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Capsaicin-sensitive nerves and energy homeostasis

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CHAPTER 7

SUMMARY OF RESULTS AND GENERAL DISCUSSION

I SUMMARY OF RESULTS

The aim of this thesis was to investigate the involvement of capsaicin-sensitive vagal afferent C-fibers in the regulation of energy homeostasis. To this end, systemic capsaicin treatment was used as a tool to destroy these vagal afferents. We investigated the involvement of vagal C- afferent fibers both in the control of food intake and the regulation of glucose homeostasis.

In **Chapter 2**, the involvement of capsaicin-sensitive nerves in the regulation of body weight and the control of food intake was studied. Overall, there were no differences in chow intake and high-fat intake. Body weight increase did not differ significantly between CAP and VEH rats. When palatable high-fat diet was offered; CAP rats ate more of this diet only on the first day of exposure but intake of regular chow was decreased the first day in CAP rats compared to VEH controls. This indicates that capsaicin-sensitive nerves are not crucial for long-term body weight maintenance and long-term food intake control. It was found that CAP rats overconsume sucrose water in short-term feeding tests in comparison with VEH controls. This overingestion was independent of the sucrose concentration or the novelty of the food. It does indicate however that capsaicin-sensitive afferents are involved in short-term food intake control and the process of satiation.

In **Chapter 3**, the relative contribution of capsaicin sensitive C-fibers and capsaicin-insensitive A-fibers in the integration of CCK signaling and gastric distension signals was studied. We demonstrated that CCK acts through capsaicin-sensitive C-fibers because CCK-induced NTS Fos is abolished in CAP rats. In contrast, distension-induced NTS Fos is visible both in CAP and VEH animals. However, low levels of distension activated significantly less NTS neurons compared to VEH controls. There were no significant differences in NTS Fos with high levels of distension. Capsaicin-insensitive A-type fibers also responded to CCK when it was combined with distension. Distension induced fos was significantly enhanced when it was combined with CCK both in CAP and VEH rats. CCK's enhancement of

distension-induced Fos in CAP rats was reversed by the selective CCK-A receptor antagonist, lorglumide. It is concluded that CCK directly activates capsaicin-sensitive C-type vagal afferents. However, in capsaicin-resistant A-type afferents, CCK's principal action is facilitation of responses to gastric distension.

In **Chapter 4**, we investigated if capsaicin-sensitive C fibers are involved in meal induced thermogenesis (MIT) during short-term feeding tests with sucrose of different concentrations and its possible role in satiation (same set up as in **Chapter 2**). In general, CAP rats have a wider fluctuation in the MIT response starting from lower basal temperature and reaching a higher maximum temperature during MIT in comparison with VEH controls. In CAP rats, variation in maximum temperature during MIT between the trials decreases after repeated exposure to sucrose water. This suggests that CAP rats are still able to control maximum temperature (T_{max}) during MIT, indicating that temperature is also sensed in other areas, most likely in the central nervous system. VEH controls showed over time the same maximum temperature during MIT. Total MIT expressed as the area under the curve

(time 0-30 min) was constant for VEH animals over time. CAP animals showed a large variation, suggesting that temperature regulation is disturbed. IP injection with the satiety hormone CCK suppressed MIT in VEH, but not in CAP. This demonstrated that CCK-induced suppression of MIT occurs via capsaicin-sensitive C-afferents. This also indicates that CCK's effect on food intake thus probably not includes its effects on core temperature. Overall, it is concluded that CAP treated animals have disturbances in meal induced thermogenesis and hence capsaicin-sensitive nerves are involved in homeostatic control of thermogenesis during food intake.

In **Chapter 5**, the role of capsaicin-sensitive afferents in glucose homeostasis was studied. This was done by an IVGTT of 5, 10, and 15% glucose infusion to study insulin and glucagon secretion. Results indicated that in all cases CAP animals displayed a reduced insulin response, while glucose tolerance did not differ from VEH controls. Glucagon responses did not differ between CAP and VEH controls, indicating that results are not due to modifications in plasma concentration of the counterregulatory hormone glucagon. These results indicate that capsaicin-sensitive nerves are involved in insulin secretion and/or in the regulation of insulin sensitivity. It might be that capsaicin-sensitive nerves display their effects by modulating the afferent part of a neural reflex loop for regulation of glucose homeostasis.

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. Results revealed that capsaicin-treated animals are more sensitive to the effects of leptin on food intake. Corticosterone levels were strongly suppressed in capsaicin-treated rats during a 15% IVGTT. Adiponectin, resistin and FFA plasma levels were modified after the infusion with glucose. These results suggest that capsaicin-treated animals developed

compensatory mechanisms for their lack in afferent neural signaling. They also suggest that the effects on body weight and glucose homeostasis after capsaicin treatment could be (partly) explained by increased sensitivity to leptin and/or modified HPA-axis activity in CAP rats.

II GENERAL DISCUSSION

1 INTRODUCTION

1.1 General approach

Food intake and glucose homeostasis are critical aspects in energy homeostasis and often disturbed in obese subjects. In this general discussion, a critical review concerning the sensing modalities of the vagus (volume, nutrients, endocrine signals, and temperature) and the role of these modalities in the signaling of satiation is presented. The role of vagal afferents in glucose sensing and the consequences for the maintenance of glucose homeostasis is also discussed. The main goal of this thesis was to gain more understanding about the role of the afferent part of the vagus in the regulation of energy homeostasis. Therefore, I end this general discussion with some thoughts about the involvement of vagal afferents in the regulation of energy homeostasis. Finally, we emphasized the functional aspect of obesity and plead for a distinction between obesity as adaptation and obesity as a disease. This distinction is discussed in the light of the concept allostasis that was introduced by Sterling and Eyer in 1988 (81) and refers to the idea of ‘adaptation through change’. In the next paragraph, the outline of the discussion is given more specifically with relation to the different chapters.

1.2 Outline

The involvement of capsaicin-sensitive afferents in the signaling of short-term satiety (**Chapter 2, 3 and 4**) and glucose homeostasis (**Chapter 5 and 6**) was studied. First, focus will be on different hypotheses about the signaling of satiation and the role of vagal afferents in this process. The role of volume and nutrients in the process of satiation with emphasis on the involvement of vagal

afferents in this signaling pathway is discussed. A distinction will be made between afferent signals deriving from the stomach and afferent signals deriving from the intestine. Furthermore, a critical discussion about the role of temperature in meal termination as well as the role of the vagus in this process will be presented. This paragraph ends with some ideas on the conversion of peripheral and/or central signals and the contribution of the vagus in this process. Second, the role and importance of capsaicin-sensitive nerves in glucose homeostasis is discussed. We will concentrate on the disturbances in efferent pathways if the afferent part of the vagus does not function or dysfunctions. Finally, different aspects of energy homeostasis are pointed out; and also, some hypotheses as suggested from the work of this thesis; specifically about the role of vagal afferents in the regulation of energy homeostasis and the development of obesity.

2 FOOD INTAKE CONTROL

2.1 Sensing volume versus nutrients

2.1.1 Gastric signals and Intestinal signals

2.1.1.1 Gastric afferent fibers and volume detection

Results from Chapter 2 indicate that ingested volume is a major component in sucrose intake control. CAP and VEH treated animals both showed a volume depended sucrose intake during short-term feeding tests. These data indicate that volume is a major component in the signaling of satiation. These data also indicated that CAP rats are still able to sense volume. Thus, capsaicin-insensitive fibers are also involved in the detection of distension. It was previously demonstrated that CAP rats still sense gastric distension (117). Results from Chapter 3 also clearly demonstrate that capsaicin-sensitive vagal C-fibers as well as capsaicin-insensitive vagal A-fibers are able to induce fos-like immunoreactivity in the dorsal vagal complex (DVC) of the hindbrain.

Volume sensitivity has not only been identified in gastric afferents; duodenal afferents are known to have a distension modality as well (34).

Berthoud (13) demonstrated that most intraganglionic laminar endings (IGLEs) and intramuscular arrays (IMAs) –structures known to be involved in distension signaling– in the small intestine are destroyed after capsaicin treatment. This effect was restricted to the intestine, since most of the IGLEs and IMAs innervating the stomach survived capsaicin treatment (13). Considering that most of the intestinal vagal innervations are destroyed by capsaicin; and that in our experiments (**Chapter 2**) sucrose consumption was strongly volume related; we can conclude that these distension signals are probably deriving from the stomach.

