and capsaicin-desensitized rats studied at the same time. Numbers of rats per group are in Table 10 and 11.

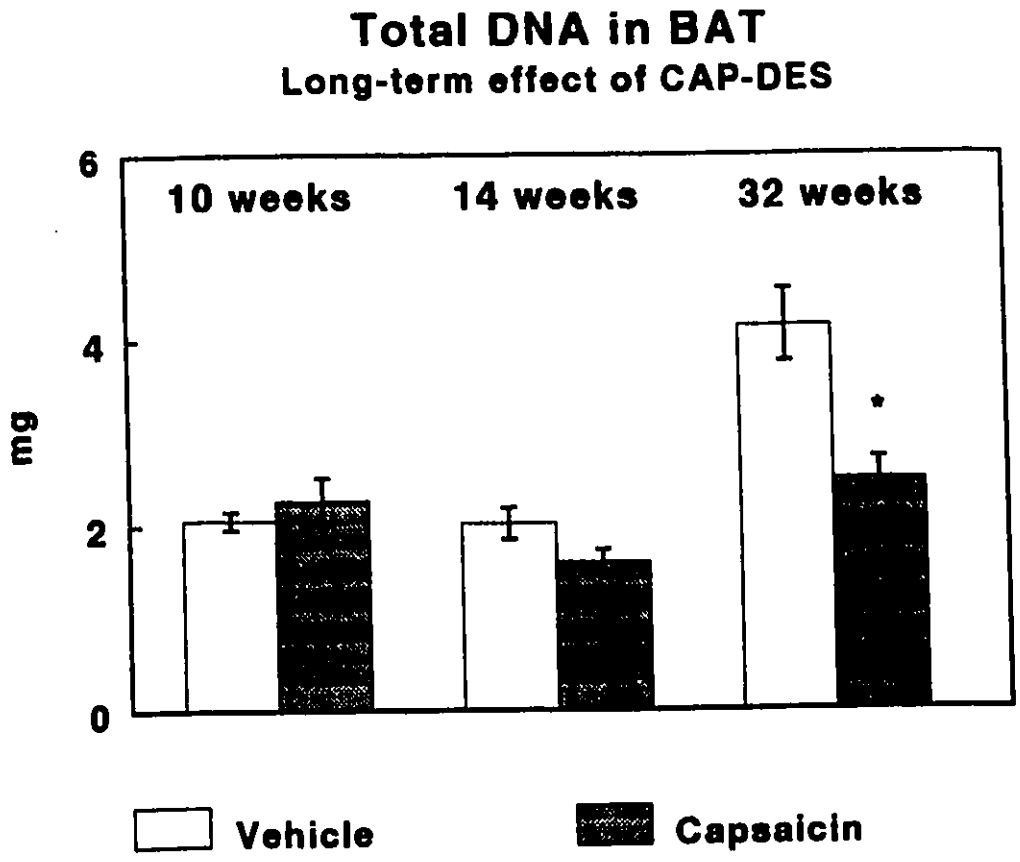


Fig. 52 Total DNA in interscapular BAT of vehicle-treated and capsaicin-desensitized rats. DNA was measured by a fluorometric method.

* denotes a significant difference between values for vehicle-treated and capsaicin-desensitized rats studied at the same time. Numbers of rats per group are in Table 10 and 11.

TABLE 14
LIVER, HEART AND KIDNEY OF VEHICLE-TREATED AND CAP-DES RATS

	14 weeks		32 weeks	
	VEHICLE (12)	CAP-DES (12)	VEHICLE (6)	CAP-DES (6)
Body Weight g	599 ±17.4	536 * ±9.9	765 ±19	622 ±22
LIVER WEIGHT g	20.3 ±0.69	18.6 ±0.51	23.5 ±1.17	19.8 * ±0.91
g/100g BW	3.39 ±0.054	3.48 ±0.052	3.07 ±0.12	3.18 ±0.07
PROTEIN g	8.0 ±0.301	7.22 ±0.253	7.93 ±0.386	7.14 ±0.317
g/100g BW	1.34 ±0.048	1.35 ±0.047	1.04 ±0.060	1.15 ±0.043
DNA mg	76.3 ±4.99	78.8 ±4.11	74.4 ±2.07	58.6 * ±4.45
mg/100g BW	12.6 ±0.60	14.7 ±0.70	9.8 ±0.44	9.5 ±0.72
COX mmol/min	14.8 ±2.64	8.7 ±1.86	11.2 ±1.86	12.8 ±1.71
mmol/100g BW	2.50 ±0.46	1.64 ±0.36	1.47 ±0.225	2.08 ±0.314
HEART WEIGHT g	1.28 ±0.030	1.21 ±0.026	1.44 ±0.05	1.30 ±0.05
g/100g BW	0.22 ±0.004	0.23 ±0.01	0.19 ±0.01	0.21 * ±0.00
PROTEIN mg	270 ±16.6	247 ±20.1	536 ±9.6	571 ±36.6
mg/100g BW	45.3 ±2.52	46.1 ±3.55	70.3 ±1.43	91.4 * ±3.80
DNA mg	4.17 ±0.10	4.00 ±0.10	3.3 ±0.18	3.1 ±0.1[illegible]
mg/100g BW	0.70 ±0.03	0.75 ±0.03	0.43 ±0.03	0.49 ±0.01

TABLE 14 (CON'D)
LIVER, HEART AND KIDNEY OF VEHICLE-TREATED
AND CAP-DES RATS

	14 weeks		32 weeks	
	VEHICLE (12)	CAP-DES (12)	VEHICLE (6)	CAP-DES (6)
COX µmol/min	1043 ±137.5	1096 ±165.8	1106 ±234.5	1142 ±321.9
µmol/100g BW	173 ±21.9	201 ±28.3	142 ±27.0	186 ±52.9
KIDNEY				
WEIGHT g	3.46 ±0.103	3.20 ±0.078	3.68 ±0.16	3.41 ±0.15
g/100g BW	0.58 ±0.007	0.60 ±0.012	0.48 ±0.02	0.55 * ±0.01
PROTEIN mg	907 ±4.6.5	873 ±31.0	1052 ±43.8	933 ±38.2
mg/100g BW	150.6 ±4.66	163.1 ±5.39	137.9 ±6.26	150.4 ±6.62
DNA mg	22.5 ±3.77	23.6 ±1.76	15.5 ±0.92	14.5 ±0.80
mg/100g BW	3.67 ±0.53	4.36 ±0.26	2.03 ±0.12	2.32 ±0.08
COX µmol/min	1515 ±360.3	1997 ±381.0	2074 ±479.4	1766 ±77.8
µmol/100g BW	255 ±62.2	367 ±66.8	273 ±64.9	286 ±17.9

* Significant difference between vehicle-treated and capsaiciin-densensitized rats studied at the same time.

Table 15: Resting oxygen consumption of vehicle-treated and CAP-DES rats studied at 30 weeks

	Vehicle	CAP-DES
	(6)	(6)
Body weight, grams	769 ±18.4	624 * ±21.4
Body surface, cm^2	675 ±9.5	596 * ±12.3
Oxygen consumption		
ml . min^{-1}	8.84 ±0.521	7.19 * ±0.285
ml/100 g . min^{-1}	1.15 ±0.042	1.16 ±0.029
ml/100 cm^2 . min^{-1}	1.31 ±0.059	1.21 ±0.031
ml/$kg^{0.75}$. min^{-1}	10.74 ±0.451	10.24 ±0.256
ml/100 g fat free mass . min^{-1}	1.42 ±0.063	1.35 ±0.040

* Significant effect of capsaicin-desensitization

significantly different from that of the control rats (Table 15). Thus, resting metabolic rate was normal for the reduced body size of the CAP-DES rats.

Discussion:

Because BAT is involved in the regulation of energy balance and is known to be atrophied in obese animals, it might have been expected that CAP-DES rats with atrophied BAT and impaired control of BAT function in relation to diet would be more susceptible to obesity than rats with a normally functioning BAT. To test this hypothesis, CAP-DES rats were fed a high fat diet for 10 weeks to study the rate at which they become obese. However, it was surprising to find that CAP-DES rats eating a high fat diet had a lower body weight and a smaller proportion of body fat. Growth curves did not show any tendency to obesity in CAP-DES rats. Retardation of BAT growth occurred and the major functional protein UCP in BAT of CAP-DES rats eating high fat diet was decreased 50% (Table 12). When UCP level in BAT of CAP-DES rats eating the high-fat diet is compared with that of CAP-DES rats eating chow, it is not significantly increased (Table 12) indicating that CAP-DES rats completely failed to respond to a high fat diet by growing more BAT. The high fat diet used in the present study was lard-based and has an effective action in normal Sprague-Dawley rats, both in promoting growth and thermogenic activation of BAT (increasing GDP binding) and in inducing obesity (Harper et al., 1991), as also seen in the present experiment.

In the physiological condition, there are age-related morphological and functional modifications in BAT (Sbarbati et al., 1991). The volume of the unilocular and connective

components increases with age. The volume of the multilocular component increases up to 24 weeks of age, and reduces thereafter (Sbarbati et al., 1991). This study implies that functional capacity of BAT is decreased with age, in association with aging-induced obesity. In the long-term study of the effect of capsaicin desensitization on rats, results were contrary to the hypothesis that atrophy of BAT would enhance aging-associated obesity. There was a lower body weight at 14 and 32 weeks, being more pronounced at the latter time at which body weight of CAP-DES was about 19% less than control rats function of BAT. The lower body weight was associated with less body fat and smaller body size. Carcass analysis showed that the CAP-DES rats at 32 weeks only contained 14.2% fat which was 25% lower than controls. This difference in fat content accounts for 46% of the difference in body weight. Other organs such as liver were also involved in the reduction of body weight in CAP-DES rats. The weight and cellularity of the liver were significantly decreased.

Function of BAT in this long-term study, as seen in the previous chapters, was seriously impeded by CAP-DES. By 32 weeks, all measures of BAT size (weight and protein), mitochondrial mass, thermogenic capacity (UCP and COX content) and cellularity (DNA content) were markedly lower than normal. The atrophied state of BAT might be expected to be associated with reduced energy expenditure in the CAP-DES rat. Although total oxygen consumption measured was less in the smaller CAP-DES rat, when expressed in terms of body mass, surface, or in a body mass independent fashion, the rate of oxygen consumption was normal in these animals. Thermoregulation in the long-term CAP-DES rat was still normal. Their lower energy intake is compatible with their smaller body weights. These findings do not explain why CAP-DES maintain a small body size and low body fat

content with age despite the atrophy of their BAT.

In summary, atrophied state of BAT and lack of response to diet, as seen in CAP-DES rats, does not in itself lead to any greater propensity to obesity. Indeed, older CAP-DES rats do not appear to develop the usual obesity that occurs with aging. Further studies are needed to see whether they do eventually become obese at a later age. A far-ranging long-term effect of CAP-DES on energy balance occurs that is not explained by the findings so far.

CHAPTER 11

GENERAL DISCUSSION AND CONCLUSIONS

General Discussion

The original objective of the work described in this thesis and started in 1987 was to study the neural control of diet-induced thermogenesis in BAT, believed at that time to be controlled solely by the sympathetic nerves which innervate it. To try to enhance diet-induced thermogenesis in BAT, rats were treated with capsaicin, a treatment known to impair warmth reception (Hori, 1984; Jancso-Gabor et al., 1970) and to enhance BAT thermogenesis during endotoxin-induced fever (Szekely and Szelenyi, 1979; Szekely and Szolcsanyi, 1979). The hypothesis was that the thermic effect of food ingestion, due to the processing of the components of the food itself, might suppress stimulated BAT thermogenesis to some extent and that the lack of this suppression, due to loss of warmth reception in the capsaicin-desensitized rat, could enhance sympathetic mediated stimulation of diet-induced thermogenesis in BAT and promote leanness. Unexpectedly, capsaicin-desensitization not only did not enhance diet-induced thermogenesis in BAT but resulted in atrophy of BAT and prevented diet-induced activation of BAT thermogenesis. This novel finding suggested some role in control of the thermogenic capacity of BAT for the subpopulation of sensory nerves known to be destroyed by the capsaicin-desensitization and to contain specific neuropeptides such as substance P and CGRP (Holzer, 1988; Maggi and Meli, 1988). The subsequent demonstration of the presence of substance P and CGRP in nerves within interscapular BAT (Norman et al., 1988; Lever et al., 1988; Mukherjee et al.,

1989; Himms-Hagen et al., 1990) reinforced the idea that sensory neuropeptides might play some role in control of BAT thermogenic capacity. Therefore, the objective of the work described in this thesis was modified. The modified objective was to elucidate the mechanism of the unexplained atrophy of BAT in the capsaicin-desensitized rat and to study the implication of this atrophy for the physiological functioning of the animal.

The principal finding in this work is that capsaicin-desensitization induces an atrophy of BAT that occurs very rapidly, within one day after the last injection, and, after a transient recovery of the tissue, reappears again in the rats at 4 to 8 months after the injections (Chapters 5, 7 and 10). The atrophy differs from that induced by surgical denervation in that it is more profound and results in both loss of mitochondria and loss of cells (Chapter 6) and prevents or retards both diet-induced and cold-induced activation of BAT thermogenesis and growth (Chapters 5,6 and 10). The effect of capsaicin-desensitization is not mediated by overheating of the BAT (Chapter 8). Preliminary results (Chapter 9) support the hypothesis that CGRP, one of the sensory neuropeptides known to be present in nerves in BAT and to be depleted after capsaicin-desensitization, may exert a trophic effect required for tissue maintenance (Chapter 9). The only major physiological consequence of capsaicin-desensitization disclosed in the present work is retardation of body weight gain for a short time after the procedure (Chapter 7) and a long-term tendency to leanness, even when an obesity-inducing diet is eaten (Chapter 10).

