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Capsaicin-sensitive nerves and energy homeostasis

Wall, Ester Henriette Eugenie Marie van de

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CHAPTER 1

GENERAL INTRODUCTION

1 BODY WEIGHT HOMEOSTASIS

1.1 The concept and the physiological evidence

The concept of homeostasis is introduced by Claude Bernard (1813-1878) and further developed by Walter B. Cannon (1871-1945). Homeostasis comes from two Greek words “homeo” meaning the same, and “stasis” meaning standing. The stability of the internal environment or “la constance du milieu intérieur”, as Claude Bernard defined it, is of crucial importance for living organisms. Another important aspect is that organisms are able to maintain this internal stability relatively constant. In other words, biological systems are capable to trigger physiological responses to maintain the constancy of the internal environment in face of disturbances of external surroundings. The ideas of Claude Bernard and Walter B. Cannon are still alive nowadays and are applied on the current hypotheses on body weight regulation. The general hypothesis is that there is a certain set point or settling point for body weight or a so-called body weight homeostasis, since an individual has a relatively constant body weight over life time while food intake and physical activity can vary considerably.

Evidence for this comes from the observation that when animals are involuntarily overfed or deprived from food and consequently gain respectively lose weight, they compensate for this until they reach the body weight of the controls again (55). This observation led to the idea that body weight is regulated. This implies that there are neurophysiological mechanisms to maintain body weight at a certain level and, if body weight increases or decreases, biological systems in the body adjust food intake and/or energy expenditure to compensate for this body weight change. The Central Nervous System (CNS) plays a crucial role in processes that regulate body weight. Lesions in the Ventromedial hypothalamus (VMH) lead to excessive overeating and obesity (42). Therefore, in the nineteen fifties, the VMH was pointed out as “satiety center” of the brain. Lesions in the Lateral Hypothalamus (LH) leads to aphagia and weight loss and was therefore also defined as “hunger center” of the brain (41).

Yet, nowadays we know that the regulation of body weight is more complex and does not involve only the VMH and LH of the hypothalamus. Other brain areas, as the paraventricular nucleus (PVN), arcuate nucleus and amygdala as well as the nucleus tractus solitarius (NTS), area postrema (AP) and the dorsal motor nucleus of the vagus (DMV) -that form together the dorsal vagal complex (DVC) of the hindbrain- are implicated in food intake control and/or the regulation of energy expenditure and hence body weight regulation. These centers receive information from the periphery either via humoral factors, sensory nerves and/or neuroendocrinological signals (see also fig.1) and are capable of initiating an adequate behavioral and/or neuroendocrinological and neural response to counterregulate challenges to energy homeostasis.

1.2 Long-term regulation versus short-term regulation

The hypothalamic areas are seen as an important site for the integration of different signals related to food intake and energy expenditure and the hypothalamus is considered to play a main role in long-term regulation of body weight homeostasis. An important hormone involved in the signaling of the peripheral energy content of the body is leptin. Leptin is produced by adipocytes proportionally to the amount of body adiposity (18, 19). It functions as a negative feedback signal to the brain (143) and plays therefore a role in the regulation of energy homeostasis. Particularly, the arcuate nucleus (ARC) of the hypothalamus is a primary site for the satiety effects of leptin (118).

The hindbrain is generally associated with the short-term control of feeding and autonomic reflex control. The gut-brain axis with vagal afferents innervating the whole gastro-intestinal (GI) tract projecting to the DVC are seen as main mediators in these short-term processes. Short-term satiation signals as stomach distension, presence of nutrients in the GI tract and endocrine signals induced by feeding are transmitted via these vagal afferents to the DVC (see figure 2) and subsequently food intake is terminated. These short-term signals are influenced by long-term signals as is demonstrated by experiments of Matson et al. (72, 73). They demonstrated that central leptin infusions increase the sensitivity to the peripheral satiety hormone

cholecystokinin (CCK). Thus, long-term signals can influence energy homeostasis by modulating short-term signals.