2.1.1.2 Gastric distension signals and satiety

Although stomach signals are generally considered to be mechanical in nature; it is still questioned whether the stomach also provides other signals that are associated with satiation. Many experiments using inflatable cuffs to occlude the pyloric sphincter; withdrawal of stomach contents or infusions with saline or nutrients all addressed this question. One of the earliest experiments to investigate the role of the stomach in satiety was done by Hull and colleagues in 1951 (55). They saw that a dog with an esophageal fistula consumed much more in a single meal compared to the condition when the fistula was closed. Additional experiments by Davis and Campbell (21) demonstrated that when food is withdrawn from the stomach after a meal is finished, the rat begins to ingest food again with a median latency of about 3 minutes. These authors suggested that this occurs through a reduction of stomach distension; or through a reduction in the amount of nutrients into the duodenum, thereby reducing duodenal satiety.

In support of the first hypothesis, Deutsch published in 1978 in *Science* that when a certain amount of milk was withdrawn from the stomach while the pyloric cuff was inflated, compensatory drinking occurred. He favored the hypothesis that termination of a meal derives from the stomach and not from the duodenum (25). Also, compensatory drinking occurred when milk escaped from the stomach into the duodenum. Thus, gastric infusions resulted in proportionate decrease in food intake and withdrawal of gastric content in compensatory ingestion. Another important indication that the stomach is predominantly involved in volume detection is the observation that infusions of

saline are as efficacious as infusions with nutrients previously withdrawn (59). A critical point that has to be made is that pyloric cuffs are usually located at parts that are richly innervated (136). Hence, these experiments could be confounded since not only the stomach is stimulated, but duodenal vagal receptors as well. However, studies done by Philips and Powley (102) and Mathis et al. (74) with more stimulus control also indicate that the stomach is more involved in volumetric control rather than nutrient sensing. An important role of gastric signals in man was reported by Schick et al. (120). They found that food consumption in man is reduced, if initiation of eating is preceded by nutrient administration into the stomach, but not into the duodenum. Thus, we can say that our results (**Chapter 2**) and data of others indicate that gastric signals play an important role in the termination of food intake and in the feeling of satiation.

Concluding, when we look more closely to the data of **Chapter 2** and **Chapter 3** it is suggested that: 1. Gastric distension-related signals are transmitted by capsaicin-resistant afferent fibers; 2. Capsaicin-sensitive fibers are also involved in distension signaling; because there is a pronounced overconsumption of sucrose water compared to VEH rats (**Chapter 2**); and low levels of gastric distension induce less Fos in the NTS of CAP rats compared to VEH controls (**Chapter 3**).

2.1.1.3 Nutrient signals and satiation

This last conclusion -that stated that gastric distension signals are transmitted via vagal afferent C- and A-fibers and that they play an important role in the control of food intake- does not exclude a role of the intestine in the process of satiation. Our own results also suggest that some kind of nutrient signal and/or metabolic signal accompanies the volumetric signal. CAP rats show a large variation during 10% sucrose intake, indicating that these rats have difficulties to control food intake when low concentrations of sucrose water are consumed. Therefore, it is likely that some kind of additional signal is missing in these deafferentated animals.

Apart from the distension signal, one would predict that glucose is sensed as well by the gut. Mei (85) demonstrated the presence of

glucoreceptors in the small intestine of the cat. Considering the possible role of glucoreceptors in the duodenum in caloric control (see also paragraph 2.2 *caloric control of food intake*) or in the detection of the total amount of carbohydrates, it would be expected that rats would decrease their intake with increasing sucrose concentration. Thus, even though a different amount of carbohydrates reached the duodenum in the described experiments in **Chapter 2**, the ingested volume was still the same not only in CAP animals, but in VEH controls as well.

Yet, forty percent sucrose water caused a small but significant drop in sucrose intake in CAP and VEH rats. This decrease is probably not due to the activation of glucose receptors; it seems more likely that the osmolality of the solution is an important determinant for satiation (54). Indeed, it has been shown that hyperosmotic saline produced more spiking activity than normal saline; hyperosmotic glucose produced more spiking activity than equiosmotic glucose (86). Apparently, osmoreceptors are still functional after capsaicin treatment and it is suggested that signals about the osmolality of the ingested compound is transmitted via capsaicin-insensitive fibers.

Also, there are indications that the type of nutrient has different effects on the activity of vagal afferents (123). Mathis et al. (74) reported that intraduodenal infusion of peptone was more effective than equicaloric glucose in eliciting gastric vagal afferent activity. Fat infusions into the duodenum are also more potent to reduce food intake compared to carbohydrates (33). Moreover, the reduction of food intake by intestinal carbohydrate, some amino acids and fat are mediated by capsaicin-sensitive vagal afferents (139, 140). Studies demonstrated that damage to the afferent vagus by application of the neurotoxin capsaicin blocks the ability of some duodenal nutrients to suppress sham feeding (131, 139, 140). Data of others (36, 39, 53, 71, 97, 112) also indicate that satiety is produced when food enters the duodenum. However, one critical remark has to be made concerning the data obtained by intestinal infusions; it has been demonstrated that intestinal infusions may also produce nausea (24). Hence, satiety could not (only) be due to satiation but because of a feeling of discomfort. Yet, a role of the duodenum in the sensing of nutrients would be expected; during ingestion, food enters rapidly to the duodenum(114,

138). These effects of nutrients on food intake could either be direct by the stimulation of vagal nerve ending or indirect by stimulation of (neuro)endocrine factors. Moreover, intestinal signals and gastric signals interact and enhance thereby their effect on food intake (*see next paragraph*).

Concluding, we can say that intestinal capsaicin-sensitive vagal afferent C-fibers are involved in nutrient signaling. The activity of these vagal afferents appears to depend on the type of nutrient. However, our studies indicate that in short-term sucrose water feeding tests, nutrient detection is not the major component for the determination of satiation. Apparently, capsaicin-sensitive nerves are necessary to detect low sucrose concentrations for food intake control.

2.1.2 Interaction of gastric and gut signals

2.1.2.1 Neuroendocrine signals and gastric distension

Neuroendocrine factors are released in response to the arrival of food in the stomach and/or duodenum. One of the most well-known factors is the satiety hormone cholecystinin (CCK), which induces satiety by binding on CCK-A receptors located on capsaicin-sensitive fibers (see also **Chapter 3 and 4**; for review see (91)).

Gastric distension, intestinal nutrients and gut hormones are all potential contributors to vagal afferent activation. Therefore, vagal afferents constitute a potential location for integration and modulation of these multiple meal related signals. Interactions between gastric distension and CCK and other gastrointestinal stimuli have been reported. For example, a recent publication by Mazda and colleagues (78) suggested that gastric distension stimulates serotonin release from the enterochromaffin cells and this may activate 5-HT₃ receptors located on capsaicin-sensitive vagal afferent nerve terminals.

Another example is leptin that can directly activate vagal afferents in culture (100, 137). Moreover, the majority of vagal afferent neurons that reacted to the gut hormone cholecystokinin (CCK) also reacted to leptin (100). This interaction was earlier demonstrated by Fos studies by Barrachina et al. (10). The latter showed also that this interaction occurred through capsaicin-sensitive afferents.

Results of **Chapter 3** indicate that there is an interaction between gastric distension and CCK by the use of Fos-like immunoreactivity. CCK clearly enhanced distension induced Fos in the NTS. This enhancement occurs apparently via capsaicin-insensitive vagal A-fibers at the peripheral site. This is in agreement with previous reports about CCK's sensitization of gastric and duodenal mechanosensitive vagal afferents (22, 30). These are specific gastric afferents in control of food intake. This is illustrated in the fact that some stimuli that reduce food intake e.g. CCK increase gastric tension and vagal activity; yet, CCK is also associated with decrease in gastric tension (43). This is contradictory with the idea that increased tension of the stomach wall is usually associated with satiation. Thus, neuroendocrine factors may interact with gastric signals to control food intake; the vagus appears to be an important nerve integrating these signals (*see also paragraph Location of integration*).