(1) Role of sensory nerves in control of BAT thermogenic function capacity

The atrophy produced by capsaicin-desensitization involves not only loss of

mitochondrial protein, but also loss of cells. This pattern of atrophy is different from most types of atrophy produced by low activity or absence of sympathetic nerves. Atrophy occurring in surgical denervation, fasting, lactation and diabetes have been demonstrated to decrease mitochondrial mass, but not cells (Himms-Hagen, 1989 for review). Compared with the atrophy produced by surgical denervation, atrophy produced by CAP-DES was much more profound, as indicated by the marked decrease in total protein content and UCP content. UCP, a key functional protein, had decreased by about 90 % (Chapters 5 and 6). At present, only de-acclimation to cold has been found to induce loss of cells in addition to loss of mitochondrial content in BAT. Thus, atrophy induced by capsaicin and atrophy induced by de-acclimation to cold have in common the loss of cells. This loss may be due to disappearance of a maintenance factor. The factor could conceivably arise from the innervation of the tissue. Whether the factor is the same in both cases or whether more than one factor is involved is unknown.

Retardation of BAT growth in response to diet or cold exposure (Chapters 5, 6 and 10) in CAP-DES rat strongly suggested a role of sensory nerves in control of BAT thermogenic capacity and growth. Surgical denervation can only partially prevent the trophic response to cold (Park and Himms-Hagen, 1988). In the case of absence of both the sympathetic neurotransmitter, noradrenaline, and sensory neurotransmitters, CGRP and SP, atrophy of BAT was greater (Chapter 6).

At present, the mechanism of the atrophy of BAT induced by capsaicin is not understood. From the available evidence, the atrophy could be explained by one or more of the following reasons:

First, neuropeptides CGRP and SP would be released from sensory nerves after systemic administration of capsaicin. The role of CGRP in BAT function is emphasized by Himms-Hagen et al (1990) who showed that CGRP is lost from nerves of BAT of CAP-DES rats. The absence of these neuropeptides may result in loss of a trophic effect on synthesis of BAT UCP and mitochondria. A trophic effect of sensory neuropeptides in other system has been reported. Loss of sensory neuropeptides produces parotid gland atrophy (Mansson et al., 1990). CGRP increases survival of a musculocutaneous critical flap (Kjartansson and Dalsgaard 1987). Capsaicin, known to deplete CGRP and other sensory neuropeptides, has an opposite effect, decreasing the survival of ischemic tissue (Kjartansson et al., 1986). Trophic effects of sensory nerves and/or CGRP have also been implicated in muscle and motoneuron morphogenesis (Garcia et al., 1990; Hashimoto et al., 1989; New and Mudge, 1986; Fontaine et al., 1986), in development of dopaminergic neurons in mouse brain (Denis-Donini, 1989) and in proliferation of human endothelial cells in culture (Haegerstrand and Dalsgaard, 1990). Trophic effects of sensory nerves on corneal epithelium are mediated by neuropeptides contained in peripheral nerve terminals (Gallar et al., 1990). Thus, neuropeptides of sensory nerves in BAT could conceivably be involved in the control of synthesis of protein, and of mitochondria or proliferation of cells. On the other hand, since changes in tissue protein mass always depend on the balance between rates of protein synthesis and degradation, the absence of CGRP or SP due to capsaicin treatment may result in the disappearance of an inhibitory influence of these neuropeptides on degradation of

BAT UCP ar · mitochondria. It is reported that BAT contains proteases which can rapidly redu · ' ·ermogenic capacity of the tissue during fasting (Desautels et al., 1990). CGRP or other sensory neuropeptides might normally restrain those reactions in BAT that promote degradation of mitochondria. Such reactions do occur as a normal physiological event, for example, BAT atrophy caused by fasting (Desautels et al., 1990).

Second, large amount of neuropeptides CGRP and SP may accumulate locally to damage cells and mitochondria.

Third, capsaicin itself may exert a direct and destructive effect on BAT that damages the cells and mitochondria.

Fourth, the combined actions of noradrenaline and sensory neuropeptides generate the initial atrophy, since capsaicin can activate sympathetic nerves as well as sensory nerves (Watanabe et al., 1987).

Atrophy of BAT seen in CAP-DES rats was not due to destruction or impairment of the sympathetic innervation to this tissue. This is supported by several studies. First of all, CAP-DES rats had a normal noradrenaline content in BAT, as measured by HPLC (Himms-Hagen et al., 1990). Secondly, in CAP-DES rats living at 26°C, BAT had lost 90% of its UCP and 50% of its total protein, but its thyroxine 5'-deiodinase concentration was not changed (Chapter 5). This enzyme is known to be totally dependent on the sympathetic innervation (Park and Himms-Hagen, 1988). Thirdly, after cold exposure or cold acclimation, there were increases in protein, UCP and T_4 5'-deiodinase in BAT of CAP-DES

rats. These changes are believed to be mediated by noradrenaline.

There is a complex relationship between neuropeptides of sensory nerves and noradrenaline of sympathetic nerves. Some studies have shown that sympathetic ganglionectomy in rat iris can increase sensory neuropeptide SP content (Kessler et al., 1983) and CGRP may inhibit the release of noradrenaline during adrenergic nerve stimulation in rat vas deferens (Ohhashi and Jacobowitz, 1985). In a recent review by Maggi (1991), interaction between sensory and noradrenergic nerve terminals has been summarized as reciprocal modulation of transmitters release. However, results of the present study make it seem unlikely that there is an opposing relationship between noradrenaline and sensory neuropeptides in BAT. Instead, activation of the sympathetic nervous system in BAT by cold would be expected to be accompanied by release of sensory neuropeptides. This idea can be supported by the finding that noradrenaline enhances the excitability of peripheral sensory nerves (Maggi and Meli, 1988) and noradrenergic sympathetic outflow can be stimulated by CGRP (Fisher et al., 1983). Thus, a partnership between noradrenaline and sensory neuropeptides in control of the trophic response of BAT to stimulation is an attractive possibility.

Since CGRP is the most powerful vasodilator known, it is possible that CGRP is involved acutely in the vasodilation that occurs in stimulated BAT and in angiogenesis during stimulated BAT growth. Both cold-induced nonshivering thermogenesis and diet-induced thermogenesis increase blood flow in BAT. However, noradrenaline itself does not regulate vasodilation in rat BAT (Foster and Depocas, 1980). The production of an endogenous vasodilator substance during noradrenaline-stimulated thermogenesis in BAT

has been suggested. Moreover, CGRP is known to promote the proliferation of endothelial cells (Haegerstrand and Dalsgaard, 1990). Thus a dual role for CGRP in control of BAT blood flow is suggested.

(2) Role of structures destroyed by capsaicin in the control of overall growth and energy balance

Energy expenditure for thermogenesis in BAT has been implicated in energy balance (Himms-Hagen, 1989, 1991 for review). Studies of a number of different types of obese rodents have demonstrated atrophy of BAT in most of them, and it has been suggested that this could contribute to the deficit in energy expenditure and the development of obesity. However, the present study did not show any agreement with this hypothesis. There was no direct correlation between atrophy of BAT function and body weight gain in CAP-DES rats.

BAT of CAP-DES rats did not show a trophic response to cafeteria feeding but the rats did not become obese (Chapter 5). This contrasts with the adult genetically obese, fa/fa, fatty rat, in which failure of diet-induced activation of BAT thermogenesis is associated with development of obesity. The food intake of the CAP-DES rats was normal. This finding agreed with the observation by Castonguay and Bellinger (1987). Body weight gain in CAP-DES rats was the same as control rats during short time study of the effect of a cafeteria diet.

In the time course study, CAP-DES caused atrophy of BAT, rapidly and extensively. The greatest atrophy of BAT occurred at 1 day and persisted up to 28 days. The recovery of BAT was very slow. Again, CAP-DES rats eating chow did not show any tendency to be

obese by 52 days after injections. In the study of the long term effect of CAP-DES, BAT was still atrophied at 32 weeks, but these animals were not obese. Unexpectedly, CAP-DES showed a significantly lower body weight, associated with lower body fat. In other words, CAP-DES rats had a tendency to leanness. To test the long-term susceptibility of CAP-DES rat to obesity, a high fat lard-based diet, known to promote growth of BAT and induce obesity in normal rats, was fed. This did not significantly increase BAT protein and UCP content in CAP-DES rat. CAP-DES rats eating the high fat diet showed lower body weight gain and body fat than that of control rats eating the same diet. Again, CAP-DES rats had no tendency to obesity.

From the above, it can be clearly seen that the atrophied thermogenic function of BAT has little contribution to energy balance in CAP-DES rat.

Three speculative explanations for why CAP-DES rats maintain a small body size and low body fat content with age despite the atrophy of their BAT can be suggested, namely partial recovery of function of BAT, a central lesion and a loss of sensory neuropeptides in other organs, as well as in BAT. Evidence was provided in the time course study for partial recovery of BAT function (Chapter 7). Atrophy of BAT by CAP-DES occurred starting at 1 day and persisted for up to 14 days. By 28 days, function of BAT began to recover. Together with long term study of effect of CAP-DES on BAT (Chapter 10), temporary recovery from the initial atrophy state can be seen from time period 4 to 10 weeks. This might possibly be due to restoration of the influence of the sensory neuropeptides. The rate of recovery of the neuropeptide CGRP is not known yet, although it can be depleted by capsaicin treatment (Himms-Hagen et al., 1990). As explained in Chapter 7, another

possibility for the temporary recovery of BAT would be the compensation by noradrenaline secreted from sympathetic nerve system which functions normally after CAP-DES (Himms-Hagen, 1990). Once animals become old, activity of sympathetic nerves is decreased. Thus, the compensation will be lost with age. However, this relationship between sensory nerves and sympathetic nerves in BAT is speculative.

It is possible that CAP-DES produces a central lesion that influences energy balance. Many studies have reported that CAP-DES impaired both central and peripheral sensory nervous system (Holzer, 1988; Maggi and Meli; 1988, Holzer, 1991). In the studies by Ritter and Dinh (1988, 1990), administration of capsaicin to adult or neonatal rats caused extensive degeneration not only in areas innervated by primary sensory neurons, but also in central nervous structures not previously associated with capsaicin-induced damage. Thus capsaicin's neurotoxicity is not limited to primary sensory neurons. Changes in energy balance in the long term CAP-DES rat could be due to alteration in one or more other systems involved in body weight and composition regulation. Some observations agree with this hypothesis. That is, the lower body weight regulation in CAP-DES was very similar to rats recovering from lateral hypothalamic lesions or to with dorsomedial hypothalamic lesions. These animals maintained lower body weight than normal. It is interesting to note that capsaicin-induced degeneration was not observed in the anterior hypothalamic or preoptic regions in adult rats, areas that are thought to be of special importance in thermoregulation (Boulant, 1980). This could explain why CAP-DES rats can maintain their body temperature during cold exposure (Chapters 5 and 6).

Distribution of sensory nerves is widespread in every organ and tissue in the body.

If sensory neuropeptides indeed exert a trophic effect on BAT, as suggested by the present study, it is also conceivable that they exert a trophic effect on other organs and tissues including white adipose tissue. Innervation by sensory nerves in both white adipose tissue (Fishman and Dark, 1987) and BAT has been found (Norman et al., 1988; Lever et al., 1988, 1989; Mukherjee et al., 1989). Moreover, trophic effects of CGRP and SP have been detected in a variety of different cell systems (Haegerstrand et al., 1990; Kjartansson and Dalsgaard 1987; Nilsson et al., 1985). Thus, CAP-DES may be associated with more than just atrophy of BAT. Retardation of growth of other organs and tissues, including white adipose tissue, could occur, as seen in liver, heart and kidney in the present experiments, which could contribute to the reduced body weight. Furthermore, many neuropeptides have been reported to function as cellular growth factors (Rozengurt, 1991; Woll and Rozengurt, 1989). The loss of neuropeptides of sensory nerves could be widely involved in atrophy of organs and tissues.

Many effects of CGRP have been demonstrated to be exerted via activation of adenylate cyclase in the cell systems studied (Haegerstrand et al., 1990; Hashimoto et al., 1989; Miles et al., 1989). If CGRP does do so, then removal of this neuropeptide by CAP-DES could inhibit proliferation of cells, eventually retarding whole organ or whole body growth. However, infusion of CGRP intravenously into pentobarbital anaesthetized rats did not produce an acute thermogenic effect on BAT (Chapter 8) indicating that CGRP did not activate adenylate cyclase in mature BAT cells. It is known that the acute thermogenic effect of noradrenaline on BAT is mediated by activation of adenylate cyclase. But, infusion of CGRP with mini pump directed towards BAT increased DNA content, indicating that

CGRP might act upon precursor cells or endothelial cells in BAT to stimulate growth. Therefore, it can be suggested that the lack of trophic influence of sensory neuropeptides on BAT as well as on other organs, including white adipose tissue, may result in the atrophy of BAT and in the reduced adiposity and stunting of the long-term CAP-DES rat.