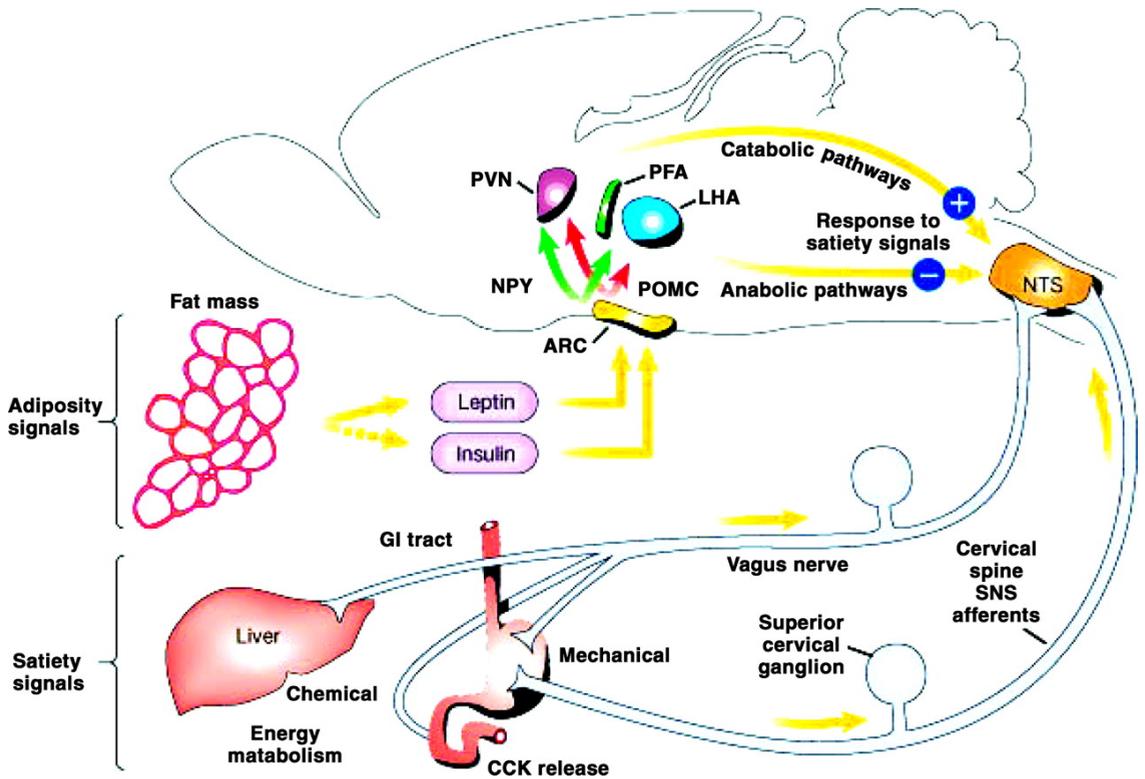


Figure 1
Schematic representation of the integration of peripheral and central signals in the regulation of energy homeostasis (121).

Although many processes and factors involved in energy homeostasis were discovered during the last decades, there is still only little insight in what the autonomic nervous system is telling the brain about the energy content in the periphery. Therefore, in this thesis, the focus will be on the vagal afferent sensory nerves and their involvement in different aspects of energy homeostasis. In particular, the involvement of sensory C- fibers and small myelinated δ -fibers in satiety and glucose metabolism will be studied. These fibers can be selectively destroyed by systemic administration of high doses of the neurotoxin capsaicin. Therefore, capsaicin was used as a tool to eliminate a part of vagal afferent sensory fibers.

The following paragraphs will discuss the involvement of vagal afferents in satiety. The emphasis will be on neuroendocrinological mechanisms as well as the involvement of meal induced thermogenic response to a meal. The role of these sensory nerves in glucose homeostasis will be discussed and a short review will be given on capsaicin and its effects after systemic administration. The introduction ends with the aim of the thesis and outline of the present dissertation.

2 SATIETY

2.1 Gut-Brain axis: anatomy and physiology

2.1.1 Innervation of the gastro-intestinal tract

Afferent signals from the upper gastro-intestinal (GI) tract are transmitted by the vagus nerve, nonvagal splanchnic mesenteric nerves, and pelvic afferents. Together, these afferent nerves provide the peripheral extrinsic neural part of the gut-brain axis. The upper GI tract is largely innervated by vagal afferents; the pelvic afferents are limited to the lower bowel, and splanchnic afferents innervate the whole GI tract. Intrinsic, enteric neural systems as the myenteric and submucosal plexuses also exist; these may mediate between GI mucosal and muscular events and extrinsic neural signaling (for review see (120)).

INTERMEZZO

Obesity, globesity and diabetes

Obesity is seemingly in conflict with the idea of body weight homeostasis. An obese individual has an excess body fat and this increase in adiposity is the net result of an excess of energy consumption over expenditure. Currently, obesity is considered as a disease and obesity is generally diagnosed when the Body Mass Index (BMI) -that is the ratio between body weight and square height exceeds- 30 kg/m². Patients with a BMI between 25 and 29.9 are considered overweight, but not obese.

Nowadays, there are more than 1 billion adults overweight and at least 300 million of these people are considered clinically obese. Obesity has increased three-fold or more since 1980 in some areas of North America, the United Kingdom, Eastern Europe, the Middle East, the Pacific Islands, Australasia and China. Of special concern is the increasing incidence of child obesity; 17.6 million children under five are estimated to be overweight worldwide. World Health Organization (1) reported that obesity has taken epidemic proportions globally (globesity) but, although clearly visible, it is still one of the most neglected problems. Reasons for the alarming increase in obesity are multifactorial. The large availability of (junk) food; more energy-dense, nutrient-poor foods with high levels of sugar and saturated fats combined with a more sedentary lifestyle; genetic predisposition; altered metabolism of adipose tissue, defective or decreased thermogenesis and certain prescribed medications are highly promoting factors for the development of obesity.

Being overweight or obese increases the risk of developing conditions and pathologies such as high blood pressure, cardio-vascular disease, stroke, gallbladder disease, certain types of cancer, and insulin resistance and type 2 diabetes. The non-fatal health problems associated with obesity include respiratory difficulties, chronic musculoskeletal problems, skin problems and infertility. In other words, obesity contributes to premature mortality. Of all obesity-related diseases non-insulin dependent diabetes mellitus (NIDDM, diabetes 2) is most clearly and strongly associated with obesity, even in children. This explains the recent coining of the term 'diabetes'. The frequency of central obesity, hypertension and elevated blood lipids is dramatically increased in persons with diabetes and this growing problem really needs attention (1).