2.1.2.2 Direct and indirect effects of nutrients; interaction with gastric signals

As mentioned before, neuroendocrine factors can be released by nutrients in the duodenum. Dietary carbohydrates release a number of different substances from enterochromaffin (EC) cells in the small intestine including glucagon-like peptide, glucose dependent insulinotropic polypeptide, and serotonin; which may be involved in mediating carbohydrate induced changes in gastric function and food intake (14, 107). Likewise, (long-chain) fatty acids induce release of CCK (56, 70) and lipid absorption stimulates synthesis of Apolipoprotein IV by enterocytes (48).

It was also discussed that there are indications that nutrients can directly activate vagal afferent endings. The interaction between signals

deriving from stomach and the presence of nutrients in the duodenum has been demonstrated in a number of studies. Experiments in humans have shown that intestinal nutrient infusions interact with gastric distension to produce the satisfying feelings of satiety experienced after the consumption of a meal (32, 46, 110). Reports of interaction between gastric and intestinal signals demonstrated that duodenal nutrient exposure and CCK elicits reductions in gastric pressure (109, 118), gastric emptying (93), and stimulates pyloric motility (118). These modulations are mediated via capsaicin-sensitive sensory fibers (108).

Vagotomy attenuates suppression of sham feeding induced by intestinal nutrients (141). Moreover, duodenal carbohydrate and protein exposure have been demonstrated to stimulate both duodenal and gastric motility in the rat (125). Walls and colleagues (135) have shown that surgical transection of afferent components of the vagus nerve that partially supply the proximal duodenum blocks the suppression of sham feeding produced by duodenal nutrients. There are indications that gastroduodenal load-sensitive vagal afferents indirectly transduce nutrient chemical information (74, 123); antral load-sensitive vagal afferents were differentially responsive to the type of macronutrient infused into the duodenum. Thus activity in these antral load-sensitive vagal afferents could indirectly signal nutrient composition and/or caloric content of the gastric lumen.

This is supported by immunohistochemical analyses demonstrating that combined gastric load and duodenal nutrient elicited significantly greater fos induced immunoreactivity than either gastric or duodenal infusions alone (29). Therefore, we could say that the presence of nutrients in the duodenum directly or indirectly interact with gastric signals.

Taken together, a variety of experiments using different techniques suggests that interactions between gastric and duodenal factors may play an important role in the control of food intake. These factors involve the interaction of gastric distension with intestinal neuroendocrine factors (**Chapter 4**) as well as the interaction of nutrient/mechanical gut signals with humoral/mechanical signals from the stomach.

2.1.3 Vagal gastric and gut signals and behavior

Experiments that indicate a variety of interactions between gut and gastric signals are not always extended to the level of behavior or actual ingestion. The results obtained with electrophysiological and immunohistochemical techniques do not necessarily predict the precise effects on food intake. For example, McHugh and Moran (82) found that in fasted monkeys there is little effect on feeding when small volumes of glucose enter the intestine from the stomach. Yet, direct infusions into the intestine of a similar volume of glucose strongly inhibited feeding. This demonstrates that the route of administration and the delivery rate and amount of food to the stomach and duodenum is crucial in the way a behavioral response is triggered. This demonstrates also that the techniques used have to be critically evaluated before general conclusions can be drawn concerning mechanisms involved in the physiological control of food intake.

The studies described in **Chapter 3** indicate that CCK and gastric distension interact and that vagal afferent A-fibers are involved in this effect. From a behavioral perspective, it has been demonstrated that CCK and gastric distension interact to reduce food intake in a variety of species, including rats, monkeys and humans (62, 65, 83, 88, 126) (see also **Chapter 3 introduction**).

We performed a pilot study to investigate the possible role of capsaicin-insensitive A-fibers in the enhancement of gastric distension and process of satiation. To this end, animals were treated either with capsaicin or vehicle and implanted with a gastric fistula. After an overnight fast, animals were exposed to a 15% sucrose solution and allowed to drink for 30 minutes. The animals were sham fed and received either control treatment (no distension + saline), no distension with CCK or distension with CCK. Results are presented in figure 7.1 (A) and the effects of the different treatment on sucrose intake are shown. We found that VEH animals reduced significantly their sham sucrose intake after CCK injection. However, there was no dose-response effect of IP CCK (2 or 4 $\mu\text{g}/\text{kg}$) in VEH rats. Distension alone also reduced sucrose intake significantly in VEH rats. In addition, CCK in combination with distension significantly enhanced the suppressive effect of distension on sucrose intake in VEH animals. However, the effect was less than the sum of the CCK-induced suppression and distension-induced suppression.

Although CCK did not have a significant effect on sucrose intake in CAP rats, there was still a strong reduction in intake after IP CCK. Distension alone induced a significant reduction in sham-feeding in CAP rats as well. Yet, the combination of CCK and distension did not significantly enhance the reduction in CAP animals. This was in contrast with our expectations, since CCK potentiated the effect of distension on NTS induced Fos. In order to confirm the successful deletion of capsaicin-sensitive vagal C-fibers, the CCK suppression-test was performed. As expected, CAP rats did not show any response to CCK. This in contrast to the VEH controls (see fig. 7.1 B).

In view of the large standard error bars in the control situation the approach was not sensitive enough to detect subtle differences between the various experimental conditions. Thus, even though the Fos data show that the interaction between CCK and distension occurs via capsaicin-insensitive A-fibers (**Chapter 4**), it is still not convincingly demonstrated that this plays a role in normal food intake. In the absence of clear behavioral data one cannot conclude that manipulations that induce changes in vagal activity or hindbrain activity demonstrate mechanisms involved in the process of satiation under physiological conditions.

Another question is whether the interaction of the various GI signals are additive or synergistic in their action on satiety. In **Chapter 3**, it was clearly demonstrated that in VEH control animals this interaction between CCK and gastric distension was an addition of the separate effects. This is in contrast to the results in CAP animals where this interaction effect was more than an addition of the separate effects of CCK and distension. One would expect the opposite. However, after chemical ablation of capsaicin-sensitive sensory nerves, there is a more specific population of nerves that is stimulated in these rats compared to VEH animals. In conclusion, it can be said that it is therefore still an open question whether the interaction of GI signals are just additive or whether they are really synergistic.

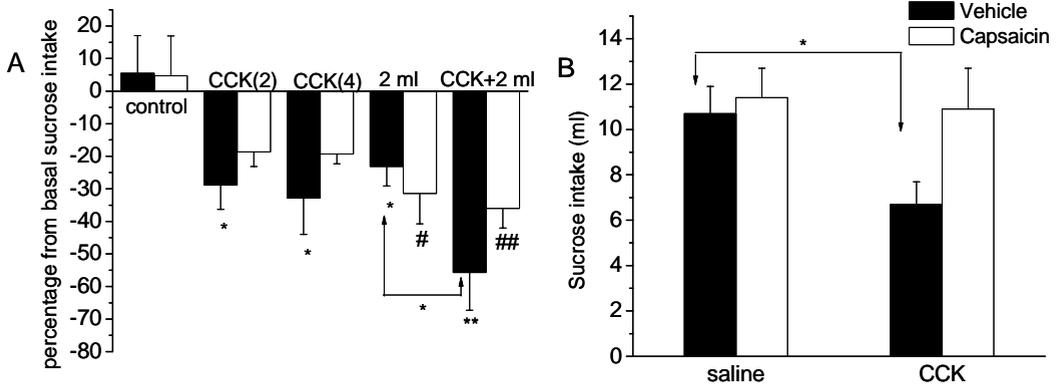


Figure 1

A) sucrose intake during sham-feeding after IP treatment with 2 or 4 $\mu\text{g}/\text{kg}$ CCK, 2 ml of gastric distension or the combination of CCK (4 $\mu\text{g}/\text{kg}$) with distension. B) CCK suppression test in CAP and VEH rats. Rats received 2 $\mu\text{g}/\text{kg}$ CCK IP and sucrose intake was measured. * and **; significant differences in VEH, $p < 0.05$ and $p < 0.01$ resp.; # and ##; significant differences in CAP, $p < 0.05$ and $p < 0.01$ resp. In contrast to VEH animals, CAP rats do not show a significant reduction in sucrose intake after CCK. There is a significant enhancement of distension induced reduction in sucrose intake in VEH rats, but not in CAP rats. Experiments performed by E.H.E.M. van de Wall, P. Duffy and R.C. Ritter (2004).

