Capsaicin-sensitive nerves and energy homeostasis

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CHAPTER 4

INVOLVEMENT OF CAPSAICIN-SENSITIVE AFFERENTS IN MEAL INDUCED THERMOGENESIS

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Submitted to Physiology and Behavior

ABSTRACT

It is known that vagal afferents are involved in thermoregulation. There is also some evidence that vagal fibers are involved in meal induced thermogenesis (MIT). Yet, the exact involvement of capsaicin-sensitive afferents in the regulation of MIT is still unknown. Therefore, in the present study we investigated MIT during sucrose intake in neonatal capsaicin-treated (CAP) and vehicle-treated (VEH) rats. To this end, CAP rats and VEH rats were offered different concentrations of sucrose during different trials. Temperature was measured by use of a telemetry device in the abdominal cavity. Results indicated that CAP rats have more variation in the MIT response during sucrose intake than CAP rats. However, capsaicin-treated rats are still able to control maximum temperature (Tmax) during MIT, indicating that temperature is also sensed in other areas, most likely in the central nervous system. Since the satiety gut hormone cholecystokinin (CCK) has profound effects on vagal afferent activity and food intake, we were interested to know the effect of CCK on MIT in capsaicin-treated animals. CCK completely suppressed MIT in intact rats. This effect of CCK was completely abolished in CAP animals. This demonstrates that capsaicin-sensitive afferents are required for this effect. CCK also suppressed sucrose ingestion. Thus, the inhibition of food intake by CCK is not induced by high core temperature. Overall, we concluded that CAP animals have disturbances in meal induced thermogenesis and hence capsaicin-sensitive nerves are involved in homeostatic control of thermogenesis during food intake.

1 INTRODUCTION

It is known that many areas – centrally as well as in the periphery- are involved in temperature sensing. In the central nervous system, not only the hypothalamus is important for thermoregulation, but also the spinal cord, medulla (6), pons and midbrain (2), and brain stem areas (3). Also, it has been demonstrated that vagal nerves play a role in thermoregulation (4, 13, 17-19, 22, 23). Anatomical evidence for the involvement of vagal afferents temperature sensing comes from El Ouazzani and Mei (10), who found

evidence for the presence of thermoreceptors on the vagus innervating the lower esophagus and the stomach. In addition, results of Adachi and Niijima indicate that hepatic vagal afferents are also thermosensitive (1). Capsaicin is often used as a tool to destroy primary C-afferents and small myelinated Aδ-afferents (11). This pungent compound binds to vanilloid 1 receptors (VR1) expressed on dorsal root ganglion primary afferents and nodosal-vagal primary afferents. The VR1 receptor has also been related to thermosensitivity (27) and indeed after capsaicin treatment, rats have disturbances in thermoregulation (4, 12, 15, 17, 19, 22, 23). Thus, the vagal thermosensor modality appears to be important for an adequate thermoregulatory response.

Temperature and food intake are closely correlated. During ingestion, temperature rises and this has been defined as meal induced thermogenesis (MIT). The thermostatic hypothesis for control of food intake was proposed by Brobeck (5) and initially defined as '*animals eat to keep warm and stop eating to prevent hyperthermia".* Indeed, de Vries et al. (7) found strong indications that liver temperature reached always a similar peak value just above 39 degrees ^{o}C at the end of a solid meal irrespective of meal size. Also, Di Bella et al. (9) demonstrated that artificial heating of the liver inhibits food intake. Moreover, this inhibition is lost when the liver was denervated (8).

Thus, it appears that liver temperature is a critical factor in the termination of food intake and that this inhibition is neurally mediated via the hepatic vagal branch. However, to our knowledge, there is no information about the effect on MIT when only the afferent nerves are destroyed. Therefore, in the present experiments we investigated the effect of systemic neonatal capsaicin treatment on MIT. Since the satiety gut hormone cholecystokinin (CCK) has profound effects on vagal afferent activity and food intake, we were interested in the effect of CCK on MIT in capsaicin-treated animals.

2 MATERIAL AND METHODS

2.1 Animals

Thirty male Wistar rats, bred in our own laboratory were used and housed in climate controlled rooms (22 °C ± 2) under a 12h:12h light-dark cycle (lights on at 8:00 am). These rats were previously treated with capsaicin or vehicle capsaicin at the age of 2 days (see below). Food and water were ad libitum available, unless mentioned otherwise. Capsaicin treated pups and vehicle treated pups grew up separately -to avoid selective mother care- in litters of 5-9 pups, in the proportion 5-7 male, 2 female (non-treated). After weaning at the age of 23 days, rats were individually housed in clear plexiglas cages (25x25x30 cm).