General conclusions

There are two principal conclusions which can be drawn from the work described in this thesis. First, that sensory nerves play an important role in the control of BAT thermogenic capacity. This conclusion contradicts the previous assumption that sympathetic nerves and their neurotransmitter noradrenaline play the major, if not the only, role in control of both BAT thermogenesis and BAT thermogenic capacity. Second, that some effect of capsaicin-desensitization, whether central or peripheral, results in altered energy balance that is conductive to leanness. This association of leanness with atrophy of BAT would argue against the hypothesis of a major determining role for BAT thermogenesis in regulation of energy balance that has prevailed over the last twelve years.

Future Prospects

Since the neuropeptide CGRP plays a trophic role in BAT thermogenesis and growth, it will be very useful in the near future to determine the change of CGRP concentration in BAT at different times after capsaicin-desensitization, which could then be correlated with the changes in BAT function. Results from this study would provide some information about whether atrophy in CAP-DES rats is indeed related to low levels of CGRP in BAT. Studies

of other neuropeptides characteristic of sensory nerves (substance P, tachykinins) should also be done.

In addition, it is important to measure both blood flow and oxygen uptake of BAT in CAP-DES rats and in VEH-treated rats and the changes in blood flow and oxygen uptake resulting from infusion of CGRP, of noradrenaline, of SP and CGRP+noradrenaline, SP+noradrenaline. The findings from these studies would either support or refute the contention that CGRP plays a vasodilatory role in BAT thermogenesis. As well, these studies will also assess whether synergism can occur between noradrenaline and CGRP, SP and CGRP.

It would also be useful to investigate the relationship of two major neuropeptides, CGRP and SP, of sensory nerves, which might provide some information on how they interact to regulate BAT function. It was reported that CGRP is a potent inhibitor of SP degradation (Greves et al, 1985). As well, SP can regulated the vasodilator activity of CGRP (Brain and Williams, 1988). Thus, an interaction between the two might be expected.

It would be necessary to find out whether the loss of mitochondria in CAP-DES was due to cessation of synthesis of mitochondrial protein, to increased destruction of mitochondrial protein, or to both of these processes. Thus, it is required to develop a cell culture system and use techniques of molecular biology to study levels of specific mRNAs (cDNA probes for the mRNA for UCP and for COX are available). These studies would be useful in determining possible changes in synthesis.

The use of BAT cells in culture and histological assessment of BAT would be expected to provide important information about the central mechanisms involved in the

neural and endocrine coordination of BAT hypertrophy and atrophy. In this way, the cell types damaged by capsaicin-desensitization and responsible for the later impairment in aging associated growth of BAT could be identified.

Further study is also needed to investigate the potential role of sensory neuropeptides in regulation of energy balance. There is evidence that neuropeptides are involved in the processes of hunger, satiety and the maintenance of body weight (Morley, 1987). One of the sensory neuropeptides, CGRP may act as modulating agent of feeding behaviour (Krahn et al., 1984; Bueno et al., 1986). Administration of CGRP into different specific areas of brain would provide some insight into the role of CGRP in the central nervous system in regulation of food intake, body weight, adiposity and thermogenesis.

Finally, further long-term studies are needed to determine whether CAP-DES animals eventually develop obesity or recover to a normal body weight at later age.

REFERENCES

Afong, M., Olynyk, K.A., and Patel, H.V., Immunological studies of the uncoupling protein of brown adipose tissue, Can. J. Biochem. Cell Biol., 63:96-101, 1985.

Agius, L., and Williamson, D.H., The utilization of ketone bodies by the interscapular brown adipose tissue of the rat, Biochim. Biophys. Acta, 666:127-132, 1981.

Allard, M., and LeBlanc, J., Effects of cold acclimatization, cold exposure, and palatability on postprandial thermogenesis in rats, Int. J. Obesity, 12:169-178, 1988.

Amara, S.A., Jonas, V., Rosenfeld, M.G., Ong, E.S., and Evans, R.M., Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products, Nature, 298:240-244, 1982.

Amir, S., Lagiorgia, M., and Pollock, R., Intra-ventromedial hypothalamic injection of insulin suppresses brown fat thermogenesis in the anaesthetized rat, Brain Res., 480:340-342, 1989.

Amir, S., Intra-ventromedial hypothalamic injection of glutamate stimulates brown adipose tissue thermogenesis in the rat, Brain Res. 511:341-344, 1990.

Aquila, H., Link, T.A., and Klingenberg, M., The uncoupling protein from brown fat mitochondria is related to the mitochondrial ADP/ATP carrier. Analysis of sequence homologies and of folding of the protein in the membrance. EMBO J. 4:2369-2376, 1985.

Arase, K., York, D.A., Shimizu, H., Shargill, N., and Bray, G.A., Effects of corticotropin-releasing factor on food intake and brown adipose tissue thermogenesis in rats, Am. J. Physiol. 255:E255-E259, 1988.

Arnold, J., LeBlanc, J., Cote, J., Lalonde, J., and Richard, D., Exercise suppression of thermogenesis in 24- and 4°C- acclimated rats, Can. J. Physiol. Pharmacol. 64:922-926, 1986.

Arnold, J., and Richard, D., Exercise during intermittent cold exposure prevents aclimation to cold in rats, J. Physiol. 390:45-54, 1987.

Baker, R., Trophic role of neurotransmitters, Trends in Neurol. Sci. 11:533, 1988.

Bartness, T.J., and Wade, G.N., Effects of interscapular brown adipose tissue denervation on body weight and energy metabolism in ovariectomized and estradiol-treated rats, Behav. Neurosci. 98:674-685, 1984.

Berthoud, H.R., Bereiter, D.A., Trimble, E.R., Siegel, E.G., and Jeanrenaud, B., Cephalic phase, reflex insulin secretion, Diabetologia, 20:393-401, 1981.

Berthoud, H.R., and Jeanrenaud, B., Sham feeding-induced cephalic phase insulin release in the rat, Am. J. Physiol., 242:E280-E285, 1982.

Bevan, S., and Szolcsanyi, J., Sensory neuron-specific actions of capsaicin: mechanisms and applications, TiPS, 11:330-333, 1990.

Bianco, A.C., and Silva, J.E., Optimal response of key enzymes and uncoupling protein to cold in BAT depends on local T_3 generation, Am. J. Physiol., 253:E255-E263, 1987.

Bianco, A.C., and Silva, J.E., Cold exposure rapidly induces virtual saturation of brown adipose tissue nuclear T_3 receptors, Am. J. Physiol., 255:E496-E503, 1988.

Bianco, A.C., Sheng, X., and Silva, J.E., Triiodothyronine amplifies norepinephrine stimulation of uncoupling protein gene transcription by a mechanism not requiring protein synthesis, J. Biol. Chem., 263:18168-18175, 1988.

Billington, C.J., Bartness, T.J., Briggs, J., Levine, A.S., and Morley, J.E., Glucagon stimulation of brown adipose tissue growth and thermogenesis, Am. J. Physiol. 252:R160-R165, 1987.

Billington, C.J., Briggs, J.E., Link, J.G., and Levine, A.S., Glucagon in physiological concentrations stimulates brown fat thermogenesis in vivo, Am. J. Physiol. 261:R501-507, 1991.

Bouillaud, F., Ricquier, D., Mory, G., and Thibault, J., Increased level of mRNA for the uncoupling protein in brown adipose tissue of rats during thermogenesis induced by cold exposure or norepinephrine infusion, J. Biol. Chem., 259:11583-11586, 1984.

Bouillaud, F., Weissenbach, J., and Ricquier, D., Complete cDNA-derived amino acid sequence of rat brown fat uncoupling protein, J. Biol. Chem., 261:1487-1490, 1986.

Bouillaud, F., Raimbault, S., and Ricquier, D., The gene for rat uncoupling protein: complete sequence, structure of primary transcript and evolutionary relationship between exons, Biochem. Biophys. Res. Commun., 157:783-792, 1988.

Boulant, J.A., Hypothalamic control of thermoregulation, P.J. Morgane and J. Panksepp (eds): Handbook of the Hypothalamus, New, York: Dekker, pp. 1-82, 1980.

Brain, S.D., Williams, T.J., Tippins, J.R., Morris, H.R., and MacIntyre, I., Calcitonin gene-related peptide is a potent vasodilator, Nature, 313:54-56, 1985.

Brain, S.D., Williams, T.J., Substance P regulates the vasodilator activity of calcitonin gene-related peptide, Nature, 335:73-75, 1988.

Buck, S.H., and Burks, T.F., The neuropharmacology of capsaicin: review of some recent observations, Pharmacol. Rev., 179-226, 1986.

Bueno, L., Fargeas, M.J., and Jukie, P., Effects of calcitonin and CGRP alone or in combination on food intake and forestomach (reticulum) motility in sheep, Physiol. Behav. 36:907-911, 1986.

Bukowiecki, L.J., Follea, N., Paradis, A., and Collet, A., Stereospecific stimulation of brown adipocyte respiration by catecholamines via β_1-adrenoreceptors, Am. J. Physiol., 238:E552-E563, 1980.

Bukowiecki, L.J., Collet, A.J., Follea, N., Guay, G., and Jahjah, L., Brown adipose tissue hyperplasia: a fundamental mechanism of adaptation to cold and hyperphagia, Am. J. Physiol., 242:E353-E359, 1982.

Bukowiecki, L.J., Mechanisms of stimulus-calorigenesis coupling in brown adipose tissue, Can. J. Biochem. Cell Biol. 62:623-630, 1984.

Bukowiecki, L.J., Lipid metabolism in brown adipose tissue, In Brown Adipose Tissue, pp. 105-121, (Trayhurn, P., and Nicholls, D.G., eds) Arnold, London, 1986.

Bukowiecki, L.J., Géloën, A., and Collet, A.J., Proliferation and differentiation of brown adipocytes from interstitial cells during cold acclimation, Am. J. Physiol., 250:C880-C887, 1986.

Cannon, B., and Lindberg, O., Mitochondria from brown adipose tissue: isolation and properties, in Methods in Enzymology, (Fleischer, S., and Parker, N., eds), Academic Press, New York, 15:65-78, 1979.

Cannon, B., Alexson, S., and Nedergaard, J., Peroxisomal ß-oxidation in brown fat, Ann. N.Y. Acad. Sci., 386:40-57, 1982.

Cannon, B., Nedergaard, J., Lundberg, J.M., Hokfelt, T., Terenius, L., and Goldstein, M., 'Neuropeptide tyrosine' (NPY) is co-stored with noradrenaline in vascular but not in parenchymal sympathetic nerves of brown adipose tissue, Exp. Cell Res., 164:546-550, 1986.

Carneheim, C., Nedergaard, J., and Cannon, B., ß-adrenergic stimulation of lipoprotein lipase in rat brown adipose tissue during acclimation to cold, Am. J. Physiol., 246:E327-E333, 1984.

Carneheim, C., Nedergaard, J., and Cannon, B., Cold-induced ß-adrenergic recuitment of lipoprotein lipase in brown fat is due to increased transcription, Am. J. Physiol., 254:E155-161, 1988.

Carneheim, C.M.H., and Alexson, S.E.H., Refeeding and insulin increase lipoprotein lipase activity in rat brown adipose tissue, Am. J. Physiol., 256:E645-E650, 1989.

Castonguay, T.W, and Bellinger, L.L., Capsaicin and its effects upon meal patterns, and glucagon and epinephrine suppression of food intake, Physiol. and Behav., 40:337-342, 1987.

Chahl, L.A., Antidromic vasodilation and neurogenic inflammation, Pharmacol. Ther., 37:275-300, 1988.

Connolly, E., Nanberg, E., and Nedergaard, J., Na^+-dependent, α-adrenergic mobilization of intracellular (mitochondrial) Ca^{2+} in brown adipocytes, Eur. J. Biochem. 141:187-193, 1984.

Cooney, G., Caterson, I.D., and Newsholme, E.A., The effect of insulin and noradrenaline on the uptake of 2-[1-^{14}C]deoxyglucose in vivo by brown adipose tissue and other glucose-utilising tissue of the mouse, FEBS Lett., 188:257-261, 1985.

Cooney, G., Astbury, L.D., Williams, P.F., and Caterson, I.D., Insulin response in individual tissues of control and gold thioglucose-obese mice in vivo with [1-^{14}C]2-deoxyglucose, Diabetes, 36:152-158, 1987.

Corbett, S.W., Kaufman, L.N., and Keesey, R.E., Thermogenesis after lateral hypothalamic lesions: contributions of brown adipose tissue, Am. J. Physiol., 255:E708-715, 1988.

Cormarèche-Leydier, M., The effect of ambient temperature on rectal temperature, food intake and short term body weight in the capsaicin desensitized rat, Pfluegers Arch., 389:171-174, 1981.

Cormarèche-Leydier, M., Shimada, S.G., and Stitt, J.T., Hypothalamic thermosensitivity in capsaicin-desensitized rats, J. Physiol. (Lond.), 363:227-236, 1985.

Cunningham, S.A., Wiesinger, H., and Nicholls, D.G., Quantification of fatty acid activation of the uncoupling protein in brown adipocytes and mitochondria from the guinea-pig, Eur. J. Biochem., 157:415-420, 1986.