2.2 Caloric Control of food intake

Although calories are generally considered to play a major role in the control of food intake; we did not find a caloric control of feeding during short-term feeding tests with sucrose water (**Chapter 2**). Increasing concentrations lead to an increasing consumption of calories. Hence, the question rises whether caloric control occurs since there are numerous indications from literature that calories are sensed by the GI tract.

Different hypotheses about the control of short-term food intake have been postulated. Mechanical signals, nutrient signals, metabolic sensing as well as caloric monitoring are all suggested playing a role to define the end of the meal and/or beginning of the next meal. As discussed before, all macronutrient groups alter gastric vagal activity and gastric secretomotor function and food intake. Also, each macronutrient group acts via activation of separate and distinct pathways and mechanisms. These latter observations suggest that the responses to nutrients are not secondary to detection of caloric content (107). A number of studies show a direct involvement of extrinsic capsaicin-sensitive primary afferents in mediating inhibition of gastric emptying, secretion, and food intake in response to chemical stimulation of the gastrointestinal tract wall (107, 108, 115). An indication for caloric sensing by the GI tract is that animals can learn to prefer a flavor associated with calories. This preference for a non-nutritive flavor can be induced by a caloric infusion into the intestine (99) or hepatic portal vein, but not by infusion in the jugular vein (132). Also, it has been reported that infusions of glucose as low as 3% are effective to reduce real feeding ((135); for review see (114)). Thus, real feeding is inhibited when caloric contents are infused in the intestine.

The emptying rate of the stomach to the duodenum appears to be controlled by caloric content, texture of the diet (solid or liquid), type of nutrient as well as concentration of a consumed meal or an infused compound (52, 64, 72, 84, 89, 92, 95). McHugh and Moran (82-84) demonstrated that gastric emptying of glucose occurred at a constant rate in terms of caloric content of the stomach. It has been demonstrated that the greater the caloric/nutritive density of a meal, the less volume was transferred to the duodenum in 30 minutes (57). Yet, the caloric emptying rate as well as the

total caloric load delivered to the intestine by the end of the meal, remained constant over the range of nutrient densities tested (16, 57, 73). However, this relationship between calories and gastric emptying rate does not always count, since fructose empties much faster as glucose, though they both contain the same amount of energy (94). Moran and colleagues (95) demonstrated in rhesus monkeys that gastric emptying increases when gastric volume content increases. They also showed that duodenal glucose infusions shifted this relationship to the right. Thus, there is an interaction between gastric volume and duodenal nutrient exposure to control gastric emptying. This observation still supports the hypothesis that the rate of gastric emptying is determined by caloric density of the diet. It has also been demonstrated that the rates of gastric emptying where calories are delivered to the small intestine following ingestion of liquid food are sufficient to elicit postgastric satiety in the absence of gastric distension (112).

Although gastric emptying appears to have a relationship with the caloric density of the diet; it appears that when rats are allowed to drink to satiety, caloric intake is not controlled by caloric density. Caloric intake increased with increasing diet nutrient density (57). We also found that short-term food intake was not correlated with caloric content of a meal (**Chapter 2**). We did not measure gastric emptying and gastric filling. Therefore, we cannot make conclusions about the caloric control of gastric emptying.

Duodenal caloric feedback to the stomach to regulate gastric emptying appears to occur via vagal afferents nerves (122). There are indications that the effects of intestinal glucose on gastric motor function occurs via capsaicin-sensitive nerves (106). This suggests that CAP treated animals do not have such a feedback and that their gastric emptying rate is different compared to VEH controls. This also suggests that short-term meal signaling occurs predominantly from the stomach since most intestinal vagal C-afferents are destroyed by capsaicin (13). Therefore, these results indicate that in capsaicin-treated animals gastric volume plays a major role in meal termination at higher sucrose concentrations.

If gastric emptying is under caloric control in VEH animals, gastric filling would be different during the ingestion of different sucrose concentrations (**Chapter 2**). Hence, the question is what signals are involved in the signaling of meal termination in VEH controls. It is very clear from our results that VEH controls regulate predominantly on volume intake. Probably, these signals are a combination of mechanical stimuli deriving from the stomach as well as from the intestine. We do not know 1. if caloric control of gastric emptying occurs in our experimental set-up and 2. how this caloric control would be related to actual food intake.

It has to be mentioned that this short-term food intake tests with palatable sucrose water is probably not completely representative for normal food intake. First, animals consume a liquid and not a solid food. Second, high palatable food might stimulate rewarding mechanisms, thereby modulating normal satiety signals. Third, the tests were performed during the light period. Although these experiments may give a good indication for mechanisms involved in the process of satiation, experiments with solid food and during the active period are required to elucidate the fine tuned mechanisms involved in meal termination.

Therefore, we can say that although calories may be sensed in a way; it appears that calories per se are not the signal for the control of food intake. Gastric emptying appears to be controlled by caloric content or nutrient density of the food. Yet, the control of meal size does not seem to depend on the regulation of gastric emptying.

2.3 Meal induced thermogenesis and food intake

2.3.1 Meal induced thermogenesis and satiety

Results from **Chapter 4** do support that in VEH controls, the end of a meal is related to a certain temperature. During the different sucrose trials, maximum temperature (T_{max}) remains constant in line with the volume intake. However, animals do ingest different amount of calories, indicating that the amount of nutrients or calories does not seem to be related to the temperature response. Also, the total thermogenic response was relatively constant in VEH controls.

Only in the last trial set of 10%, meal induced thermogenesis (MIT) decreases. This indicates that during the ingestion of a meal the same energy is required for digestion, absorption and disposal of the ingested food. It also suggests that after repeated exposure, the animal 'learns' to deal more efficiently with the ingested liquid. Interestingly, in CAP animals T_{max} during a meal appears to be controlled as well. Although there is a large variation during the first 5 trials, the variation between trials gets smaller and smaller in CAP animals as trial experience increases (**Chapter 4**). Also, temperature never becomes higher than 37.9 °C in CAP. The total thermogenic response to a sucrose meal shows a large variation in CAP rats and suggests that this is disturbed after chemical ablation with capsaicin-sensitive nerves.

Thus, it appears that critical factors -such as avoiding hyperthermia- are still controlled after capsaicin treatment. However, disturbances in temperature regulation are illustrated by the large variation in total thermogenic response to a meal. It poses questions though how and if temperature functions as satiety factor.

MIT depends on the composition and amount of food ingested and typically 10-30% increase in energy expenditure is seen in the hours after a meal (Jequier et al., 1988). A hypothesis that involves food intake control is the thermostatic hypothesis (see also **Chapter 1**). The liver appears to be crucial in this process (1, 23). According to de Vries et al. (23) this specific liver temperature functions as a satiety signal and consequently the animal stops eating to prevent hyperthermia. The involvement of vagal afferents in this process is a.o. demonstrated by El Ouazzani and Mei (27) who identified thermoreceptors on unmyelinated vagal afferents that were only stimulated by temperature and not by mechanical or chemical stimuli (see also **Chapter 1**, **Chapter 4**). They also found indications that these receptors might be involved in the coordination of digestive activity as well as thermoregulation.

Although there is a strong relationship between temperature and food intake, this is certainly not a linear one. One critical point towards this hypothesis can be made. Even in the results of de Vries et al. (23) it is shown that meal sizes vary; however, the temperature rise is not always linearly

related to the amount of food consumption. This suggests that during food intake, temperature is regulated to avoid hyperthermia. Of course, when temperature rises to high, this will make the animal stop eating. This may be considered as an emergency situation and seems to be more comparable to the situation of e.g. fever. Apparently, capsaicin-treated animals are still able to cope with this kind of threats, since they are still functioning quite well under stable, predictable circumstances. This suggests that mechanisms to avoid hyperthermia are controlled in higher centres, probably localized in the brain. This does not implicate that vagal afferents are not important in VEH controls in the avoidance of hyperthermia. Probably, there are several defense lines to protect the organism from 'overheating'.