The vagus nerve consists of both sensory and motor axons. In the periphery, it enters the abdomen with 2 trunks (the dorsal and the ventral) along the esophagus. When the vagi cross the diaphragm, they divide in 5 distinctive branches: the paired gastric branches, the paired celiac branches and a single hepatic branch that originates from the ventral trunk (107-109). Ingestive and visceral reflexes are mediated by nociceptive or chemosensitive C-fibers which have their cell bodies in the nodose ganglia and show a viscerotopic distribution in the nucleus of the solitary tract (NTS) (6) (see figure 2). Anterograde tracing after injections into the individual subdiaphragmatic vagal branches (celiac, accessory celiac, hepatic, dorsal gastric, and ventral gastric) also show discrete, yet somewhat overlapping, NTS termination fields for each branch (93). Thus, the NTS contains viscerotopic and branch specific information. Phillips et al. (100) showed that hepatic-branch vagal afferents supply the forestomach, antrum, pylorus, duodenum, and caecum. This is also consistent with the general notion that individual gut vagal branches innervate multiple gastrointestinal-tract segments. Thus, in analogy with the efferent innervation of the GI tract (13) one gastrointestinal target can be innervated by vagal afferents from more than one gut vagal branch.

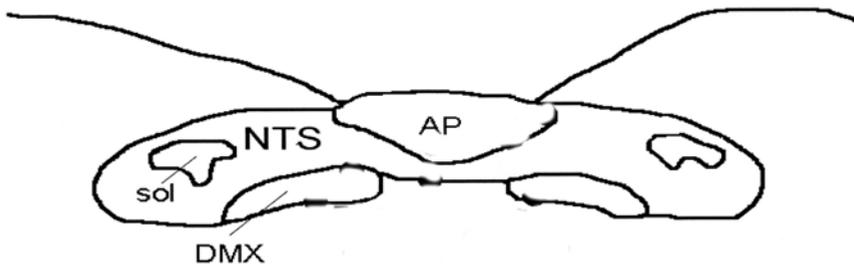


Figure 2

Structures of the dorsal vagal complex (DVC): Nucleus of the solitary tract (NTS); Area Postrema (AP), Dorsal Motor Nucleus of the vagus (DMX). Adapted from (30).

The vagus consists largely (80-90%) of unmyelinated C-fibers (106, 107) and its central projections enter the brainstem where they make synaptic connections with second order neurons to other areas in the CNS as the hypothalamus and limbic system (142). The most important projection site for efferent motor neurons of the NTS is the Dorsal Motor Nucleus of the Vagus (DMN), a structure just ventral of the NTS. The DMN contains numerous dendrites penetrating the NTS and the Area Postrema (AP) (122). About 95% of preganglionic neurons in the DMN contribute to projections to the stomach (64) suggesting that the efferent part of the vagus nerve is highly involved in the motor innervation of the stomach. As in the afferent projections, each vagal branch contains the axons of a topographically distinct column of cells within the dorsal motor nucleus of the vagus (DMN) (32). Therefore, the afferent-efferent viscerotopic and branch specific organization of the vagus could reflect distinct neurophysiological reflexes and functions involved in ingestion.

Neuronal tracing techniques using carbocyanine dyes as DiI, wheatgerm agglutinin-conjugated horseradish peroxidase or dextran tracers have given a lot of understanding about the anatomy of vagal innervation of different layers and regions of the gut and stomach (10, 11, 31). Different populations of vagal afferents terminations in the gut and stomach indicate also a different sensory modality which can react to mechanical as well as chemical stimulation. Vagal endings in the longitudinal and circular muscle layers are also defined as intramuscular arrays (IMAs) (144) and have been suggested to be in-series tension receptors (102). Vagal afferents in the myenteric plexus throughout the GI tract are called intraganglionic laminar endings (IGLEs) (90) and have characteristics of a tension receptor (102). Together, all these observations provide the anatomical hardware for a role of vagal afferent fibers in volume detection of the stomach and gut which may contribute to the process of satiation.

2.1.2 Vagal afferents and neuro-endocrine signals

Numerous neurophysiological studies give strong support for an important role of the vagus in the process of satiation and subsequent meal termination. In these studies the mediational role of cholecystokinin (CCK) in this process was

established. CCK acts as a satiety hormone and is produced by a population of I cells –which are mucosal endocrine cells- in the small intestine by presence of nutrients in the duodenum (65). CCK is known to induce satiety after peripheral administration (9, 27, 83, 84, 87); and it is clear now that vagal afferents are required for the effects of CCK on food intake (33, 125). These vagal afferents are capsaicin-sensitive since ablation of these vagal afferents by systemic capsaicin treatment abolishes the action of CCK (75, 112, 128). There is also neuroanatomical evidence for neuroendocrinological interaction with gut hormones as cholecystokinin (CCK). Berthoud and Patterson (12) demonstrated an anatomical relationship between vagal afferent fibers and CCK-immunoreactive entero-endocrine cells in the small intestinal mucosa of the rat. Also, the CCK-A receptor – which is the receptor type CCK acts on to induce satiation (8, 85, 86) - is abundantly present on vagal afferents and in the nodose ganglion (131). These observations suggest that vagal afferents are an ideal location for interaction with humoral satiety factors such as CCK and neural signals from the upper GI tract.