Cunningham, S.A., and Nicholls, D.G., Induction of functional uncoupling protein in guinea pigs infused with noradrenaline. Studies with isolated brown adipocytes, Biochem. J., 245:485-491, 1987.

Denis-Donini, S., Expression of dopaminergic phenotypes in the mouse olfactory bulb induced by the calcitonin gene-related peptide, Nature, 339:701-703, 1989.

Desautels, M., Zaror-Behrens, G., and Himms-Hagen, J., Increased purine nucleotide binding, altered polypeptide composition, and thermogenesis in brown adipose tissue

mitochondria of cold-acclimated rats, Can. J. Biochem., 56:378-383, 1978.

Desautels, M., Dulos, R.A., and Mozaffari, B., Selective loss of uncoupling protein from mitochondria of surgically denervated brown adipose tissue of cold-acclimated mice, Biochem. Cell Biol., 64:1125-1134, 1986.

Desautels, M., and Dulos, R.A., Effects of repeated cycles of fasting-refeeding on brown adipose tissue composition in mice, Am. J. Physiol., 955:E120-E128, 1988.

Desautels, M., Michelska, E., and Mozaffari, B., Role of acid proteases in brown adipose tissue atrophy caused by fasting in mice, Biochem. Cell Biol. 68:441-447, 1990.

Desautels, M., and Dulos, R.A., Is adrenergic innervation essential for maintenance of UCP in hamster BAT mitochondria ?, Am. J. Physiol. 254:R1035-1042, 1990.

Diamond, P., and LeBlanc, J., A role of autonomic nervous system in postprandial thermogenesis in dogs, Am. J. Physiol., 252:E719-E726, 1985.

Diamond, P., Brondel, L., and LeBlanc, J., Palatability and postprandial thermogenesis in dogs, Am. J. Physiol., 248: E75-E79, 1985.

Diamond, P., and LeBlanc, J., Hormonal control of postprandial thermogenesis in dogs, Am. J. Physiol., 252:E512-E529, 1987.

Diamond, P., and LeBlanc, J., A role for insulin in cephalic phase of postprandial thermogenesis in dogs, Am. J. Physiol., 254:E625-E632, 1988.

Downs, T.R., and Wilfinger, W.W., Fluorometric quantification of DNA in cells and tissue, Analyt. Biochem., 131:538-547, 1983.

Ebner, S., Burnol, A.F., Ferré, P., Saintaurin, M.A., and Girard, J., Effects of insulin and norepinephrine on glucose transport and metabolism in rat brown adipocytes, Eur. J. Biochem., 170:469-474, 1987.

Eckerskorn, C., and Klingenberg, M., In the uncoupling protein from brown adipose tissue the C-terminus protrudes to the c-side of the membrance as shown by tryptic cleavage, FEBS Lett., 226:166-170, 1987.

Ekstrom, J., Ekman, R., Hakanson, R., Luts, A., Sundler, F., and Tobin, G., Effects of capsaicin pretreatment on neuropeptides and salivary secretion of rat parotid glands, Br. J. Pharmacol. 97:1031-1038, 1989.

Etienne, T., Girgis, S., MacIntyre, I., Morris, H.R., Panico, M., and Tippins, F.R., An investigation of the activity of human and rat calcitonin gene-related peptides (CGRP) on

isolated tissues from the rat and guinea pig, J. Physiol. 351:489, 1984.

Falcou, R., Bouillaud, F., Mory, G., Apfelbaum, M., and Ricquier, D., Increase of uncoupling protein and its mRNA in brown adipose tissue of rats fed on 'cafeteria diet, Biochem. 231:241-244, 1985.

Fernandez, M, Nicholls, D.G., and Rial, E., The uncoupling protein from brown-adipose-tissue mitochondria. Chymotrypsin-induced structural and functional modifications, Eur. J. Biochem., 164:675-680, 1987.

Fisher, L.A., Kikkawa, D.O., Rivier, J.E., Amara, S.G., Evans, R.M., Rosenfeld, M.G., Vale, W.W., and Brown, M.R., Stimulation of noradrenergic sympathetic outflow by calcitonin gene-related peptide, Nature, 305:534-536, 1983.

Fishman, R.B., and Dark, J., Sensory innervation of white adipose tissue, Am. J. Physiol., 253: R942-R944, 1987.

Fisler, J.S., Lupien, J.R., Wood, R.D., Bray, G.A., and Schemmel, R.A., Brown fat thermogenesis in a rat model of dietary obesity, Am. J. Physiol. 253:R756-R762, 1982.

Fisler, J.S., Yoshida, T., and Bray, G.A., Catecholamine turnover in S 5B.P1 and Osborne-Mendel rats: response to a high fat diet, Am. J. Physiol. 247:R290-R295, 1984.

Fontaine, B., Klarsfeld, A., Hokfelt, T., and Changeux, J.P., Calcitonin gene-related peptide, a peptide present in spinal cord motoneurons, increases the number of acetylcholine receptors in primary cultures of chick embryo myotubes, Neurosci. Lett. 71:59-65, 1986.

Foster, D.O., Evidence against a mediatory role of brown adipose tissue in the calorigenic response of cold-acclimated rats to nordrenaline, Can. J. Physiol. Pharmacol., 52:1051-1062, 1974.

Foster, D.O., and Frydman, M.L., Comparison of microspheres and $^{86}Rb^{+}$ as tracers of the distribution of cardiac output in rats indicates invalidity of $^{86}Rb^{+}$-based measurements, Can. J. Physiol. Pharmacol., 56:97-109, 1978a.

Foster, D.O., and Frydman, M.L., Nonshivering thermogenesis in the rat. II Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorigenesis induced by noradrenaline, Can. J. Physiol. Pharmacol., 56:110-122, 1978b.

Foster, D.O., and Frydman, M.L., Tissue distribution of cold-induced thermogenesis in conscious warm-or cold-acclimated rats reevaluated from changes in tissue blood flow: the dominant role of brown tissue in the replacement of shivering by nonshivering thermogenesis, Can. J. Physiol. Pharmacol., 57:257-270, 1979.

Foster, D.O., and Depocas, F., Evidence againt noradrenergic regulation of vasodilation in rat brown adipose tissue, Can. J. Physiol. Pharmacol., 58:1418-1525, 1980.

Foster, D.O., Depocas, F., and Frydman, M.L., Noradrenaline-induced calorigenesis in warm- and in cold-acclimated rats: relations between concentration of noradrenaline in arterial plasma, blood flow to differently located masses of brown adipose tissue, and calorigenic response, Can. J. Physiol. Pharmacol. 58:915-924, 1980.

Foster, D.O., Depocas, F., and Zaror-Behrens, G., Unilaterality of the sympathetic innervation of each pad of rat interscapular brown adipose tissue, Can. J. Physiol. Pharmacol., 60:107-113, 1982a.

Foster, D.O., Depocas, F., and Zuker, M., Heterogeneity of the sympathetic innervation of rat interscapular brown adipose tissue via intercostal nerves, Can. J. Physiol. Pharmacol., 60:747-754, 1982b.

Foster, D.O., Quantitative role of brown adipose tissue in thermogenesis, in Brown Adipose Tissue, (Trayhurn, P., and Nicholls, D.G., eds) Arnold, London, 1986.

Franco-Cereceda, A., Henke, H., Lundberg, J.M., Petermann, J.B., Hokfelt, T., and Fischer, J.A., Calcitonin gene-related peptide (CGRP) in capsaicin-sensitive substance P-immunoreactive sensory neurons in animals and man: distribution and release by capsaicin, Peptides, 8:399-410, 1987.

Freedman, M.R., Wellman, P.J., Brown adipose tissue thermogenesis induced by low level electrical stimulation of hypothalamus in rats, Brain Res. Bull., 18:7-11, 1987.

Freeman, K.B., Heffernan, M., Dhalla, Z., and Patel, H.V., Effects of exposure temperature on brown adipose tissue uncoupling protein, Biochem. Cell. Biol. 67:147-151, 1989.

French, R.R., Gore, M.G., and York, D.A., A study of GDP binding to purified thermogenin protein from brown adipose tissue, Biochem. J., 251:385-389, 1988.

Fukushima, M., Lupien, J., and Bray, G.A., Interaction of light and corticosterone on food intake and brown adipose tissue of the rat, Am. J. Physiol. 249:R753-R757, 1985.

Fyda, D.M., Cooper, K.E., Veale, W.L., Modulation of brown adipose tissue-mediated thermogenesis by lesions to the nucleus tractus solitarius in the rat, Brain Res. 546:203-210, 1991.

Gallar, J., Pozo, M.A., Rebollo, I., and Belmonte, C., Effects of capsaicin on corneal wound healing, Invest. Ophthalmo. & Visual Sci. 31:1968-1974, 1990.

Galpin, K.S., Henderson, R.G., James, W.P.T., and Trayhurn, P., GDP binding to brown-

adipose-tissue mitochondria of mice treated chronically with corticosterone, Biochem. J., 214:265-268, 1983.

Gamse, R., Petsche, U., Lembeck, F., and Jancso, G., Capsaicin applied to peripheral nerve inhibits axoplasmic transport of substance P and somatostatin, Brain Res. 239:447-462, 1982.

Garcia, L., Pincon-Raymond, R., Romey, G., Changeux, J.P., Lazdunski, M., and Rieger, F., Induction of normal ultrastructure by CGRP treatment in dysgenic myotubes, FEBS Lett. 263:147-152, 1990.

Géloën, A., Collet, A.J., Guay, G., and Bukowiecki, L.J., ß-adrenergic stimulation of brown adipocyte proliferation, Am. J. Physiol., 254:C175-C182, 1988.

Géloën, A., Collet, A.J., Guay, G., and Bukowiecki, L.J., In vivo differentiation of brown adipocytes in adult mice: an electron microscopic study, Am. J. Anat. 188:366-372, 1990.

Géloën, A., and Trayhurn, P., Regulation of the level of uncoupling protein in brown adipose tissue by insulin, Am. J. Physiol. 258:R418-424, 1990a.

Géloën, A., and Trayhurn, P., Regulation of the level of uncoupling protein in brown adipose tissue by insulin requires the mediation of the sympathetic nervous system, FEBS Lett. 267:265-267, 1990b.

Geppetti, P., Maggi, C.A., Perretti, F., Frilli, S., and Manzini, S., Simultaneous release by bradykinin of substance P- and calcitonin gene-related peptide immunoreactivities from capsaicin-sensitive structures in guinea-pig heart, Br. J. Pharmacol. 94:288-290, 1988.

Giovannini, P., Seydoux, J., and Girardier, L., Evidence for a modulating effect of NA^+/H^+ exchange on the metabolic response of rat brown adipose tissue, Eur. J. Physiol., 411:273-277, 1988.

Girardier, L., and Seydoux, J., Neural control of brown adipose tissue thermogenesis, in Brown Adipose Tissue, (Trayhurn, P., and Nicholls, D.G., eds) pp. 122-151, Arnold, London, 1986.

Gong, T.W.L., Horwitz, B.A., and Stern, J.S., The effects of 2-deoxy-D-glucose and sympathetic denervation on brown fat GDP binding in Sprague-Dawley rats, Life Sci., 46:1037-1044, 1990.

Goodchild, A.K., Dampney, R.A.L., and Bandler, R., A method for evoking physiological responses by stimulation of cell bodies, but not axons of passage, within localized regions of the central nervous system, J. Neurosci., Methods, 6:351-363, 1982.

Granneman, J.G., MacKenzie, R.G., Fluharty, S.J., Zigmond, M.J., and Stricker, E.M., Neural control of adenylate cyclase responsiveness in brown adipose tissue, J. Pharmacol. Exper. Therapeutics, 233:163-167, 1985.

Greco-Perotto, R., Zaninetti, D., Assimacopoulos-Jeannet, F., Bobbioni, E., and Jeanrenaud, B., Stimulatory effect of cold adaptation on glucose utilization by brown adipose tissue, J. Biol. Chem., 262:7732-7736, 1987a.

Greco-Perotto, R., Assimacopoulos-Jeannet. F., and Jeanrenaud, B., Insulin modifies the properties of glucose transporters in rat brown adipose tissue, Biochem. J., 247:63-68, 1987b.

Greves, P.L., Nyberg, F., Terenius, L., and Hokfelt, T., Calcitonin gene-related peptide is a potent inhibitor of substance P degradation, Eur. J. Pharmacol. 115:309-311, 1985.

Griggio, M.A., Richard, D., and LeBlanc, J., The involvement of the sympathetic nervous system in meal-induced thermogenesis in mice, Int. J. Obesity, 15:711-715, 1991.

Guerra, F.J.D., Zaidi, M., Bevis, P., Macintyre, I., and Emson, P.C., Evidence for release of calcitonin gene-related peptide and neurokinin a from sensory nerve endings in vivo, Neuroscience, 25:839-846, 1988.

Haegerstrand, A., Dalsgaard, C-J., Jonzon, B., Larsson, O., Nilsson, J., Calcitonin gene-related peptide stimulates proliferation of human endothelial cells, Proc. Natl., Acad., Sci., USA, 87:3299-3303, 1990.

Hansen, E.S., and Knudsen, J., Parallel measurements of heat production and thermogenin content in brown fat cells during cold acclimation of rats, Biosci. Rep. 6:31-38, 1986.