Clearly, in CAP rats there is no obvious relation between the end of a meal and MIT or Tmax. In VEH controls, there is a relation between the end of a meal and MIT and Tmax. As mentioned before, different amount of calories were consumed during the different trials. It is therefore doubtful that temperature itself would function as satiety signal in these circumstances. There are factors that influence MIT response. Apart from meal size (68) composition/texture of the diet (127) and physical state of a meal (98) have effects on MIT response. We offered a liquid diet and not a solid diet. In addition, experiments were performed in the light phase of the animal. As a consequence, animals do not eat in their normal feeding period and this could give a different picture concerning MIT responses.

Thus, although from our experiments MIT or Tmax appear not to be correlated with the end of a meal; additional experiments are required to elucidate the involvement of MIT or Tmax in the control of meal size. These experiments should be performed under more physiological circumstances e.g. in the dark phase and with solid food.

It appears though that after ablation of capsaicin-sensitive nerves, the MIT response shows wider fluctuations and is less fine tuned compared to VEH controls. This suggests a role for these vagal C-type afferents in temperature homeostasis.

2.3.2 CCK and MIT

The involvement of afferent vagal activity in MIT is also demonstrated by the fact that CCK injection prior to the meal blunts the MIT completely in VEH animals, but not in CAP rats. CCK is a satiety factor and according to the thermogenic hypothesis, one would expect an increase in food intake. However, CCK induces a decrease in food intake. Of course, the dose given to the animals was supraphysiological, therefore not reflecting the involvement of CCK in MIT. Also, the IP injection with the CCK-A receptor antagonist devazepide (2 µg/kg and 200 µg/kg; generous gift of ML Laboratories Plc, UK) did not have any clear effect on temperature in VEH or CAP rats. In control circumstances, devazepide is able to increase food intake (111). Likewise, we could therefore expect an increase in MIT as well (since CCK suppressed MIT). However, we even see a decrease in MIT after administration of devazepide. Yet, it could be that CCK displayed its effects via the CCK-B receptor. It has also to be mentioned that these doses of devazepide did not had significant effects on sucrose intake.

Therefore, the role of CCK in MIT is not clear. It is not likely that CCK also displays its satiating effects via temperature. These results support the thought that there is no relationship between the end of a meal and MIT or Tmax. MIT reflects a complex interaction of gustatory sensory, hormonal, neural, neuroendocrinological and metabolic factors. Since after capsaicin-treatment these factors are modulated, this is probably represented in the MIT as well. It can be said that capsaicin-sensitive afferents do play a role in MIT, either direct, or indirect. However, it is not very likely that these afferents play a role in MIT or Tmax for the induction of satiety.

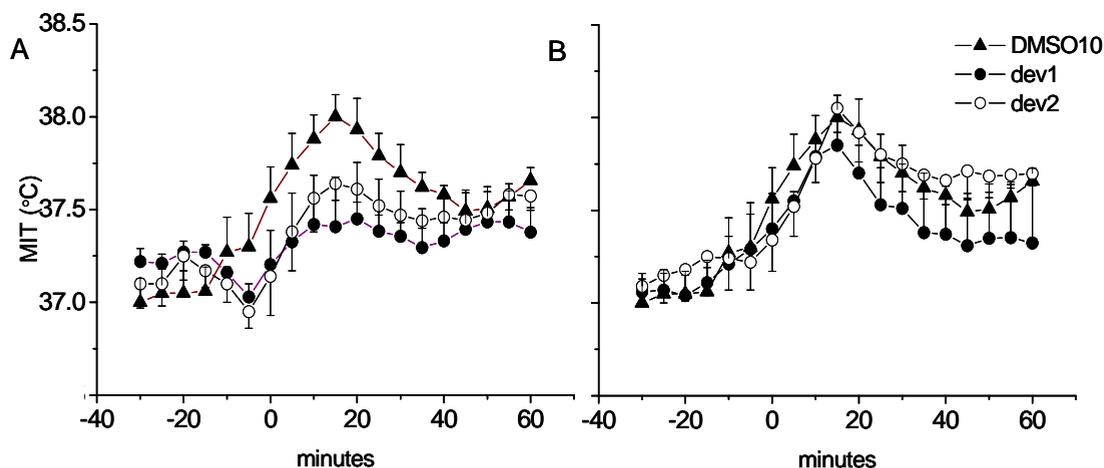


Figure 2

MIT response of VEH (A) and CAP (B) rats during 15% sucrose intake. Animals received either a control injection (DMSO 10%), devazepide (1=2 μ /kg; 2=200 μ /kg). Although the MIT response in VEH animals is reduced after injection with devazepide 2, this difference did not reach significance. Devazepide did not have any effect in CAP treated rats.

2.4 Convergence of peripheral and central signals

2.4.1 *The vagus: a peripheral site for integration?*

The vagus has a wide range of sensory modalities that derive from the GI tract as well from the liver connecting via the nodose ganglion to the dorsal vagal complex of the hindbrain. These sensory modalities are mechanical signals of the GI tract; neurendocrine signals; nutrient detection; but also temperature; glucose; and other metabolic signals. This means that the vagus transmits a huge amount of information from peripheral sites to the CNS. Therefore, it would be a candidate for the integration and modulation of peripheral signals as well. Yet, it is often not clear from experiments if convergence of peripheral signals transmitted via distinct vagal afferent fibers takes place at the level of

the NTS; or if convergence of signals occurs as well at the level of the vagus.

Chapter 3 describes experiments that strongly suggest that vagal A-fibers are involved in the integration of CCK and gastric distension; and data of **chapter 4** poses the hypothesis that capsaicin-sensitive afferents may also play a role in the integration of digestive functions and MIT. This does not necessarily mean that the vagus is the only site for convergence of these types of GI signals. Integration of GI signals occurs as well in the hindbrain at the level of the NTS and AP.

Convergence of e.g. CCK and gastric distension at the level of the hindbrain has been suggested in a number of studies (6, 117). It has indeed been demonstrated that convergence of afferent fibers occurs at the level of the hindbrain (7, 8). In support of this are results that show that CCK stimulates capsaicin-sensitive C-fibers, but gastric distension predominantly stimulates capsaicin-insensitive A-fibers (e.g. (13, 116, 117), **Chapter 3**). In addition, CCK receptors are numerous in the hindbrain and it has been reported that during a meal CCK is also produced at central levels (119), suggesting also that convergence at the level of the DVC occurs. In **Chapter 3** we found strong indications that CCK-A receptors are also present at capsaicin-insensitive A-type fibers. Moreover, stimulation of these receptors in combination with gastric distension enhances distension-induced Fos in the NTS. Therefore, there are strong indications that the integration of CCK and gastric distension occurs both at peripheral and central level.

Another example of central and peripheral interaction is the enhancement of NTS responses to gastric loads by leptin (124). Also, the synergistic action between leptin and CCK has potential peripheral as well as central integration sites. Recently, it has been demonstrated that leptin also can display its effects at vagal afferent neurons in culture (100, 101). Also, it has been demonstrated that some vagal afferent neurons that respond to leptin respond to CCK as well (35, 100). Together, these data suggest that the vagus is a potential site of integration for gastric leptin and gut CCK. In addition, central effects of leptin with CCK are also reported. Other experiments demonstrated that central leptin infusions sensitized peripheral action of CCK (28, 75-77).

Thus, there are strong indications that there are both peripheral as well as central integration sites for GI signals. Yet, more research is needed to actually demonstrate the interaction of different signals at the level of the vagus. Also, more specific studies to investigate which type of fibers are involved in these integration processes would be valuable and interesting subjects for future research.

2.4.2 The brain: a central site for integration?

2.4.2.1 Long-term versus short-term signals

The hypothalamus has been the feeding-center model since the 1950s as defined by Eliot Stellar (40) and is considered as an important center for the integration of long-term-signals as leptin and short-term signals –signals from the GI tract. However, the interesting experiments performed by Harvey Grill and colleagues (41) demonstrated that decerebrated rats show similar discriminative responses as intact animals to gustatory signals and feedback signals from the gut. In other words, the animals could eat ‘on their hindbrain’. It is also known that the DVC is reciprocally connected with numerous forebrain regions implicated in the central control of energy balance, including the paraventricular nucleus of the hypothalamus (PVN) (105, 115). Thus, not only convergence from peripheral sites occurs in the brain. Within the brain, there are complex networks that converge in nuclei from different parts of the brain. This is among others demonstrated by a study by Monnikes et al. (90), who reported that intraduodenal lipid infusion markedly increased Fos protein-like immunoreactivity in locus coeruleus complex (LCC), NTS, AP and paraventricular nucleus (PVN) of the hypothalamus. Moreover, perivagal capsaicin pretreatment reduced the increase of Fos in the LCC, NTS, AP and PVN. Other reports for evidence are 1. the anatomical studies that indicate convergence of the gastric vagal and cerebellar fastigial nuclear afferent inputs to single glycaemia-sensitive neurons of the lateral hypothalamic area (LHA) of the hypothalamus (143) and 2. the observation that medial hypothalamic hyperphagia and obesity syndrome is associated with damage to PVN projections to NTS/DMV complex (61).