Meal-related stimuli such as mechanical distension, chemical properties of the luminal contents, gut peptides and neurotransmitters can be sensed by vagal afferents and play a direct role in reducing meal size (84, 87, 88, 111, 121). Pyloric cuff experiments and sham feeding preparations have given strong support for the idea that the stomach detects volume. Hungry animals eat significantly longer and more when the food is drained from the stomach (24, 25). Likewise, when rats eat a very large meal (25) or receive gastric preloads (101) with occlusion of the pyloric cuff rats eat less than with the cuff open. In addition, saline was as effective as a liquid diet to induce satiation when gastric emptying was prevented by pyloric cuffs. This indicates that the stomach is primarily involved in volume detection which is confirmed by the observation in humans that reduction of stomach capacity by banding is often used to treat severe obesity.

Most absorption and enzymatic digestion is located in the small intestine (111). Also, humoral factors involved in the process of satiation (see before) are secreted by the small intestine. The small intestine is thought to be primarily involved in nutrient sensing (105, 111). Some vagal afferents

respond to infusions of specific nutrients as carbohydrate (76), fatty acids (22, 59, 110) or amino acids (99). Thus, vagal afferents innervating the intestine are able to respond to different nutrients. There are indications that specific nutrients may be sensed by anatomically distinct populations of visceral afferent neurons (153).

In a normal meal, gastric stimulation occurs simultaneously with intestinal stimulation. Therefore, it is likely that these responses are modulated by humoral factors of the duodenum (e.g. CCK) and the stomach (e.g. leptin and grehlin).

The term 'gut-brain axis' refers to the observation that most visceral primary afferents have their nerve endings in the DVC of the hindbrain. The process of satiation appears to be controlled by a reflex mechanism also called vago-vagal reflexes, since forebrain structures are not required for the inhibition of food intake. Experiments of Grill and colleagues demonstrated that decerebrated rats show still satiation to food and injection of CCK (38, 39). In other words, visceral feedback from the GI tract to brainstem areas is sufficient to induce satiation. I refer to the introductions of chapter 2 and chapter 3, and chapter 7 for more information about the involvement of vagal afferents and hindbrain structures in the termination of feeding.

2.1.3 Vagal afferents and long-term regulation of energy homeostasis

Although it is clear that vagal afferents play a role in the process of satiation, there is not much data on the involvement of these afferents on the long-term. In other words, could it be that vagal afferents play a role in the development of obesity? Animal models used in obesity research with mutations in certain receptors or knock-out studies could give more understanding about this question.

Evidence for a role of vagal afferents in the regulation of energy homeostasis comes from the Otsuka Long-Evans Tokushima fatty (OLETF) rat. This OLETF animal lacks CCK-A receptors and is an animal model of type 2 diabetes, characterized by abdominal obesity, hyperphagia, insulin resistance,

hypertension, and dyslipidemia (43). Thus, the phenotype of the OLETF rat points at an important role of the CCK-A receptor in the regulation of energy homeostasis. More specific, it points at an important role of CCK in food intake, since pair-feeding prevented the increased body weight, but also normalized the elevated levels of leptin and insulin and the alterations in arcuate nucleus neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) gene expression in OLETF rats (14). As mentioned before, CCK-A receptors are abundant on vagal afferents, so this suggests indirectly a role of vagal afferents in the long-term regulation of energy balance.

A result which contradicts this hypothesis is the observation that the CCK-A receptor knockout mice are not obese and their food intake is at control levels. These mice do not show a response to CCK on the short-term. Kopin and colleagues interpreted these observations that the CCK-A receptor is not essential for the maintenance of body weight (57). However, recent data demonstrated that the distribution of CCK-A receptor differs in mice and rats (84). This could explain the difference in phenotype between rats and mice lacking CCK-A receptor. All together this shows that the role of CCK and the CCK-A receptor in the regulation of long-term energy homeostasis is yet unclear. Therefore, the involvement of vagal afferents in the development of obesity is undefined, since the CCK-A receptor is not limited to vagal afferent fibers, but is also widely distributed throughout the brain and spinal cord (78). Indications which support the thought of the involvement of vagal afferents in energy homeostasis are studies that demonstrated that treatment with microchip vagal afferent pacing reduces food intake and body mass in rats (58, 61) and rabbits (127). This suggests that reduced activity of vagal afferent fibers could be involved in weight gain.

It is clear that animals with disturbances in long-term control of energy balance have also reduced sensitivity to short-term signals related to food intake. The obese Zucker rat has a point mutation in the gene coding for the leptin receptor in both alleles and this mutation results in a shortened receptor. Therefore, leptin is less effective and this results in a massive obesity in the Zucker rat (17). It has been demonstrated that the obese Zucker rat is

less sensitive to CCK than its lean controls (91). As pointed out before, CCK acts on vagal afferents. Thus, the suppressed action of CCK in the Zucker rat could indirectly point at a decreased sensitivity of the CCK-A receptor that is a.o. located on vagal afferents and the nodose ganglion. Other evidence for modulations of vagal signaling during changes in energy homeostasis is that rats maintained on a high fat diet – which are exposed to increased levels of endogenous CCK- show also a reduced sensitivity to exogenous CCK (21). Thus, there are several lines of evidence that suggest that vagal afferents are involved in the long-term regulation of energy homeostasis.