Harper, M.E., Patrick, J., and Himms-Hagen, J., Altered Na/K transport and brown adipose tisue (BAT) activity in diet induced obesity (dio) and food restriction, FASEB J. 5:A1654, 1991.

Hashimoto, K., Uchida, S., and Yoshida, H., Effects of calcitonin gene-related peptide on the adenylate cyclase system in cultured rat skeletal muscle cells, Life Sci. 45:2183-2192, 1989.

Henry, C.J.K., and Emery, B., Effect of spiced food on metabolic rate, Human Nutrition: Clinical Nutrition, 40C:165-168, 1985.

Himms-Hagen, J., and Park, I.R.A., Role of the sympathetic nervous system in the growth and maintenance of brown adipose tissue in cold-adapted and cafeteria-fed rats, In: Hales JRS, ed., Thermal Physiology, New York: Raven Press, 193-196, 1984.

Himms-Hagen, J., The role of brown adipose tissue in the calorigenic effect of adrenaline

and noradrenaline in cold-acclimated rats, J. Physiol. Lond., 205:393-403, 1969.

Himms-Hagen, J., Nonshivering thermogenesis, Brain Res. Bull., 12:151-160, 1984.

Himms-Hagen, J., Brown adipose tissue and cold-acclimation, in Brown Adipose Tissue, (Trayhurn, P., and Nicholls, D.G., eds) pp. 214-268, Arnold, London, 1986.

Himms-Hagen, J., Brown adipose tissue thermogenesis and obesity, Progr. Lipid Res., 28:67-115, 1989.

Himms-Hagen, J., Brown adipose metabolism, Obesity, (Björntorp, P., and Brodoff, B.N., eds), Lippincott, Philadelphia, in press.

Himms-Hagen, J., Cui, J., Park, I.R.A., Sigurdson, L., and Zaror-Behrens, G., Neural control of brown adipose tissue growth, Living in the cold II, (Malan, A., and Canguihem, B., eds), pp. 367-375, 1989.

Himms-Hagen, J., Cui, J., and Sigurdson, S.L., Sympathetic and sensory nerves in control of growth of brown adipose tissue: effect of denervation and of capsaicin, Neurochemistry International, 17:271-280, 1990.

Himms-Hagen, J., Neural control of brown adipose tissue thermogenesis, hypertrophy, and atrophy, Frontiers in Neuroendocrinology, 12:38-93, 1991.

Hogaboam, C.M., and Wallace, J.L., Inhibition of platelet aggregation by capsaicin. An effect unrelated to actions on sensory afferent neurons, Eur. J. Pharmaco. 202:129-131, 1991.

Hogan, S., Coscina, D.V., and Himms-Hagen, J., Brown adipose tissue of rats with obesity-inducing ventromedial hypothalamic lesions, Am. J. of Physiol. 243:E338-E344, 1982.

Holt, S.J., Wheal, H.V., and York, D.A., Hypothalamic control of brown adipose tissue in Zucker lean and obese rats. Effect of electrical stimulation of the ventromedial nucleus and other hypothalamic centres, Brain Res., 405:227-233, 1987.

Holt, S.J., and York, D.A., Effect of lateral hypothalamic lesion on brown adipose tissue of Zucker lean and obese rats, Physiol. Behav. 43:292-300, 1988.

Holzer, P., Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides, Neuroscience, 24:739-768, 1988.

Holzer, P., Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons, Pharmac. Rev., 43:143-201, 1991.

Hori, T., Capsaicin and central control of thermoregulation, Pharmacol. Ther., 26:389-416, 1984.

Imai-Matsumura, K., and Nakayama, A., The central efferent mechanism of brown adipose tissue thermogenesis induced by preoptic cooling in rats, Can. J. Physiol. Pharmacol., 65:1299-1303, 1987.

Imai-Matsumura, K., Matsumura, K., Tsai, C.L., and Nakayama, T., Thermal responses of ventromedial hypothalamic neurons in vivo and in vitro, Brain Res., 445:193-197, 1988.

Iwai, M., Hell, N.S., and Shimazu, T., Effect of ventromedial hypothalamic stimulation on blood flow of brown adipose tissue in rats, Pflugers Arch. 410:44-47, 1987.

Jacobsson, A., Stadler, U., Glotzer, M.A., and Kozak, L.P., Mitochondrial uncoupling protein from brown fat, J. Biol. Chem., 260:16250-16254, 1985.

Jacobsson, A., Cannon, B., Nedergaard, J., Physiological activation of brown adipose tissue destabilizes thermogenin mRNA, FEBS Lett., 224:353-356, 1987.

Jamal, Z., and Saggerson, E.D., Enzymes involved in adenosine metabolism in rat white and brown adipocytes. Effects of streptozotocin-diabetes, hypothyroidism, age and sex differences, Biochem. J., 245:881-886, 1987.

Jamal, Z., and Saggerson, E.D., Factors influenceing the altered thermogenic response of rat brown adipose tissue in streptozotocin-diabetes, Biochem. J., 249:415-421, 1988a.

Jamal, Z., and Saggerson, E.D., Changes in brown-adipose-tissue mitochondrial processes in streptozotocin-diabetes, Biochem. J., 252:293-296, 1988b.

Jancso, G., Kiraly, E., and Jancso-Gabor, A., Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones, Nature, 270:741-743, 1977.

Jessell, T.M., Iversen, L.L., and Cuello, A.C., Capsaicin-induced depletion of substance P from primary sensory neurons, Brain Res., 152:183-188, 1978.

Jin, J.G., Takaki, M., and Nakayama, S., Ruthenium red prevents capsaicin-induced neurotoxic action on sensory fibers of the guinea pig ileum, Neurosci. Lett. 106:152-156, 1989.

Johansson, B., Brown fat: a review, Metabolism 8:221-240, 1959.

Johnston, J.L., and Balachandran, A.V., Effects of dietary protein, energy and tyrosine on central and peripheral norepinephrine turnover in mice, J. Nutr., 117:2046-2053, 1987.

Kawada, T., Watanabe, T., Takaishi, T., Tanaka, T., and Iwai, K., Capsaicin-induced ß-adrenergic action on energy metabolism in rats: influence of capsaicin on oxygen consumption, the respiratory quotient and substrate utilization, Proc. Soc. Exp. biol. Med., 183:250-256, 1986.

Kawada, T., Sakabe, S., Aoki, N., Watanabe, T., Higeta, K., Iwai, K., and Sugimoto., E., Intake of sweeteners and pungent ingredients increase the thermogenin content in brown adipose tissue of rat, J. Agric. Food Chem., 39:651-654, 1991.

Kessler, J.A., Bell, W.O., and Black, I.B., Interactions between the sympathetic and sensory innervation of the iris, J. Neurosci., 3:1301-1307, 1983.

Kiraly, E., Jancso, G., and Hajos, M., Possible morphological correlates of capsaicin desensitization, Brain Res. 540:279-282, 1991.

Kjartansson, J., Dalsgaard, C.J., and Jonsson, C.E., Decreased survival of experimental critical flaps in rats after sensory denervation with capsaicin, Plast. Reconstr. Surg. 79:218-221, 1986.

Kjartansson, J., and Dalsgaard, C.J., Calcitonin-gene related peptide increases survival of a musculocutaneous critical flap in the rat, Eur. J. Pharmacol., 142:355-358, 1987.

Klingenberg, M., Characteristics of the uncoupling protein from brown fat mitochondria, Biochem. Soc. Trans. 12:390-393, 1984.

Klingenberg, M., and Winkler, E., Reconstitution of an H^+ translocator, the 'uncoupling protein' from brown adipose tissue mitochondria, in phospholipid vesicles, Methods in Enzymology, 127:772-779, 1986.

Klingenberg, M., Nucleotide binding to uncoupling protein: mechanism of control by protonation, Biochemistry, 27:781-791, 1988.

Kopecky, J., Sigurdson, L., Park, I.R.A., and Himms-Hagen, J., Thyroxine 5'-deiodinase in hamster and rat brown adipose tissue: effect of cold and diet, Am. J. Physiol., 251:E1-E7, 1986.

Kopecky, J., Jezek, P., Drahota, Z., and Houstek, J., Control of uncoupling protein in brown-fat mitochondria by purine nucleotides, Chemical modification by diazobenzenesulfonate, Eur. J. Biochem., 164:687-694, 1987.

Kozak, L.P., Brittona, L.H., Kozak, U.C., and Well, J.M., The mitochondrial uncoupling protein gene, J. Biol. Chem., 263:12274-12277, 1988.

Kraegen, E.W., James, D.E., Storlien, L.H., Burleigh, K.M., and Chisholm, D.J., In vivo

insulin resistance in individual peripheral tissues of the high fat fed rat: assessment by euglycaemic clamp plus deoxyglucose administration, Diabetologia, 29:192-198, 1986.

Krahn, D.D., Gosnell, B.A., Levine, A.S., and Morley, J.E., Effects of calcitonin gene-related peptide on food intake, Peptides, 5:861-864, 1984.

Kubota, M., Moseley, J.M., Butera, L., Dusting, G.J., MacDonald, P.S., and Martin, T.J., Calcitonin gene-related peptide stimulates cyclic AMP formation in rat aortic smooth muscle cells, Biochem. Biophy. Res. Commun., 132:88-94, 1985.

Kuroshima, A., Yahata, T., and Habara, Y., Hormonal regulation brown adipose tissue with special reference to the participation of endocrine pancreas, J. Therm. Biol. 9:81-86, 1984.

LaNoue, K.F., Strzelecki, T., Strzelecka, D., and Koch, C., Regulation of the uncoupling protein in brown adipose tissue, J. Biol. Chem., 261:298-305, 1986a.

LaNoue, K.F., Koch, C., Strzelecki, D., and Kobylski, T.P., Regulation of Na^+ transport in brown adipose tissue, Biochem. J., 235:545-552, 1986b.

Larsen, P.R., Radioimmunoassay of thyroxine, triiodothyronine, and thyrotropin and human serum, In Manual of Clinical Immunology, (Rose, N.R., and Friedman, H., eds), Washington, D.C., pp . 222-230, 1976.

Laufer, R., and Changeux, J.P., Calcitonin gene-related peptide and cyclic AMP stimulate phosphoinositide turnover in skeletal muscle cells, J. Biol. Chem. 264:2683-2689, 1989.

Lean, M.E.J., Branch, W.J., James, W.P.T., Jennings, G., and Ashwell, M., Measurement of rat brown-adipose-tissue mitochondrial uncoupling protein by radiommunoassay: increased concentration after cold acclimation, Biosci. Rep., 3:61-71, 1983.

Lean, M.E.J., James, W.P.T., Jennings, G., and Trayhurn, P., Brown adipose tissue uncoupling protein content in infants, children and adult humans, Clin. Sci., 72:291-297, 1986.

Lean, M.E.J., and Trayhurn, P., Brown adipose tissue in humans, J. Obesity Weight Regulation, 6:234-253, 1987.

LeBlanc, J., Lupien, D., Diamond, P., Macari, M., and Richard, D., Thermogenesis in response to various intakes of palatable food, Can. J. Physiol. Pharmacol., 64:976-982, 1986.

LeBlanc, J., and Diamond, P., Efffect of meal size and frequency on postprandial thermogenesis in dogs, Am. J. Physiol. 250: E144-E147, 1986.

Lee, M.O., Determination of the surface area of the white rat with its application to the

expression of metabolic results, Am. J. Physiol., 89:24-33, 1929.

Leeman, S.E., and Gamse, R., Substance P in sensory neurons, TIPS, 119-121, May, 1981.

LeFeuvre, R.A., Rothwell, N.J., and Stock, M.J., Activation of brown fat thermogenesis in response to central injection of corticotropin releasing hormone in the rat, Neuropharmacology, 26:1217-1221, 1987.

Lefkowitz, R.J., Hoffman, B.B., and Taylor, P., Neurohumoral transmission: the autonomic and somatic motor nervous systems, in The pharmacological basis of therapeutics, (Gilman, A.G., Rall, T.W., Nies, A.S., and Taylor, P., eds), Pergamon Press Inc., 1990.

Lenz, H.J., Mortrud, M.T., Vale, W.W., Rivier, J.E., and Brown, M.R., Calcitonin gene-related peptide acts within the central nervous system to inhibit gastric acid secretion, Regul. Pept. 9:271-277, 1984.

Lenz, H.J., Mortrud, M.T., Rivier, J.E., and Brown, M.R., Central nervous system actions of calcitonin gene-related peptide on gastric acid secretion in the rat, Gastroenterology, 88:539-544, 1985.

Leonard, J.L., Mellen, S.A., and Larsen, P.R., Thyroxine 5'-deiodinase activity in brown adipose tissue, Endocrinology 112:1153-1155, 1983.

Lever, J.D., Mukherjee, S., Norman, D., Symons., D., and Jung, R.T., Neuropeptide and noradrenaline distributions in rat interscapular brown fat and in its intact and obstructed nerves of supply, J. Auton. Nerv. Syst., 25:15-25, 1988.

Lever, J.D., Mukherjee, S., Norman, D., Symons., D., Wheeler, M.H., Connacher, A., and Jung, R.T., Catecholaminergic and peptidergic nerves in naturally occuring and pheochromocytoma-associated human brown adipose tissue, Clin. Anat., 2:157-166, 1989.