In addition to this, leptin receptors –associated with long-term regulation of energy homeostasis– are localized in hypothalamic subnuclei but also in the DVC, parabrachial nucleus (PBN) as well as other brain stem areas as the LCC, lateral reticular, cochlear nuclei, inferior olive and hypoglossal (40, 42). The MC4 receptor –typically seen as a downstream target of leptin– is also localized in the hindbrain and has interaction with CCK (31). Thus, putting things together, we could say that long-term satiety signals as well as short-term satiety signals are activating both hindbrain structures as well as hypothalamic structures; thereby providing anatomical substrates for the integration of long- and short term signals related to food intake and energy homeostasis.

2.4.2.2 The role of the parabrachial nucleus in the control of feeding behavior

The PBN has gained prominence as a site of potential importance in the control of feeding behavior. In general, medial (gustatory) and lateral (visceral) subdivisions of the PBN have been implicated in a variety of ingestive behaviors. Signals related to feeding, including taste, gastric, duodenal, hepatic, and osmotic stimuli, have PBN representation (37, 47, 49). Also, there is convergence of vagal and gustatory afferent input within the parabrachial nucleus of the rat (50). Gastric projections to the PBN originate in the greater splanchnic and vagal afferents (142) and relay to the PBN through the caudal region of the nucleus of the solitary tract. Indeed, it has been demonstrated that gastric distension activated PBN neurons (5). Also, lesions of the lateral PBN abolished the suppressive effect of CCK (133). However, Rinaman (113) found that after lesioning noradrenergic neurons in the NTS CCK was not able to induce suppression of food intake. Yet, CCK-induced neural activation in the PBN and amygdala appeared normal; hypothalamic *c-Fos* expression after CCK injection was markedly attenuated in these lesioned rats. These results by Trifunovic and Rinaman raise some questions about the exact involvement of the PBN in the mediation of the CCK signal and also about the role of the PBN in the process of satiation.

Concluding, it is obvious that numerous brain areas are involved in the control of food intake and also in the regulation of energy homeostasis. Also, vagal afferents play an important role in the transmission of information from

the periphery to central sites and subsequently also for the communication between different brain areas. The decision to terminate feeding is dependent on a variety of motivational, metabolic, and hormonal factors. Therefore, this suggests that the interaction between different brain areas is necessary for an adequate behavioral response to challenges of homeostasis. As proposed by Grill and Kaplan (40), a distributionist model as opposed to hierarchical model probably describes better food intake control. Concerning the role of vagal afferents, results from this thesis (**Chapter 2, Chapter 3, Chapter 4**) indicate that vagal afferents and the DVC in the hindbrain are directly involved in short-term food intake control. Moreover, the results from **Chapter 2** do also suggest that reactions to long-term challenges to energy balance do not solely depend on vagal afferents. Apparently, compensatory metabolic, hormonal and neural routes are used to control food intake and regulate body weight increase.

3 CAPSAICIN-SENSITIVE AFFERENTS AND GLUCOSE HOMEOSTASIS

It was demonstrated that CAP treated rats show a reduced insulin response to an intravenous glucose tolerance test (IVGTT) compared to VEH controls. This difference was most pronounced when high concentrations of glucose (15%) were infused, but persistent during infusion with low concentration of glucose (5%). Therefore, it seems that CAP treated rats do not have a problem with the detection of glucose. Further analysis of blood parameters revealed that these differences may be related to modulations in adrenergic, adipocyte hormones and metabolic factors. Adiponectin levels were increased whereas resistin and FFA levels were decreased after infusion in CAP treated rats compared to their VEH controls. Also, corticosterone levels were suppressed in CAP rats during infusion. There were no differences observed in leptin levels and the counterregulatory hormone glucagon between CAP and VEH rats. Thus, our studies indicate that after capsaicin treatment, different hormones and metabolic factors related to glucose homeostasis are modulated.

3.1 Insulin response to an IVGTT

Studies with neonatal capsaicin treatment indicated that glucose tolerance is improved after ablation of primary afferents and an increased insulin response to an IVGTT or OGTT is associated with this phenomenon (44, 60). Gram (38) demonstrated that neonatal capsaicin treatment or treatment with the ultra-potent capsaicin analogue resiniferatoxin (RTX) in fatty Zucker rats prevented the development of diabetes 2, improved fasting blood glucose over 24 hours, as well as the oral glucose tolerance during an OGTT. In agreement with this, it had previously been reported that CAP rats show resistance to aging-associated insulin-resistance (87).

As in other studies, glucose-stimulated-insulin release was increased in CAP and RTX rats (38). Thus, from literature it appears that deletion of capsaicin-sensitive nerves increases glucose tolerance as well as insulin secretion in response to a single intra venous glucose injection. This suggests that these sensory nerves modulate efferent loops involved in insulin secretion and/or insulin sensitivity. Indeed, stimulation of sensory nerves in skeletal muscle *in vitro* causes the efferent release of CGRP (69). Also, it is known that CGRP inhibits insulin action in skeletal muscle (17). Our own results, presented in **Chapter 5** and **Chapter 6**, demonstrated that after capsaicin treatment, insulin response is reduced while glucose tolerance is comparable to VEH controls during a glucose infusion. The outcome of both studies suggests that insulin secretion is modulated by capsaicin sensitive nerves. This result in combination with reports of Koopmans et al. (63) and Zhou et al. (144) suggest that insulin sensitivity might be increased (see also discussion of **Chapter 5** and **Chapter 6**). However, there is some debate about the underlying mechanisms. Capsaicin-pretreatment of rats resulted in a reduced adrenaline release during insulin-induced hypoglycaemia for up to 30 min and, as a consequence, generated a greater fall in blood glucose (26). In addition, Koopmans et al. (63) also reported a decline in insulin antagonistic hormones as adrenaline, noradrenalin, glucagon and corticosterone. We found no effect of glucagon after capsaicin treatment, but corticosterone levels were decreased during glucose infusions. Trudeau and Milot (134) also found decreased catecholamine output during exercise till exhaustion. They did not find

differences in catecholamine levels after 60 minutes of swimming. Also, glucose levels did not differ between both groups. They did not measure insulin, but liver glycogen was much higher in CAP treated rats. We also did not find significant differences between the catecholamine responses between CAP and VEH rats during 20 min exercise. Glucose levels were comparable between CAP and VEH animals. In these studies, CAP rats also had significant lower insulin levels (data not shown).

3.2 Hormonal/metabolic factors and glucose homeostasis

Results of chapter 6 indicate that CAP rats have similar leptin plasma levels compared to VEH controls. However, leptin has stronger effects on food intake in CAP rats in comparison with VEH controls. This suggests that CAP animals are more sensitive to leptin which is often seen as a factor involved in the regulation of (long-term) energy homeostasis. This may also implicate that part of the effects on glucose homeostasis after capsaicin treatment are due to increased leptin sensitivity. Leptin has effects on and interactions with other hormones as adiponectin, resistin, corticosteron and lipid metabolism (see **Chapter 6**). However, the fatty Zucker rat has a leptin receptor deficiency and does subsequently not react to leptin and also shows similar effects after capsaicin treatment on glucose homeostasis. Yet, it has been reported that leptin retains some efficacy in the obese Zucker rat, even though these rats are less responsive than their lean controls (3). Therefore, increased leptin sensitivity cannot be ruled out.