2.2 Meal induced thermogenesis, obesity, and vagal afferents

2.2.1 Meal induced thermogenesis and the liver

The thermostatic hypothesis has been proposed by Brobeck in 1948. This theory includes that core temperature is involved in the termination of feeding; “an animal eats to keep warm and stops to prevent hyperthermia” (15). Typically, eating begins shortly after temperature starts to rise and stops when the temperature reaches its peak. This increased thermogenesis is thought to be caused by an increase in intensity of stimulation of brown adipose tissue (BAT) via its sympathetic innervations (44). In support of the thermostatic theory, Glick et al. (34) demonstrated an inverse relationship between BAT thermogenesis and meal size. A study done by de Vries et al. (26) shows that a liver temperature of 39.3°C is associated with the end of a meal. They also showed that skin temperature rises when core temperature increases and is longer elevated suggesting there is a heat flow from core to skin. Thus, both heat production and heat loss mechanisms appear to be activated during and after a meal.

There is some evidence that sensors in the liver are able to signal the elevation in core temperature. This would mean that there is a neural inhibition of feeding when temperature increases. Indeed, Di Bella and colleagues found evidence for this hypothesis in 1981 (28). They found that feeding was inhibited after application of external heat to the liver.

Subdiaphragmatic denervation of the liver abolishes this inhibition in food intake (28) indicating the involvement of the hepatic vagal afferent in this mechanism. Thus, all these studies demonstrate that there is a strong relation between (liver) temperature and meal termination and suggest that vagal afferents are important in thermosensitivity.

The thought that the vagus nerve could be involved in meal induced thermogenesis (MIT) is supported by anatomical data. A study of Adachi and Nijijima demonstrated that afferent fibers in the hepatic branch of the vagus are thermosensitive (3). More specifically, three types of thermosensitive unmyelinated fibers can be distinguished by cold (10–36°C), warm (39–50°C), and mixed (10–35°C and 40–50°C) temperatures (29, 154). This suggests that the vagus nerve may mediate thermosensitivity.

2.2.2 Meal induced thermogenesis and obesity

The link between reduced (meal induced) thermogenesis and obesity started to get a lot of attention since the nineteen eighties (for review see (136)). This would be in line with the thermostatic hypothesis, namely, that reduced thermogenesis due to a lower liver temperature causes a delay in the termination of feeding. Numerous studies have been performed in men and women and human biologists have reported that lower thermogenic responses to feeding may be a factor for increased energy storage in (some) obese subjects (53, 103, 119, 123). Diabetic subjects show also a reduced thermogenic response to a glucose or insulin infusion compared to their controls. The authors hypothesized that this may also contribute to a decreased meal induced thermogenic response (36, 37).

More recent data of Matsumoto et al. (74) also indicate that obese subjects have decreased thermogenesis and energy expenditure after mixed food intake. In contrast, Tentolouris et al. (135) did not find differences in thermogenic response to a carbohydrate-rich meal between lean and obese subjects. Also, high carbohydrate meal induced thermogenesis (MIT) did not differ between lean and overweight men (71). However, it appears that type of

diet plays an important role in MIT as is described by different groups (see also (66, 71, 96, 126, 146)). Therefore, differences in MIT between obese and lean subjects may depend on the nutrient(s), the composition or even the texture of a meal.

Studies on thermogenesis are performed in obese animal models as the obese ob/ob mouse (137, 141), diabetic-obese db/db (138, 139), the diabetic-obese KKAY mouse (95) and the fatty (fa/fa) rat (69). In all mutants there was clearly a decrease in metabolic activity. Moreover, most if not all obese rodents show a reduced capacity for thermogenesis. In an experiment using a cafeteria diet it was found that lean mice deposit only 4% of their excess energy intake during overfeeding. In contrast, obese mice deposited 55% of their extra energy intake on the cafeteria diet. This suggests that lean mice have a very substantial capacity to dissipate excess energy by MIT and thereby regulate energy balance (140). Thus, it appears that regulatory MIT is defective in the ob/ob mutant and could contribute to the development of obesity. Also in other obese models as in the fatty rat appears to be an impairment in MIT (69, 151, 152).

Although in most experiments, the relation between satiety and thermogenesis is not directly studied, it could be that reduced thermogenesis in obese subjects causes a delay in satiety. Andrews et al. (7) found that in vagotomized rats the rise in thermic response following gastric intubation with a carbohydrate meal was diminished. Thus, it could be that a reduced afferent signaling could contribute to a decreased thermogenesis and thereby promoting obesity.

3 GLUCOSE HOMEOSTASIS

Another aspect of energy balance is the metabolic changes that occur during and after food intake. Accurate regulation of blood glucose levels is critical for maintenance of homeostasis. Often, obesity is associated with insulin resistance and type 2 diabetes. It is obvious that vagal afferent fibers are

involved in meal termination as well as thermogenesis. The question rises if modified vagal signaling also affects glucose homeostatic mechanisms.