Levin, N., Shinsako, J., and Dallman, M.F., Corticosterone acts on the brain to inhibit adrenalectomy-induced adrenocorticotropin secretion, Endocrinology, 122:694-701, 1988.

Lin, C., and Klingenberg, M., Isolation of the uncoupling protein from brown adipose tissue mitochondria, FEBS Let., 113:299-303, 1980.

Lin, C., and Klingenberg, M., Characteristics of the isolated purine nucleotide binding protein from brown fat mitochondria, Biochemistry, 21:2950-2956, 1982.

Liposits, Z.S., Uht, R.M., Harrison, R.W., Gibbs, F.P., Paull, W.K., and Bohn, M.C., Ultrastructural localization of glucocorticoid receptor (GR) in hypothalamic paraventricular neurons synthesizing corticotropin releasing factor (CRF), Histochemistry, 87:407-412, 1987.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randal, R.J., Protein measurement with Folin phenol reagent, J. Biol. Chem., 193:265-275, 1951.

Lupien, J.R., Tokunaga, K., Kemnitz, J.W., Groos, E., and Bray, G.A., Lateral hypothalamic lesions and fenfluramine increase thermogenesis in brown adipose tissue, Physiol. Behav. 38:15-20, 1986.

Ma, S.W.Y., and Foster, D.O., Redox state of brown adipose tissue as a possible determinant of its blood flow, Can. J. Physiol. Pharmacol., 62:949-956, 1984.

Ma, S.W.Y., Nadeau, B.E., and Foster, D.O., A nonocclusive cannula for sampling venous blood from the interscapular brown fat of conscious rats, Can. J. Physiol. Pharmacol, 64:1434-1439, 1986.

Ma. S.W.Y., Nadeau, B.E., and Foster, D.O., Evidence for liver as the major site of the diet-induced thermogenesis of rats fed a 'cafeteria' diet, Can. J. Physiol. Pharmacol., 65:1802-1804, 1987.

Ma, S.W.Y., Foster, D.O., and Nadeau, B.E., Absence of increased oxygen consumption in brown adipose tissue of rats exhibiting 'cafeteria' diet-induced thermogenesis, Can. J. Physiol. Pharmacol. 66:1347-1354, 1988.

Maggi, C.A., and Meli, A., Review: the sensory-efferent function of capsaicin-sensitive sensory neurons, Gen. Pharmac. 19:1-43, 1988.

Maggi, C.A., Santiciolc, P., Geppetti, P., Parlani, M., Astolfi, M., Pradelles, P., Patacchini, R., and Meli, A., The antagonism induced by Ruthenium Red of the actions of capsaicin on the peripheral terminals of sensory neurons: further studies, Eur. J. Pharmacol. 154:1-10, 1988a.

Maggi, C.A., Patacchini, R., Santiciolc, P., Giuliani, S., Geppetti, P., and Meli, A., Protective action of Ruthenium red toward capsaicin desensitization of sensory fibers, Neurosci. Lett. 88:201-205, 1988b.

Maggi, C.A., Patacchini, R., Santiciolc, P., Giuliani, S., Geppetti, P., Bianco, E.D., Geppetti, P., and Meli, A., The 'efferent' function of capsaicin-sensitive nerves: Ruthenium Red discriminates between different mechanisms of activation, Eur. J. Pharmacol. 170:167-177, 1989.

Maggi, C.A., and Pierau, Fr.K., Recent advances in research on sensory peptides and capsaicin mechanisms. A conference report, Neurosci. Lett. 122:199-201, 1991.

Maggi, C.A., Review: The pharmacology of the efferent function of sensory nerves, J. Autonom. Pharmacol., 11:173-208, 1991.

Mansson, N., Ekman, R., Hakanson, R., Ekstrom, J., Neuropeptides and disuse of the rat parotid gland, Experiment. Physiol. 75:597-599, 1990.

Marchington, D., Rothwell, N.J., Stock, M.J., and York, D.A., Thermogenesis and sympathetic activity in BAT of overfed rats after adrenalectomy, Am. J. Physiol. 250:E362-E366, 1986.

Marsh, S.J., Stansfeld, C.E., Brown, D.A., Davey, R., and McCarthy, D., The mechanism of action of capsaicin on sensory C-type neurons and their axons in vitro, Neurosci. 23:275-289, 1987.

Marshall, I., Hollman, J.J., Al-Kazwini, S.J., and Craig, R.K., Three day intravenous infusion of human α-calcitonin gene-related peptide (human α-CGRP) causes sustained hypotension in rats, Br. J. Pharmacol., 91 (Proc. Suppl.):354P, 1987.

McDonald, R.B., Stern, J.S., and Horwitz, B.A., Thermogenic responses of younger and older rats to cold exposure: comparison of two strains, J. Gerontology: Biological Science, 44:B37-B42, 1989.

McDonald, R.B., Day, C., Carlson, K., Stern, J.S., and Horwitz, B.A., Effect of age and gender on thermoregulation, Am. J. Physiol., 257:R700-R704, 1989.

McDonald, R.B., Stern, J.S., and Horwitz, B., Cold-induced metabolism and brown fat GDP binding in young and old rats, Exp. Gerontol., 22:409-420, 1987.

Meddings, J.B., Hogaboam, C.M., Tran, K., Reynolds, J.D., and Wallace, J.L., Capsaicin effects on non-neuronal plasama membranes, Biochimica et Biophysica Acta, 1070:43-50, 1991.

Mercer, S.W., and Trayhurn, P., Effects of high fat diets on the thermogenic activity of brown adipose tissue in cold-acclimated mice, J. Nutr., 114:1151-1158, 1984a.

Mercer, S.W., and Trayhurn, P., The development of insulin resistance in brown adipose tissue may impair the acute cold-induced activation of theremogenesis in genetically obese (ob/ob) mice, Biosci. Rep., 4:933-940, 1984b.

Mercer, S.W., and Trayhurn, P., Effects of ciglitazone on insulin resistance and thermogenic responsiveness to acute cold in brown adipose tissue of genetically obese (ob/ob) mice, FEBS Lett., 195:12-16, 1986.

Mercer, S.W., and Trayhurn, P., Effect of high fat diets on energy balance and thermogenesis in brown adipose tissue of lean and genetically obese ob/ob mice, J Nutr., 117:2147-2153, 1987.

Miller, M.S., Buck, S.H., Sipes, I.G., Yamamura, H.I., and Burks, T.F., Regulation of substance P by nerve growth factor: Disruption by capsaicin, Brain Res. 250:193-196, 1982.

Milner, R.E., and Trayhurn, P., Evidence that the acute unmasking of GDP-binding sites in brown adipose tissue mitochondria is not dependent on mitochondrial swelling, Biochem. Cell Biol. 66:1226-1230, 1988.

Miles, K., Greengard, P., and Huganir, R.L., Calcitonin gene-related peptide regulates phosphorylation of the nicotinic acetylcholine receptor in rat myotubes, Neuron 2:1517-1524, 1989.

Mohell, N., Nedergaard, J., and Cannon, B., Quantitative differentiation of α- and ß-adrenergic respiratory responses in isolated hamster brown fat cells: evidence for the presence of an α-adrenergic component, Eur. J. Pharmacol., 93:183-193, 1983.

Mohell, N., Wallace, M., and Fain, J.N., Alpha1-adrenergic stimulation of phosphatidylinositol turnover and respiration of brown fat cells, Mol. Pharmacol., 25:64-69, 1984.

Mohell, N., Connolly, E., and Nedergaard, J., Distinction between mechanisms underlying α_1- and ß-adrenergic respiratory stimulation in brown fat cells, Am. J. Physiol. 253:C301-C308, 1987.

Morimoto, A., and Murakami, N., [^{14}C]deoxyglucose incorporation into rat brain regions during hypothalamic or peripheral thermal stimulation, Am. J. Physiol., 248:R84-92, 1985.

Morley, J.E., Neuropeptide regulation of appetite and weight, Endocr. Rev. 8:256-287, 1987.

Mory, G., Bouillaud, F., Combes-George, M., and Ricquier, D., Noradrenaline controls the concentration of the uncoupling protein in brown adipose tissue, FEBS Lett., 166:393-396, 1984.

Mukherjee, S., Lever, J.D., Norman, D., Symons, D., Spriggs, T.L.B., and Jung, R.T., A comparison of the effects of 6-hydroxydopamine and reserpine on noradrenergic and peptidergic nerves in rat brown adipose tissue, J. Anat. 167:189-193, 1989.

Nagy, J.I., Iversen, L.L., Goedert, M., Chapman, D., and Hunt, S.P., Dose-dependent effects of capsaicin on primary sensory neurons in the neonatal rat, J. Neurosci., 3:399-406, 1983.

Nanberg, E., Nedergaard, J., and Cannon, B., α-adrenergic effects on $^{86}Rb^+(K^+)$ potentials and fluxes in brown fat cells, Biochim. Biophys. Acta, 804:291-300, 1984.

Nanberg, E., Connolly, E., and Nedergaard, J., Presence of a Ca^{2+}-dependent K^+ channel in brown adipocytes. Possible role in maintenance of α1-adrenergic stimulation, Biochim. Biophys. 844:42-49, 1985.

Nanberg, E., and Putney, Jr J., α1-adrenergic activation of brown adipocytes leads to an increased formation of inositol polyphosphates, FEBS Lett., 195:319-322, 1986.

Nanberg, E., and Nedergaard, J., α_1-adrenergic inositol trisphosphate production in brown adipocytes is Na^+ dependent, Biochimica et Biophysica Acta, 930:438-445, 1987.

Nechad, M., Structure and development of brown adipose tissue, in Brown Adipose Tissue, (Trayhurn, P., and Nicholls, D.G., eds), pp. 1-30, Arnold, London, 1986.

Nedergaard, J., and Lindberg, O., The brown fat cell, Int. Rev. Cytol., 74:187-284, 1982.

Nedergaard, J., Mohell, N., Nanberg, E., Connolly, E., Raasmaja, A., and Henschen, L., α_1-adrenergic pathways in brown adipose tissue: mode of action and recruitment pattern, In: Living in the Cold, (Heller, H.C., Musacchia, X.J., and Wang, L.C.H., eds), New York: Elsevier, pp. 83-91, 1986.

New, H.V., and Mudge, A.W., Calcitonin gene-related peptide regulates muscle acetylcholine receptor synthesis, Nature 323:809-811, 1986.

Nicholls, D., Hamster brown adipose tissue mitochondria. Purine nucleotide control of the ion conductance binding site, Eur. J. Biochem., 62:223-228, 1976.

Nicholls, D., The thermogenic mechanism of brown adipose tissue, Biosci. Rep., 3:431-441, 1983.

Nicholls, D.G., Cunningham, S.A., and Rial, E., The bioenergetic mechanisms of brown adipose tissue mitochondria, in Brown Adipose Tissue, (Trayhurn, P., and Nicholls, D.G., eds), pp. 52-85, Arnold, London, 1986.

Nielsch, U., and Keen, P., Effects of neonatal 6-hydroxydopamine admistration on different substance P-containing sensory neurones, Eur. J. Pharmacol. 138:193-197, 1987.

Niijima, A., Effect of glucose and other hexoses on efferent discharges of brown adipose tissue nerves, Am. J. Physiol. 251:R240-R242, 1986.

Niijima, A., Effects of taste stimulation on the efferent activity of the autonomic nerves in the rat, Brain Res. Bull. 26(1):165-167, 1991.

Nilsson, J., Euler, A.M., and Dalsgaard, C.J., Stimulation of connective tissue cell growth by substance P and substance K, Nature, 315:61-63, 1985.

Nissenbaum, J.W., and Sclafani, A., Qualitative differences in polysaccharide and sugar tastes in the rat: a two-carbohydrate taste model, Neurosci. Biobehav. Rev., 11(2):187-196, 1987.

Nnodim, J.O., and Lever, J.D., Neural and vascular provisions of rat interscapular brown adipose tissue, Am. J. Anat., 182:283-293, 1988.

Norman, D., Mukherjee, S., Symons, D., Jung, R.T., and Lever, J.D., Neuropeptides in interscapular and perirenal brown adipose tissue in the rat: a plurality of innervation, J. Neurocytol., 17:305-311, 1988.

Ohhashi, T., and Jacobowitz, D.M., Effect of calcitonin gene-related peptide on the neuroeffector mechanism of sympathetic nerve terminals in rat vas deferens, Peptide, 4:987-991, 1985.

Park, I.R.A., Himms-Hagen, J., and Coscina, D.V., Long-term effects of lateral hypothalamic lesions on brown adipose tissue in rats, Brain Res. Bull., 17:643-651, 1986.

Park, I.R.R., and Himms-Hagen, J., Neural influences on trophic changes in brown adipose tissue during cold acclimation, Am. J. Physiol., R1-R8, 1988.

Park, I.R.R., Mount, D.B., and Himms-Hagen, J., Role of T3 in thermogenic and trophic responses of brown adipose tissue to cold, Am. J. Physiol. 257:E81-E87, 1989.

Payan, D.G., Neuropeptides and inflammation: the role of substance P, Ann. Rev. Med. 40:341-352, 1989.

Peachey, T., French, R.R., and York, D.A., Regulation of GDP binding and uncoupling-protein concentration in brown-adipose-tissue mitochondria, Biochem. J., 249:451-457, 1988.