Our results and that of others (58, 63) suggest that capsaicin-sensitive afferents may be involved in the regulation of HPA-axis activity. The suppression of corticosterone could display beneficial effects on glucose homeostasis. Higher levels of circulating glucocorticoids are associated with insulin resistance (4). Therefore, the reduced level of corticosterone could be involved in the enhanced glucose disposal during an IVGTT in CAP animals as well as display effects on other factors associated with insulin sensitivity. Furthermore, dysregulation of the hypothalamic-pituitary-adrenal axis is strongly associated with obesity as well as the development of diabetes (15). Thus, effects on glucose homeostasis after neonatal capsaicin treatment could

be either direct (by modulation of afferent signaling) or indirect (by increasing sensitivity to or 'dysregulation' of other factors).

Results in **Chapter 6** indicated that CAP rats have decreased levels of FFA after infusion. This could point in the direction of increased oxidation of FFA and result in accelerated FFA clearance from the blood. This decrease in FFA after infusion might also be involved in the effects on glucose homeostasis after capsaicin treatment, since higher levels of FFA are usually associated with disturbances in glucose homeostatic mechanisms (96). Koopmans et al. (63) demonstrated that glucose and fat metabolism are modified after capsaicin treatment. Muscle glycogen synthesis and whole body de novo lipogenesis was increased in CAP animals and hepatic glucose production was decreased in animals during a moderate euglycaemic insulin clamp. It is thought that decreased skeletal muscle glucose disposal and increased endogenous glucose production contribute to postprandial hyperglycaemia in type 2 diabetes. Specifically, increased hepatic glucose production is often associated with type 2 diabetes (9).

These observations raise the hypothesis that the vagus nerve may be more involved in the measurement of energy fluxes than absolute energy content per se. These energy fluxes may have profound effects on glucose homeostatic reflex mechanisms. Changes in adipocyte hormones as leptin, resistin and adiponectin, metabolic factors and corticosterone after neonatal capsaicin treatment have also effects on metabolism. Leptin has also effects on these glucose fluxes partly by modulation of the melanocortin pathway in the hypothalamus. Leptin stimulates gluconeogenesis through the melanocortin pathway; while an inhibition of glucose production appears to occur via a melanocortin independent pathway (45). Our results of **Chapter 6** do suggest that it is unlikely that the increased leptin sensitivity in CAP rats occurs through the melanocortin system. Therefore, it could be hypothesized that in CAP rats there is a stronger inhibition of glucose production because of an increased leptin sensitivity which could contribute to the observed effects on glucose homeostasis.

3.3 Concluding remarks on capsaicin-sensitive nerves and glucose homeostasis

All together, these observations suggest that animals have an increased insulin sensitivity after neonatal capsaicin treatment. This may be tissue specific, since local capsaicin-induced desensitization of the anterior hepatic plexus in rabbit results in decreased insulin sensitivity (103, 104). Also, we can conclude that sensory nerves are involved in the modulation of the efferent loop of glucose homeostasis; this effect could occur either via a local reflex loop or via a longer loop including the brain. These modulations appear to depend on the way in which glucose homeostasis is challenged. For example, an insulin induced hypoglycaemia gives other effects on catecholamine output compared to swimming (see before). Indications for the efferent function of capsaicin-sensitive sensory nerve fibers are the recent reports of Porszasz et al. (103, 104) who demonstrated that hepatic sensory nerves are involved in the secretion of a hormone-like substance from the liver termed hepatic insulin sensitizing substance (HISS). Also, the results of Ahren (2) suggest that GLP-1-induced insulin secretion at a low dose in mice is dependent on intact sensory nerves. These factors probably also contribute to the effects of capsaicin treatment on insulin secretion.

Finally, it is pointed out that it is maybe more likely that sensory nerves are involved in the regulation of insulin sensitivity rather than the prevention of insulin resistance. Also, insulin sensitivity should be investigated at the level of the receptor in CAP animals; since physiological techniques to investigate insulin sensitivity as insulin induced hypoglycaemia and euglycaemic insulin clamp studies investigate the outcome between glucose uptake and glucose production. Long-term signals can be modified after capsaicin treatment involved in glucose homeostasis as leptin, adiponectin and others (**Chapter 6**). Therefore, increased insulin sensitivity can occur at multiple levels and the exact and subtle function of sensory nerves at these levels needs still to be determined.

4 ENERGY HOMEOSTASIS

4.1 Vagal afferents and energy homeostasis

One of the questions that rise is if the development of obesity has an important neural component. Certainly, hormonal, metabolic and neural factors influence each other. Therefore, it is hard sometimes to distinguish between them and is it difficult, and even impossible in some cases to point a causal factor. For example, intestinal adaptation –and thus also of intestinal afferents- occurs in response to thyroid hormone, insulin, and corticosterone as well as to obesity, pregnancy, and illness, which all may have important effects on eating behavior and energy homeostasis in these situations. Also, there is a strong correlation between ambient temperature and food intake as well as body temperature and food intake (23).

It has been demonstrated that stimulation of vagal afferents reduces food intake and body weight by increasing vagal afferent signals (67). When this stimulation is combined with systemic capsaicin treatment the effects on food intake and body weight are even enhanced (66). These results suggest that information transmitted via vagal afferents can be modulated resulting in changes in feeding behavior and body weight and may therefore be a potential therapeutic intervention in obese people. The studies in this thesis demonstrate that capsaicin-sensitive afferents are involved in food intake control. We found that vagal afferents are: involved in short-term food intake; is a location for the integration of peripheral signals; involved in MIT; and has a profound effect on insulin secretion and glucose disposal from the blood circulation. We also found that CAP rats have increased leptin sensitivity and that this probably not occurs through the melanocortin pathway. Thus, this modification in CAP rats has to be more upstream, maybe at the level of the production of POMC. These results suggest that the lack of a substantial part of afferent information is compensated by increased sensitivity of (a) signal(s) related to long-term regulation of body fat and fuel homeostasis.

The group of Himms-Hagen did also find effects of neonatal capsaicin treatment on energy homeostasis. It was reported that neonatal CAP animals showed resistance to the development of age-related obesity as well as age-

related decrease in insulin sensitivity (87). Also, CAP animals had a long-term decrease in body fat and in brown adipose tissue (BAT) (18, 19). Atrophy of BAT is characteristic of many different kinds of obesity in laboratory rodents and is usually thought to contribute to the high metabolic efficiency, particularly with lack of diet induced thermogenesis in BAT (51). This reduction of BAT in CAP rats was also associated with a reduction of uncoupling proteins in BAT. These observations suggested that most of the mitochondria of BAT in CAP rats have been lost (20). Cui et al. (20) also report that feeding a palatable diet had a delayed thermogenic effect in CAP treated rats. Results from this thesis do not indicate such thermogenic effects on MIT when temperature is measured in the abdominal cavity. However, this may not be representative for BAT thermogenesis. We did not find differences in fat pads of white adipose tissue between both groups (data not shown); although we did not measure interscapular BAT. Thus, there seems to be some beneficial effects on energy homeostasis after systemic capsaicin treatment; yet, it is unclear what consequences the reported reduction in BAT in CAP rats exactly has on energy homeostasis

We did observe differences in short-term satiety, temperature regulation, and glucose homeostasis between CAP and VEH rats. Thus, it appears that even though CAP animals are able to maintain body weight vagal afferents are involved in the fine-tuning of responses to physiological challenges. Possibly, the integrative function of the vagus at peripheral level plays also a role in triggering adequate responses to threats of homeostasis. This does not implicate that vagal afferents are not involved in the development of obesity or that in intact animals vagal afferents are not important to maintain energy homeostasis. Especially, since these animals were denervated at neonatal age, rats could develop alternative mechanisms to deal with their lack of information, which is also suggested by results in **Chapter 6**. Also, it may very well be that under critical and/or stressful circumstances, these animals are not able to respond adequately. This was previously demonstrated by putting CAP animals in either a cold or warm environment. The CAP animals showed impairment of thermoregulation at both high and low environmental temperatures (11, 128-130). In other words,

it could be that impaired sensory signaling creates problems for the subject to defend its current homeostasis in challenging situations. Therefore, obesity could be an adaptation to these physiological, psychological and socio-economic challenges resulting in the creation of a new 'homeostasis' (or allostasis, see next paragraph). Disturbances in signal transduction in vagal afferent nerves could work as a catalyser for this development and contribute in this way to obesity with all its complications.