3.1 Glucose homeostasis, obesity, and the vagus

It is known since the 19th century that the autonomic nervous system plays an important role in the regulation of glucose metabolism. The importance of the nervous system was already observed by Langerhans in 1869, who found that rabbit and cat pancreas contain unmyelinated fibers, which have a tube connection to intrapancreatic ganglia which form a rich nervous plexus joining the specific cellular system (4). These islets of Langerhans, or also called the β - or B- cells are crucial for insulin secretion in response to food ingestion and are therefore necessary in order to maintain glucose homeostasis. Hormonal gut factors have effects on insulin secretion, but as already pointed out, also the neural system is involved (132).

The communication between gut and B-cells and its effects on insulin secretion is also known as the entero-insular axis (23, 97). For the neural component, the vagus nerve system has been thought to play important roles for this regulation (62). The glucose-sensitive afferents from the liver seem to initiate a reflex control of blood glucose level. In support of this, it has been reported (130) that there is an early insulin response following gustatory information. Moreover, a study by Mei (77) also indicated the importance of information from the intestinal glucoreceptors in the reflex control of insulin secretion. The importance of the vagus in this reflex loop has been demonstrated electrophysiologically.

It has been reported that hyperglycaemia in the portal venous blood suppresses afferent activity of the vagus nerve (82, 92) and this would increase the activity of the celiac branch of the vagus as well as the pancreatic efferent branch. Activation of the pancreatic efferent vagus stimulates insulin secretion. However, intracarotic glucose infusion leads to a reduction in gastric efferent vagal activity (45) and intra venous (134) glucose infusion suppressed the activity of the dorsal motor nucleus of the vagus. Thus, depending on the location of infusion in the system, different effects can be elicited. Also,

different branches may have different activity patterns in response to the same stimulus.

Neuromodulation of the vagus by implantation of a microchip reduced fasting glucose levels (58, 60, 134) and this effect was even enhanced when this was combined with capsaicin treatment (60). This observation also supports a role for the vagus in glucose homeostasis.

Obesity is often associated with disturbances in glucose homeostasis and most obese animal models (ob, db, fa, Ay, Zucker) are characterized by hyperinsulinemia. Subjects have problems to control their glucose levels due to a diminished ability of tissues to respond to the action of insulin. Consequently, the pancreas produces a lot of insulin to compensate for this situation. This hyperinsulinemic response is also defined as the insulin resistance syndrome and apart from increased insulin secretion it is characterized by an increased prevalence of obesity, hypertension, dyslipemia and type 2 diabetes mellitus (70). Insulin resistance, without the confounding factors of obesity, diabetes, and significant hypertension, is usually associated with a large reduction in efferent vagal activity, which occurs via attenuation in reflex activity (81). Vagus nerve-mediated regulation of insulin secretion appears to be impaired in Wistar fatty rats (94) and this appears to start from an early stage of life (150). VMH-induced obesity is also characterized by increased insulin secretion. This increase is reduced after vagotomy in conjunction with the development of obesity (52).

All together, these studies suggest that the vagus nerve plays an important role in glucose homeostasis by glucose sensing and/or insulin secretion and/or insulin sensitivity. An inadequate response of afferent nerves could lead to a disturbance in the reflex loop of insulin secretion. Consequently, an insufficient insulin secretion in response to nutrient ingestion could be the result followed by a delayed peak. Therefore, these abnormalities may contribute to the development of glucose homeostatic disturbances in obese subjects such as insulin resistance and fasting hyperinsulinemia (150).

3.2 Sensory nerves and glucose homeostasis

The pancreatic B-cell is extensively innervated by a network of sensory nerves. Hepatic glucoreceptors have been postulated to be coupled with capsaicin-sensitive afferent nerves and they are thought to transmit sensory signals of blood glucose concentration to the central nervous system (149). The role of these sensory nerves in glucose homeostasis has been investigated by using the neurotoxin capsaicin as a tool (see also paragraph Capsaicin for more information).

Glucose tolerance is usually tested by an intravenous glucose tolerance test (IVGTT) or oral glucose tolerance test (OGTT). Insulin sensitivity is usually studied by the performance of either an insulin induced hypoglycaemia or by an euglycaemic hyperinsulinemic clamp.

In general, mice or rats treated with capsaicin show an increased insulin response to an intravenous (54) or oral glucose load (40). This could mean that sensory nerves are involved in a tonic inhibition of insulin secretion. It could also mean that these nerves are involved in a neural reflex, by activation of glucose receptors in the portal area (92). Furthermore, capsaicin-treated rats show increased glucose elimination (56). This could be explained by the potentiated early insulin response – the insulin peak seen 1 minute after intravenous glucose injection. However, streptozocin diabetic rat show the same phenomenon, indicating that there is an increased insulin action as well. The latter is confirmed by findings of Koopmans et al. (56), who found that capsaicin-treated rats have an increased glucose disposal compared to their controls in an euglycaemic hyperinsulinemic clamp. Moreover, Zhou et al. (155) found that capsaicin treated animals showed a slower recovery from an insulin induced hypoglycaemia. These results suggest that insulin sensitivity is increased after capsaicin treatment.