Pennington, J.A.T., Bowes & Church's Food values of portions commonly used, J.B. Lippincott Company, 1989.

Pernow, B., Substance P, Pharmacol. Rev., 35:85-141, 1983.

Petersen, S., Feeding, blood glucose and plasma insulin of mice at dusk, Nature, 275:647-669, 1978.

Porikos, K.P., and Koopmans, H.S., The effect of non-nutritive sweeteners on body weight in rats, Appetite, 11:12-15, 1988.

Rafael, J., Fesser, W., and Nicholls, D.G., Cold adaptation in guinea pig at level of isolated brown adipocyte, Am. J. Physiol. 250:C228-235, 1986.

Rial, E., Poustie, A., and Nicholls, D.G., Brown-adipose-tissue mitochondria: the regulation of the 32000-Mr uncoupling protein by fatty acids and purine nucleotides, FEBS Lett., 137:197-203, 1983.

Ricquier, D., Mory, G., Bouillaud, F., Thibault, J., and Weissenbach, J., Rapid increase of mitochondrial uncoupling protein and its mRNA in stimulated brown adipose tissue, FEBS Lett., 178:240-244, 1984.

Ricquier, D., and Bouillaud, F., The brown adipose tissue mitochondrial uncoupling protein, in Brown Adipose Tissue, (Trayhurn, P., and Nicholls, D.G., eds), pp. 86-104, Arnold, London, 1986.

Ricquier, D., Bouillaud, F., and Toumelin, P., Expression of uncoupling protein mRNA in thermogenic or weakly thermogenic brown adipose tissue, J. Biol. Chem. 261:13905-13910, 1986.

Ritter, S., and Dinh, T.T., Capsaicin-induced neuronal degeneration: Silver impregnation of cell bodies, axons, and terminals in the central nervous system of the adult rat, J. Comp. Neurol., 271:79-90, 1988.

Ritter, S., and Dinh, T.T., Capsaicin-induced neuronal degeneration in the brain and retina of preweanling rats, J. Comp. Neurol., 296:447-461, 1990.

Rozengurt, E., Neuropeptides as cellular growth factors: role of multiple signalling pathways, Eur. J. Clin. Invest., 21:123-134, 1991.

Rosenfeld, M.G., Mermod, J.J., Amara, S.G., Swanson, L.W., Sawchenko, P.E., Rivier, J., Vale, W.W., and Evans, R.M., Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing, Nature, 304:129-135, 1983.

Rothwell, N.J., and Stock, M.J., A role for brown adipose tissue in diet-induced thermogenesis, Nature, 281:31-35, 1979.

Rothwell, N., and Stock, M.J., A role for insulin in the diet-induced thermogenesis of cafeteria-fed rats, Metabolism, 30:673-678, 1981.

Rothwell, N.J., and Stock, M., Effects of age on diet-induced thermogenesis and brown adipose tissue metabolism in the rat, Int. J. Obesity, 7:583-589, 1983.

Rothwell, N.J., and Stock, M., Effect of denervating brown adipose tissue on the responses to cold, hyperphagia and noradrenaline treatment in the rat, J. Physiol. (Lond.), 355:457-463, 1984a.

Rothwell, N.J., and Stock, M., Energy balance, thermogenesis and brown adipose tissue activity in tube-fed rats, J. Nutr. 114: 1965-1970, 1984b.

Rothwell, N., and Stock, M.J., Energy balance and brown fat activity in adrenalectomized male, female, and castrated male rats, Metabolism, 35:657-660, 1986.

Rothwell, N.J., and Stock, M.J., Influence of adrenalectomy on age-related changes in energy balance, thermogenesis and brown fat activity in the rat, Comp. Biochem. Physiol., 89A:265-269, 1988.

Rothwell, E., Neuropeptides as cellular growth factors: role of multiple signalling pathways, Eur. J. Clin. Invest. 21:123-134, 1991.

Russell, L.C., and Burchiel, K.J., Neurophysiological effects of capsaicin, Brain Res. Rev., 8:165-176, 1984.

Saggerson, D., McAllister, W.J., and Baht, H.S., Lipogenesis in rat brown adipocytes. Effects of insulin and noradrenaline, contributions from glucose and lactate as precursors and comparisons with white adipocytes, Biochem. J., 251:701-709, 1988.

Saito, M., Minokoshi, Y., and Shimazu, T., Ventromedial hypothalamic stimulation accelerates norepinephrine turnover in brown adipose tissue of rats, Life Sci., 41:193-197, 1987.

Saito, M., Minokoshi, Y., and Shimazu, T., Accelerated norepinephrine turnover in peripheral tissues after ventromedial hypothalamic stimulation in rats, Brain Res., 481:298-303, 1989.

Sakaguchi, T., and Bray, G.A., The effect of intrahypothalamic injections of glucose on sympathetic efferent firing rate, Brain Res. Bull., 18:591-595, 1987a.

Sakaguchi, T., and Bray, G.A., Intrahypothalamic injection of insulin decreases firing rate of sympathetic nerves, Proc. Nat. Acad. Sci., U.S.A. 84:2012-2014, 1987b.

Sakaguchi, T., and Bray, G.A., Sympathetic activity following paraventricular injections of glucose and insulin, Brain Res. Bull., 21:25-29, 1988.

Sakaguchi, T., Takahashi, M., and Bray, G.A., Lateral hypothalamus and sympathetic firing rate, Am. J. Physiol. 255:R507-512, 1988a.

Sakaguchi, T., Takahashi, M., and Bray, G.A., Diurnal change in sympathetic activity. Relation to food intake and to insulin injection in to the ventromedial or suprachiasmatic nucleus, J. Clin. Invest. 82:282-286, 1988b.

Sakaguchi, T., Bray, G.A., and Eddlestone, G., Sympathetic activity following paraventricular or ventromedial hypothalamic lesions in rats, Brain Res. Bull. 20:461-465, 1988c.

Sakaguchi, T., and Bray, G.A., Effect of norepinephrine, serotonin and tryptophan on the firing rate of sympathetic nerves, Brain Res., 492:271-280, 1989.

Sakaguchi, T., Arase, K., Fisler, J.S., and Bray, G.A., Effect of a high-fat diet on firing rate of sympathetic nerves innervating brown adipose tissue in anesthetized rats, Physiol. and Behav., 45:1177-1182, 1989.

Sakaguchi, T., and Bray, G.A., Ventromedial hypothalamic lesions attenuate response of sympathetic nerves to carotid arterial infusions of glucose and insulin, Int. J. Obesity 14:127-134, 1990.

Saria, A.G., Skofitsch, G., and Lembeck, F., Distribution of capsaicin in rat tissue after systemic administration, J. Pharm. Pharmacol., 34:273-275, 1982.

Sbarbati, A., Morroni, M., Zancanaro, C., and Cinti., S., Rat interscapular brown adipose tissue at different ages: a morphometric study, Int. J. Obesity, 15:581-588, 1991.

Scarpace, P.J., Mooradian, A.D., and Morley, J.E., Age-associated decrease in beta-adrenergic receptors and adenylate cyclase activity in rat brown adipose tissue, J. Gerontology: Biological Sciences, 43:B65-70, 1988.

Schacterle, G., and Pollack, R., A simplified method for the quantitative assay of small amounts of protein in biological material, Anal. Biochem., 51:654-655, 1973.

Schimmel, R.J., McCarthy, L., and McMahon, K.K., α_1-adrenergic stimulation of hamster brown adipocyte respiration, Am. J. Physiol. 244:C362-C368, 1983.

Schimmel, R.J., Dzierzanowski, D., Elliott, M.E., and Honeyman, T.W., Stimulation of phosphoinositide metabolism in hamster brown adipocytes exposed to α_1-adrenergic agents and its inhibition with phorbol esters, Biochem. J., 236:757-764, 1986.

Schneider-Picard, G., and Girardier, L., Post-natal development of sympathetic innervation of rat brown adipose tissue reevaluated with a method allowing for monitoring flavoprotein redox state, J. Physiol. (Paris), 78:151-157, 1982.

Sclafani, A., and Abrams, M., Rats show only a weak preference for the artificial sweetener aspartame, Physiol. Behav., 37(2):253-256, 1986.

Seydoux, J., Giacobino, J.P., and Girardier, L., Impaired metabolic response to nerve stimulation in brown adipose tissue of hypothyroid rats, Mol. Cell. Endocrinol., 25:213-226, 1982a.

Seydoux, J., Ricquier, D., Rohner-Jeanrenaud, F., Assimacopoulos-Jeannet, F., Giacobino, J.P., Jeanrenaud, B., and Girardier, L., Decreased guanine nucleotide binding and reduced equivalent production by brown adipose tissue in hypothalamic obesity, FEBS Lett. 146:161-164, 1982b.

Seydoux, J., Chinet, A., Schneider-Picard, G., Bas, S., Imesch, E., Assimacopoulos-Jeannet, F., Giacobino, J.P., and Girardier, L., Brown adipose tissue metabolism in streptozotocin-diabetic rats, Endocrinology, 113:604-610, 1983.

Shimazu, T., and Takahashi, A., Stimulation of hypothalamic nuclei has differential effects on lipid synthesis in brown and white adipose tissue, Nature, 284:62-63, 1980.

Shimomura, Y., Bray, G.A., and Lee, M., Adrenalectomy and steroid treatment in obese (ob/ob) and diabetic (db/db) mice, Horm. Metabol. Res., 19:295-299, 1987.

Siemen, D., and Reuhl, T., Non-selective cationic channel in primary cultured cells of brown adipose tissue, Pflugers Arch, 408:534-536, 1987.

Silva, J.E., and Larsen, P.R., Adrenergic activation of triiodothyronine production in brown adipose tissue, Nature, 305:712-713, 1983.

Silva, J.E., and Larsen, P.R., Potential of brown adipose tissue type II thyroxine 5'-deiodinase as a local and systemic source of triiodothyronine in rats, J. Clin. Invest., 76:2296-2305, 1985.

Silva, J.E., and Larsen, P.R., Interrelationships among thyroxine, growth hormone, and the sympathetic nervous system in the regulation of 5'-iodothyronine deiodinase in rat brown adipose tissue, J. Clin. Invest., 77:1214-1223, 1986.

Silva, J.E., Full expression of uncoupling protein gene requires the concurrence of norepinephrine and triiodothyronine, Mol. Endocrinol, 2:706-713, 1988.

Silva, W.L., Mason, J.R., Marshall, D.A., and Maruniak, J.A., Rat trigeminal, olfactory and taste responses after capsaicin desensitization, Brain Res. 333:45-54, 1985.

Slinde, E., Pedersen, J.I., and Flatmark, T., Sedimentation coefficient and buoyant density of brown adipose tissue mitochondria from guinea pig, Anal. Biochem., 65:581-585, 1975.

Smith, R.E., and Hock, R.J., Brown fat: thermogenic effector of arousal in hibernators, Science 140: 199-200, 1963.

Smith, R., and Roberts, J.C., Thermogenesis of brown adipose tissue in cold-acclimated rats, Am. J. Physiol., 206:143-148, 1964.

Smith, R., and Horwitz, B., Brown fat and thermogenesis, Physiol. Rev., 49:330-425, 1969.

Smith, J.C., Castonguay, T.W., Foster, D.F., and Bloom, L.M., A detailed analysis of glucose and saccharin drinking in the rat, Physiol. Behav., vol. 24, pp. 173-176, 1980.

Steffens, A.B., Van Der Gugten, J., Godeke, J., Luiten, P.G.M., and Strubbe, J.H., Meal-induced increases in parasympathetic and sympathetic activity elicit simultaneous rises in plasma insulin and free fatty acids, Physiol. Behav. 37:119-122, 1986.

Storlien, L.H., James, D.E., Burleigh, K.M., and Chisholm, D.J., and Kraegen, E.W., Fat feeding causes widespread in vivo insulin resistance, decreased energy expenditure, and obesity in rats, Am. J. Physiol. 251:E576-E583, 1986.

Sudo, M., Minokoshi, Y., and Shimazu, T., Ventromedial hypothalamic stimulation enhances peripheral glucose uptake in anesthetized rats, Am. J. Physiol. 261:E298-E303, 1991.

Szekely, M., and Szolcsanyi, J., Endotoxin fever in capsaicin treated rats, Acta Physiol. Acad. Sci. Hung., 53:469-477, 1979.

Szekely, M., and Szolcsanyi, J., Endotoxin fever in rats, Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 53:265-277, 1979.

Szolcsanyi, J., Joo, F., and Jancso-Gabor, A., Mitochondrial changes in preoptic neurones after capsaicin desensitization of the hypothalamic thermodetectors in rats, Nature, Lond., 229:116-117, 1971.

Szolcsanyi, J., and Jancso-Gabor, A., Capsaicin and other pungent agents as pharmacological tools in studies on thermoregulation, In The Pharmacology of thermoregulation, (Schobaum, E., and Lomax, P., edss), Basel:Karger, 395-409, 1973.

Tache, Y., Pappas, T., Lauffenburger, M., Goto, Y., Walsh, J.H., and Debas, H., Calcitonin gene-related peptide: Potent peripheral inhibitor of gastric acid secretion in rats and dogs, Gastroenterology, 87:344-349, 1984.

Tippins, J.R., Morris, H.R., Panico, M., Etienne, T., Bevis, P., Birgis, S., MacIntyre, I., Azri, M., and Attinger, M., The myotropic and plasma-calcium modulating effects of calcitonin gene-related peptide (CGRP), Neuropeptides, 4:425-434, 1984.