4.2 Allostasis and obesity- obesity: adaptation or pathology?

Allostasis means literally: "maintaining stability (or homeostasis) through change". This concept was first introduced by Sterling and Eyer (Fisher S., Reason J. (eds): Handbook of Life Stress, Cognition and Health. J. Wiley Ltd. 1988, p. 631) (81) and has recently gained more attention in biobehavioral as well as physiological research. While *homeostasis* refers predominantly to the internal state of the body, *allostasis* refers to the relationship between environmental challenge and biological responses of the intern milieu. Therefore, the concept allostasis is broader and more complete compared to the concept homeostasis to explain how a subject deals with a variety of challenges. Allostatic load refers to the cumulative negative effects (79); or in other words the price that the body pays for being forced to adapt to various psychosocial challenges and adverse environments. Mediators of allostasis are the neuroendocrine system, autonomic nervous system, and immune system in order to adapt to challenges of daily life (79). Among the many factors that contribute to allostatic load are genes and early development, as well as learned behaviors reflecting life style choices of diet, exercise, smoking and drinking. All of these factors influence the reactivity of the systems that produce the physiological stress mediators.

In general, there are both short-term adaptive actions (allostasis) that are protective and long-term effects that can be damaging (allostatic load). In its extreme, allostatic load can involve positive feedback responses (12). The cascade in neurohormonal responses to stress is an example of this (80). Also, allostasis is described in cases of withdrawal symptoms of systems that have changed over time, but otherwise used homeostatic negative feedback

mechanisms regulate (12). For example, an individual in a 'normal and healthy' environment (no excess of food, stable life, no chronic stress) is able to maintain a relative constant body weight over time. However, the excess of food availability in combination with lack of exercise and stress induces short-term adaptations in feeding behavior. Yet, on the long term, these changes in food intake and metabolism also have their reflections in changes in physiological and neural systems. An adaptation to a positive energy balance is the accumulation of body fat and, in extreme cases, this can lead to obesity. In the case of obesity allostatic load refers to the "cost" of adaptation; and the cost of obesity would be the obesity-related complications and a failure in internal homeostasis, which could result in e.g. diabetes 2.

Also, often, the lifestyle where high-fat and fast food is consumed in large amounts is a way to cope with the environment- also called the not well defined *stress-eating*. Hence, change in nutrition as avoiding high-fat diets could be experienced as sort of 'withdrawal, which is caused by homeostatic feedback mechanism. This reinforces the cascade for the development of obesity. Of course, damage in or malfunctioning of mediators of allostasis as in neuroendocrine systems and autonomic nervous system e.g. impairment in vagal afferent signaling could be a factor in this allostatic load which leads to maladaptation to the situation.

Concluding, we could say that obesity is an allostatic load and therefore an adaptation to cope with the environment. However, on the long-term, obesity is damaging for an individual and can lead to pathological complications as is e.g. type 2 diabetes .

III CONCLUSION

In conclusion, we could say from the presented studies that capsaicin-sensitive afferents are not crucial to maintain energy homeostasis in predictable, stable environments. It appears that capsaicin-treated rats compensate their lack in afferent neural signaling by increased sensitivity of other factors as leptin which is related to long-term regulation of energy homeostasis. Yet, there are

profound effects on mechanisms involved in short-term food intake, meal induced thermogenesis and glucose homeostasis. These effects suggest a role of the vagus in the fine-tuning of responses to physiological challenges. Possibly, the vagus has an integrative function at the peripheral level necessary to trigger adequate responses to threats of homeostasis. Therefore, we speculate that in critical and threatening situations animals will have problems to respond adequately to their environment and maintain homeostasis thereby increasing their allostatic load and catalyze the development of obesity.

Figure 3 summarizes the results and ideas from this thesis.

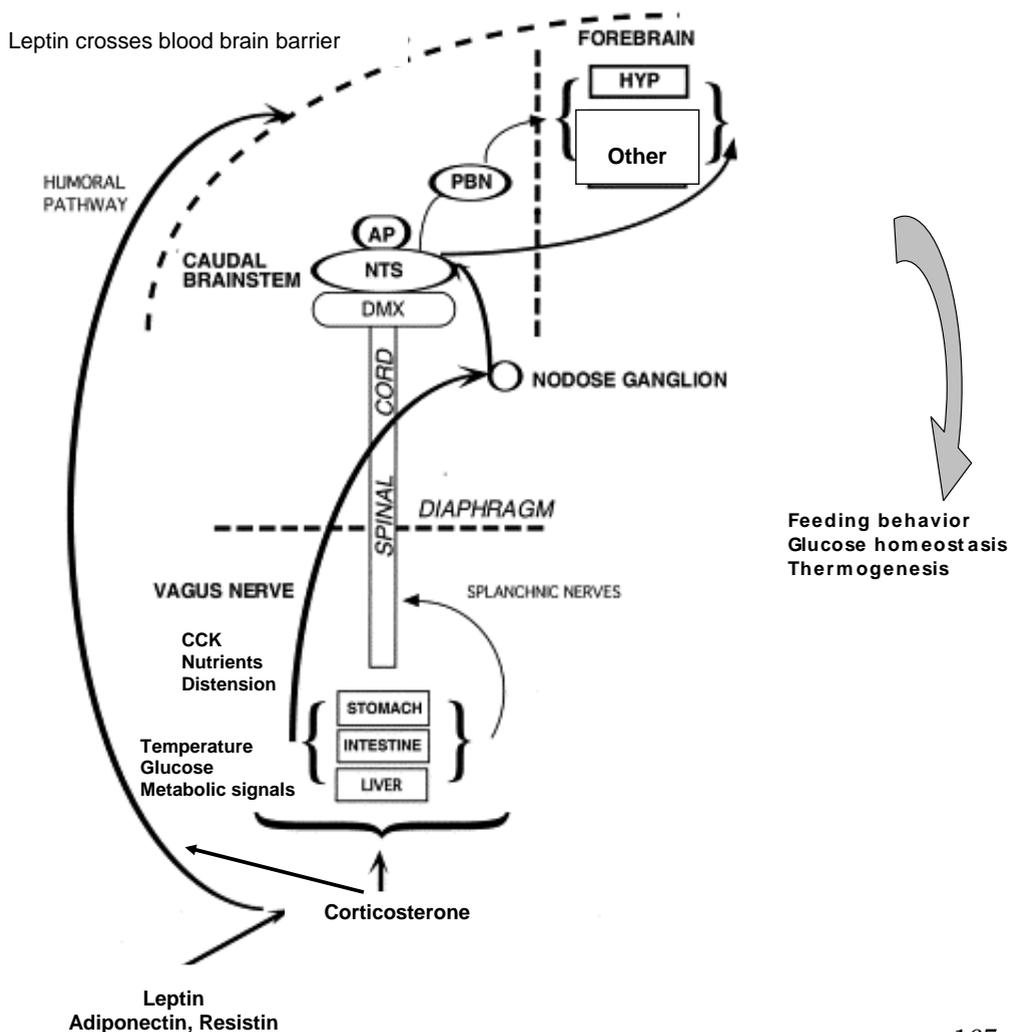


Figure 3

Schematic representation of peripheral and central factors involved in the regulation of energy homeostasis. Vagal afferent fibers have a sensory function and are involved in the transmission of peripheral signals of the GI tract and the liver. These signals include gastric distension; neuroendocrine signals as e.g. CCK; nutrient signals; temperature; metabolic signals; and glucose. A majority of of this information is transmitted via capsaicin-sensitive C-fibers; yet, also capsaicin-insensitive A-type fibers are involved. Vagal afferents project to the DVC of the hindbrain. Integration of peripheral signals occurs at the level of the vagus, but also at the level of the DVC. Long-term adiposity signals as e.g. leptin have profound effects on energy homeostasis. Moreover, leptin modulates other factors involved in food intake and metabolism as the sensitivity of CCK as well as the production of e.g. adiponectin. Peripheral information about energy homeostasis leads to adaptations of feeding behavior, glucose homeostasis and thermogenesis to maintain homeostasis.

Abbreviations: CCK: cholecystokinin; DVC: dorsal vagal complex; PBN: parabrachial nucleus; HYP: hypothalamus; Adapted from (121).

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“...To make and end is to make a beginning....”

Little Gidding (T. S. Eliot from “Four Quartets”)