The question comes up whether vagal sensory nerves could be involved in the control of insulin sensitivity. The thought behind this is that the vagus would protect the organism from hypoglycaemia. Support for this thought is found by studies of Spiridonov (129) who showed that capsaicin stimulation of intact rats decreased the hypoglycemic action of insulin and increased

hyperglycaemia following glucose dosage. In contrast to Zhou et al. (155), they found that neonatal treatment with capsaicin decreased the hypoglycemic effect of insulin but had no effect on hyperglycaemia following glucose doses. Moreover, selective sensory denervation of the anterior hepatic plexus rats leads to a decrease in insulin sensitivity during an euglycaemic hyperinsulinaemic glucose clamp, suggesting that hepatic vagal sensory nerves also play a role in insulin sensitization (104).

Concluding, it could be said that the exact role of vagal afferent C-fibers in glucose sensing and insulin sensitivity needs still to be elucidated. Recently, Ahren and colleagues (5) demonstrated that glucagonlike peptide I (GLP-1) -induced insulin secretion at a low dose in mice is dependent on intact sensory nerves. This suggests a complicated neuro-endocrinological network between gut and pancreatic B-islets. The functional implications for type 2 diabetes in this needs still to be revealed. It seems likely that neural circuits are involved in the development of insulin resistance and it might be that failure in such circuits result in the development of impaired glucose tolerance, insulin resistance or type 2 diabetes.

4 THE USE OF CAPSAICIN

The methods typically used for research of vagal involvement in biological systems are either selective or complete vagotomies or by administration of a toxin that specifically destroys these nerves. Vagotomies affect both afferent and efferent systems with several consequences; a.o. the animals have even problems with eating of solid food. Chemical unidirectal vagotomies are usually performed by systemic injections with high dosages of capsaicin. This neurotoxin destroys unmyelinated C-fibers and small myelinated δ -fibers which makes it a useful tool to investigate the involvement of vagal afferent nerves in biological systems. I decided to use the neurotoxin capsaicin to investigate the role of sensory nerves in the regulation of energy homeostasis. Yet, before discussing the aims of this thesis, I will focus on the compound capsaicin and also the use of capsaicin as a tool.

4.1 Capsaicin and the capsaicin receptor

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the pungent ingredient of hot peppers (genus *Capsicum*) and is eaten by about 25% of world's population on a daily basis. Hot pepper is pungent, affects thermoregulation, activates autonomic reflexes and is poorly absorbed. Hot pepper is a native of the Americas and the Aztecs called it chili. The active ingredient was first isolated by Thresh (1846). In the beginning of the 20th century, the exact chemical structure of capsaicin was determined by Nelson (1919) (for review see (133)). The broad use of capsaicin was already known by the Incas, who burned dried chili peppers to temporarily blind the invading Spaniards. Native Americans were familiar with the analgesic use of capsaicin and rubbed their gums with pepper to relieve their tooth ache. Hot peppers are rich in vitamin C and therefore a good additional food supplement.

In general, people living in tropical climates eat their food more hot compared to people living in temperate climates. There is a hypothesis that this is to combat the warm climate by gustatory sweating (63). Indeed, the capsaicin receptor is activated both by capsaicin and heat, indicating a possible role in thermoregulation. The capsaicin receptor belongs to the vanilloid family. The exact function of this receptor has still to be established. What is known is that the vanilloid receptor (VR) is activated by noxious heat and low pH; this makes the VR as a potential integrator of painful chemical and physical stimuli (133).

Vanilloids show species-related differences in biological actions (46). The dose of capsaicin that can kill the guinea pig almost instantaneously is well tolerated by the hamster (35). The species-related differences in responses to vanilloids can be explained by the different distribution of the capsaicin receptor, TRPV1, (transient receptor potential channel-vanilloid subfamily member 1, also called vanilloid receptor I or VR1). TRPV1 is a cation channel subunit expressed by a subset of nociceptive neurons in dorsal root and trigeminal ganglia (16). TRPV1 is also expressed at a subset of visceral afferents (46). The expression of TRPV1 is not limited to primary afferents. Jancso and colleagues in the nineteen seventies indicated that hypothalamic

preoptic (POAH) and anterior centers are also capsaicin-sensitive. Direct capsaicin administration to the rat POAH triggers hypothermia. Also, animals desensitized with capsaicin exhibit impaired thermoregulation in warm environmental temperatures or during direct hypothalamic warming (50, 51). By now, it is known that VR1 is widely expressed in CNS (see for more information also (20, 79, 116, 117)) and is among others expressed in brainstem structures that receive vagal afferent sensory endings as the DVC (2). Moreover, TRPV1 is also expressed in the nodose ganglion of the mouse (154) and rat (80, 98) as well as in neurons innervating the gastro-intestinal tract (98, 145).

The high expression of TRPV1 on sensory neurons makes capsaicin a valuable pharmacological tool to destroy these neurons in order to investigate the role of these afferents in physiology.