Tokuyama, K., and Himms-Hagen, J., Increased sensitivity of the genetically obese mouse to
corticosterone, Am. J. Physiol. 252:E202-E208, 1987.

Tokunaga, K., Fukushima, M., Kemnitz, J.W., and Bray, G.A., Comparison of ventromedial and paraventricular lesions in rats that become obese, Am. J. Physiol., 251:R1221-1227, 1986.

Tordoff, M.G., How do non-nutritive sweeteners increase food intake, Appetite, 11:5-11, 1988.

Tordoff, M.G., and Friedman, M.I., Drinking saccharin increases food intake and preference-I. Comparison with other drinks, Appetite, 12:1-10, 1989.

Tordoff, M.G., and Friedman, M.I., Drinking saccharin increases food intake and preference-II. Hydrational Fators, Appetite, 12:11-21, 1989.

Tordoff, M.G., and Friedman, M.I., Drinking saccharin increases food intake and preference-III. Sensory and associative factors, Appetite, 12:23-36, 1989.

Tordoff, M.G., and Friedman, M.I., Drinking saccharin increases food intake and preference-IV. Cephalic phase and metabolic factors, Appetite, 12:37-56, 1989.

Trayhurn, P., and Jennings, G., Evidence that fasting can induce a selective loss of uncoupling protein from brown adipose tissue mitochondria of mice, Biosci. Rep., 6:805-810, 1986.

Trayhurn, P., and Jennings, G., Nonshivering thermogenesis and the thermogenic capacity of brown fat in fasted and/or refed mice, Am. J. Physiol. 254:R11-R16, 1988.

Trayhurn, P., and Milner, R.E., A commentary on the interpretation of in vivo biochemical measures of brown adipose tissue thermogenesis, Can. J. Physiol. Pharmacol. 67:811-819, 1989.

Triandafillou, J., Hellenbrand, W., and Himms-Hagen, J., Trophic response of hamster brown adipose tissue: role of norepinephrine and pineal gland, Am. J. Physiol., 247:E793-E799, 1984.

Vallerand, A.L., Perusse, F., and Bukowiecki, L.J., Cold exposure potentiates the effect of insulin on in vivo glucose uptake, Am. J. Physiol. 253:E179-E186, 1987.

Vallerand, A.L., Perusse, F., and Bukowiecki, L.J., Stimulatory effects of cold exposure and cold acclimation on glucose uptake in rat peripheral tissues, Am. J. Physiol. 259:R1043-R1049, 1990.

Vander Tuig, J.G., Knehans, A.W., and Romsos, D.R., Reduced sympathetic nervous system activity in rats with ventromedial hypothalamic lesions, Life Sci. 30:913-920, 1982.

Vander Tuig, J.G., and Romsos, D.R., Effects of dietary carbohydrate, fat, and protein on norepinephrine turnover in rats, Metabolism, 33:26-33, 1984.

Vander Tuig, J.G., Kerner, J., and Romsos, D.R., Hypothalamic obesity, brown adipose tissue, and sympathoadrenal activity in rats, Am. J. Physiol. 248:E607-617, 1985.

Vander Tuig, J.G., Crist, K.A., and Romsos, D.R., Temporal adjustments in

sympathoadrenal activity in rats with obesity-producing hypothalamic knife cuts, Proc. Soc. Exp. Biol. Med. 185:134-140, 1987.

Varro, A., Green, T., Holmes, S., and Dockreay, G.J., Calcitonin gene-related peptide in visceral afferent nerve fibres: quantification by radioimmunoassay and determination of axonal transport rates, Neuroscience, 26:927-932, 1988.

Villarroya, F., Felipe, A., and Mampel, T., Sequential changes in brown adipose tissue composition, cytochrome oxidase activity and GDP binding throughout pregnancy and lactation in the rat, Biochim. Biophys. Acta., 882:187-191, 1986.

Visser, T.M., Leonard, J.L., Kaplan, M.M., and Larsen, P.R., Kinetic evidence suggesting two mechanisms for iodothyronine 5'-deiodination in rat cerebral cortex, Proc. Natl. Acad. Sci. U.S.A. 79:5080-5084, 1982.

Von Euler, U.S., and Gaddum, J.H., An unidentified depressor substance in certain tissue extracts, J. Physiol. 72:74-87, 1931.

Walsberg, G.E., Evaluation of a nondestructive method for determining fat stores in small birds and mammals, Physiol. Zool., 61(2):153-159, 1988.

Watanabe, T., Kawada, T., Yamamoto, M., and Iwai, K., Capsaicin, a pungent principle of hot red pepper, evokes catecholamine secretion from adrenal medulla of anaesthetized rats, Biochem. Biophys. Res. Commun. 142:259-264, 1987.

Watanabe, T., Kawada, T., Kurosawa, M., Sato, A., and Iwai, K., Adrenal sympathetic efferent nerve and catecholamine secretion excitation caused by capsaicin in rats, Am. J. Physiol., 255:E23-27, 1988.

Wimalawansa, S., Age-related increase of calcitonin gene-related peptide in rat thyroid and circulation, Peptides, 12:1143-1147, 1991.

Woll, P.J., and Rozengurt, E., Neuropeptides as growth regulators, Br. Med. Bull. 45:492-505, 1989.

Yonetani, T., and Ray, G.S., Studies on cytochrome oxidase, J. Biol. Chem., 240:3392-3398, 1965.

Yoshida, T., Kemnitz, W.K., and Bray, G.A., Lateral hypothalamic lesions and norepinephrine turnover in rats, J. Clin. Invest., 72:919-927, 1983.

Yoshida, T., and Bray, G.A., Catecholamine turnover in rats with ventromedial hypothalamic lesions, Am. J. Physiol. 246:R558-565, 1984.

Yoshida, T., Yoshioka, K., Wakabayashi, Y., Nishioka, H., and Kondo, M., Effects of capsaicin and isothiocyanate on thermogenesis of interscapular brown adipose tissue in rats, J. Nutr. Sci. Vitaminol. 34:587-594, 1988.

Yoshida, T., Yoshioka, K., Nishioka, H., and Kondo, M., Lesions of the hypothalamic paraventricular nucleus and norepinephrine turnover in rats, Endocrinol. Jpn. 36:187-194, 1989.

Zaidi, M., Bevis, P.J.R., Abeyasekera, G., Girgis, S.I., Wimalawansa, S.J., Morris, H.R., and MacIntyre, I., The origin of circulating calcitonin gene-related peptide in the rat, J. Endor., 110:185-190, 1985.

Zaidi, M., Breimer, L.H., and MacIntyre, I., Biology of peptides from the calcitonin genes, J. Exp. Physiol. , 72:371-408, 1987.

Zelissen, P.M.J., Koppeschaar, H.P.F., Lips, C.J.M., and Hackeng, W.H.L., Calcitonin gene-related peptide in human obesity, Peptides, 12:861-863, 1990.

CURRICULUM VITAE

Name: **Jingying Cui**

Addresses:

Home:
180 Lees Ave.
Apt. # 1109
Ottawa, Ontario
K1S 5J6
Tel: (613)-565-0286

Work:
Dept. of Biochemistry
University of Ottawa
451 Symth Road
Ottawa, Ontario
K1H 8M5
Tel: (613)-787-6632

Personal:

Date and Place of Birth:	Nov. 5, 1959, Fujian, China
Sex:	Female
Marital Status:	Married
Present Occupation:	Ph.D. Candidate

Education:

1987 - now: Ph.D. Candidate, Dept. of Biochemistry, Univ. of Ottawa, Canada.
1977 - 1982: M.D., Qingdao Medical School, Qingdao, China

Work Experience:

1987 - now: Graduate Student under the supervision of Dr. Jean Himms-Hagen, Dept. of Biochemistry, Univ. of Ottawa, Ottawa, Canada.
1990: Laboratory Demonstrator, Dept. of Biochemistry, Univ. of Ottawa, Ottawa, Canada.
1982 - 1986: Pediatrician, Qingdao Children's Hospital, Qingdao, China

Scholarship, Assistantship, and Award:

1987 - now: Research Assistantship, Dept. of Biochemistry, Univ. of Ottawa, Ottawa, Canada.
1990: Teaching Assistantship, Dept. of Biochemistry, Univ. of Ottawa, Ottawa, Canada.
1987: Entrance Scholarship for M.Sc. program, Graduate School, Univ. of Ottawa, Ottawa, Canada.
1989: Entrance Scholarship for Ph.D. program, Graduate School, Univ. of Ottawa, Ottawa, Canada.
1990: Entrance Scholarship for Ph.D. program (renewed), Graduate School, Univ. of Ottawa, Ottawa, Canada.
1991: Conference Travel Award, Graduate School, Univ. of Ottawa, Canada.
1991: Conference Travel Award, Dept. of Biochemistry, Univ. of Ottawa, Ottawa, Canada.

LIST OF PUBLICATIONS

Theses

1. Jingying Cui, "Role of sensory nerves in growth and thermogenesis of brown adipose tissue", Ph.D. thesis, 1992.

Papers in Refereed Journals

1. J. Cui, G. Zaror-Behrens and J. Himms-Hagen, "Capsaicin desensitization induces atrophy of brown adipose tissue in rats", American J. Physiology, 259:R324-R332, 1990.

2. J. Himms-Hagen, J. Cui and S. L. Sigurdson, "Sympathetic and sensory nerves in control of growth of brown adipose tissue: Effect of denervation and of capsaicin", Neurochemistry International, 17:271-280, 1990.

3. J. Cui and J. Himms-Hagen, "Time-course of atrophy of brown adipose tissue (BAT) in capsaicin-desensitized (CAP-DES) rats", American J. Physiology, in press, 1992.

4. J. Cui and J. Himms-Hagen, "Long term effect of capsaicin-desensitization (CAP-DES) on brown adipose tissue (BAT) in the regulation of energy balance", American J. Physiology, in press, 1992.

Chapters in Refereed Conference Proceedings

1. J. Himms-Hagen, K. Tokuyama, J. Eley, I.R.A. Park, J. Cui, G. Zaror-Behrens and D.V. Coscina, "Hypothalamic regulation of brown adipose tissue in lean and obese rodents", Proc. of the Eighteenth Steenbock Symposium on Hormones, Thermogenesis and Obesity, Lardy, H.A, and Stratman, F., eds., New York, Elsevier, pp 173-184, 1989.

2. J. Himms-Hagen, J. Cui, J. Eley, I.R.A. Park, L. Sigurdson and G. Zaror-Behrens, "Neural control of brown adipose tissue growth", Living In The Cold II, Ed. A. Malan, B. Canguilhem. Colloque. INSERM/John Libbey Eurotext Ltd., pp. 367-375, 1989.

3. J. Himms-Hagen and J. Cui, "Diet-induced obesity and brown adipose tissue", Obesity Factors and Control, Ed. D.R. Romsos, J. Himms-Hagen and M. Suzuki, Japan Sci, Soc, Press, Tokyo/S. Karger, Basel, pp. 81-95, 1991.

Abstracts and Presentations

1. J. Himms-Hagen, J. Cui and G. Zaror-Behrens, "Atrophy of brown adipose tissue (BAT) in capsaicin-desensitized rats: role for sensory nerves in BAT function?", Proc. of International Symposium on Living In The Cold II, Le Hohwald, Strasbourg, France, 1989.

2. J. Himms-Hagen, J. Cui and G. Zaror-Behrens, "Atrophy of brown adipose tissue (BAT) in capsaicin-desensitized rats: role for sensory nerves in BAT function?", J. Cell. Biochem. Supplement, UCLA Symposium On Molecular & Cellular Biology 13E, 248, 1989.

3. J. Cui, G. Zaror-Behrens and J. Himms-Hagen, "Activation of brown adipose tissue (BAT) by saccharin (SACC.): A model for cephalic phase thermogenesis?", FASEB Journal, 3: A346, 1989.

4. J. Himms-Hagen, J. Cui and G. Zaror-Behrens, "Atrophy of brown adipose tissue (BAT) in capsaicin-desensitized rats: role for sensory nerves in BAT function?", FASEB Journal, 3: A347, 1989.

5. J. Cui and J. Himms-Hagen, "Time-course of atrophy of brown adipose tissue (BAT) in capsaicin-desensitized (CAP-DES) rats", FASEB Journal, 4: A379, 1990.

6. J. Himms-Hagen and J. Cui, "Time-course of atrophy of brown adipose tissue (BAT) in capsaicin-desensitized (CAP-DES) rats: no correlation with energy balance", International J. Obesity 14:(suppl. 2), pp. 99, 1990.

7. J. Cui and J.Himms-Hagen, "Long-term effect of capsaicin-desensitization (CAP-DES) on brown adipose tissue (BAT) in the regulation of energy balance", FASEB Journal, 5: A1654, 1991.

8. J. Himms-Hagen and J. Cui, "Long-term reduction in body weight, body fat and brown adipose tissue (BAT) thermogenic capacity in capsaicin-desensitized (CAP-DES) rats", International J. Obesity 15:(suupl. 1), pp. 67, 1991.

9. J. Himms-Hagen, and J. Cui, "Leanness in long-term capsaicin-desensitized rats", International J. Obesity 15:(suppl. 3), pp. 50, 1991.