4.2 Capsaicin as tool

Capsaicin is able to both stimulate and destroy unmyelinated primary afferent fibres. Systemic treatment with capsaicin in high doses causes permanent ablation of primary afferents in neonates as well as in adult animals. Desensitization –defined as rapid loss of activity of the receptor occupied by an agonist- by capsaicin is not a well-defined biochemical process. It is a complex phenomenon that involves different stages and probably different mechanisms of action (133). The usefulness of capsaicin as a tool for studying the function of sensory nerves has got a lot of attention since the nineteen eighties (49). Capsaicin is used to distinguish subpopulations of primary afferents (67) which is reflected by the use of terms as capsaicin-sensitive as opposed to capsaicin-resistant or capsaicin-insensitive nerves. Different approaches are used to study the involvement of capsaicin-sensitive nerves in physiological processes. Systemic treatment in neonates and adults as well as local administration of capsaicin is applied to destroy sensory fibers. The neurotoxicity of capsaicin is not limited to primary sensory neurons, but also destroys neurons in different brain areas (46-49, 68, 89, 114, 115). Likewise, high doses of systemic capsaicin may induce argyrophylia along the entire neuroaxis of the rat, including the retina (113).

It is hypothesized that neonatal capsaicin treatment kills neurons by stopping the intra-axonal transport of nerve growth factor (NGF) from the periphery to the cell bodies of dorsal root ganglia neurons (147). In adult sensory neurons in culture capsaicin is able to destroy sensory neurons through the TPVR1 receptor. This process is most likely mediated by calcium (148). Capsaicin depletes the neuropeptides –especially substance P and calcitonin gene related peptide– of sensory neurons and releases these transmitters from their central endings as well as from the periphery. Although there are certainly limitations in the use of capsaicin as tool, it is a rather selective neurotoxin destroying specific nerves and therefore useful for investigating the role of these capsaicin-sensitive vagal afferents in the regulation of energy homeostasis.

5 OUTLINE AND AIM OF THESIS

The aim of this thesis is to investigate the involvement of capsaicin-sensitive vagal afferent C-fibers in the regulation of energy homeostasis. The involvement of vagal C- afferent fibers in the control of food intake, as well as in the regulation of glucose homeostasis is studied and the different sensory modalities of the vagus implicated in the regulation energy homeostasis –as satiation, temperature and glucose– are discussed. To this end, systemic capsaicin treatment is used as a tool to destroy these vagal afferents.

In Chapter 2, the involvement of capsaicin-sensitive afferents in food intake control and body weight regulation was investigated. The focus in this chapter was on short-term satiety mechanisms as well as on longer term food intake control. One of the hypotheses is that the lack of short-term signals controlling energy homeostasis is compensated by an increased sensitivity of long-term signals. To this end, during several weeks capsaicin treated animals were subjected to one hour short-term feeding tests of increasing concentrations of sucrose. Another group received a condensed milk suspension for 5 days next to their regular chow. During the experiments food intake and

body weight gain was measured to study the effect on energy homeostasis after ablation of capsaicin-sensitive nerves.

The results of chapter 2 suggested that capsaicin-insensitive nerves may be involved in gastric volume detection. Therefore, in Chapter 3, we investigated if low and high levels of distension may activate neurons in the DVC of capsaicin-treated rats. In these experiments the early gene protein c-Fos was used as a cellular marker for activation and fos immunoreactivity (Fos) was quantified in the different nuclei of the DVC. In addition, we investigated the interaction between CCK and distension, partly because a recent *in vitro* study of Simasko and Ritter (124) demonstrated that capsaicin-insensitive nerves also respond to CCK.

In Chapter 4, the involvement of capsaicin-sensitive nerves in meal induced thermogenesis (MIT) was studied. To this end, thermogenic responses to sucrose intake (same set up as chapter 2) were measured in capsaicin-treated and vehicle treated rats. The effect of the satiety hormone CCK on MIT during sucrose intake was also investigated. In this chapter, the involvement of capsaicin-sensitive nerves in MIT as well as the function of MIT as satiety signal is discussed.

In Chapter 5, the involvement of capsaicin-treated nerves in glucose homeostasis was investigated. Capsaicin-treated rats and their vehicle controls received intravenous infusions of different concentrations of glucose to study glucose tolerance and insulin responses after chemical ablation of primary sensory nerves. Different concentrations of glucose were used to see whether glucose detection is altered in capsaicin-treated rats. Results indicated that capsaicin-treated rats have an improved glucose disposal from the blood circulation. This could be due to an increased action of insulin-dependent mechanisms after capsaicin treatment.

Results of previous chapters indicated that capsaicin-treated animals are capable to maintain their body and fuel homeostasis even though they miss a substantial part of their afferent innervation. Hence, it is anticipated that capsaicin-treated rats are more sensitive to humoral signals related to energy

homeostasis. Therefore, in Chapter 6, we investigated if capsaicin animals are more sensitive to or have modifications in humoral factors -as leptin, adiponectin, resistin and corticosterone- related to food intake and glucose homeostasis.

Finally, in Chapter 7, the results are summarized and discussed in a broader perspective. Specifically, a critical evaluation will be given at current paradigms in the control of food intake. A part of the discussion will focus on the role of vagal afferent fibers in the regulation of glucose homeostasis. To integrate everything, the role of vagal afferent fibers in energy homeostasis and in the development of obesity is discussed. Finally, I will end the discussion with the conclusion of the studies in this thesis.

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