2.2 Capsaicin treatment

Capsaicin (8-methyl-N-vallinyl 6 nonenamide, 50 mg/kg; Sigma Chemical, The Netherlands) was given neonatally when pups were 2 days old (n=15) by a subcutaneous injection. This was done under 100% O_2 conditions to avoid hypoxia. Capsaicin was dissolved in a vehicle consisting of 10% ethanol (10%) and 5% cremophore-0.9% sodium chloride solution (90%). As a control, vehicle solution was injected s.c. (n=15). Each animal was given the same volume of 50 µl based on an average weight of the pups of 8 grams. After treatment, body weight was measured weekly. At the age of 15 weeks efficacy of the capsaicin treatment was assessed by testing the corneal chemosensory response (20, 21). This test involved application of one drop of 0.1% capsaicin solution into the left eye. One vehicle-treated animal was tested to see if the solution was effective to trigger a behavioral response. The vehicle-treated animal immediately exhibited a strong reaction (> 10 wipes) of the affected eye, while capsaicin-treated animals did not exhibit any response to the application of 0.1% capsaicin solution. Absence of the corneal chemosensory response indicated that the small unmyelinated afferent fibers of the trigeminal nerve, and presumably all other small unmyelinated primary afferents, were successfully destroyed by capsaicin.

2.3 Surgery

Rats were provided with a telemetry device (Dataquest) in the abdominal cavity to allow continuous temperature registration. Rats were anesthetized with isoflurane in combination with oxygen inhalation. After surgery, rats received 0.1 ml/kg finadyne as a post surgical analgesia. Thereafter, rats were allowed to recover for at least 10 days before experiments started.

2.4 Experimental procedure

2.4.1 Experiment 1: MIT during short-term sucrose intake test

Rats received different concentrations of sucrose in the following order: 10%-15%-20%-40%. Training included exposure to 10% sucrose and rats were allowed to lick for 10 seconds. In this way, rats became familiar with the taste and the experimental set up, but did not get the experience of satiation. After the sucrose series, rats were again exposed to 10% sucrose to investigate the effect on meal induced thermogenesis (MIT) after all the trial experiences. Each concentration was offered 3 times and the interval between each trial was at least 4 days. During sucrose ingestion, body temperature was measured once every 5 minutes in CAP rats (n = 7) and VEH controls (n = 8) to determine MIT.

2.4.2 Experiment 2: Effect of CCK on MIT

Rats were trained to drink 20% sucrose water (see *experiment 1* for training procedure). Five minutes before sucrose water was introduced, animals (CAP; n = 8; VEH; n = 7) received an intra peritoneal (IP) injection of saline or CCK (Sigma, The Netherlands) in a dosage of 3 or 6 µg/kg. The experiment was performed in a cross-over design; thus, each animal received each treatment. During the sucrose ingestion, temperature was measured every 5 minutes to follow MIT. All experiments were performed during the light period (start at ZT 2).

2.5 Statistical analysis

The MIT is defined from point zero (start of the meal) till 60 minutes after the introduction of the meal. Area under the curve analysis was done with delta MIT responses, not with the absolute values. Data were analyzed with an

ANOVA repeated measurements with time and body temperature as within subject factors and group as between subject factor. Post-hoc pairwise comparisons (Least Significant Difference-test) were done based on estimated marginal means. Differences were considered significant if $p \leq 0.05$.

3 RESULTS

3.1 Meal Induced Thermogenesis (MIT) during short-term sucrose tests

Figure 1 presents the average MIT of 1 trial set –this is the average of 3 single trials- of the first and last trial set of 10% (A+E), 15% (B), 20% (C) and 40% (D) trials. The average food intake of CAP and VEH per trial set is also presented in the graph. In all cases, CAP rats consumed significantly more compared to VEH rats ($F_{1,13} = 63.17$, $p < 0.001$). ANOVA repeated measurements indicates that there is a significant effect of time on body temperature ($F_{18,684\text{-}810} = 11.65\text{-}53.59$; $p < 0.001$). Also, there is a significant time × group interaction ($F_{18,684\text{-}810} = 1.75\text{-}9.42$; $p < 0.05\text{-}0.001$). There is no significant difference between CAP and VEH animals ($F_{1,38\text{-}43} = 0.01\text{-}2.7$; NS). However, overall CAP animals have a wider fluctuation in MIT compared to VEH controls. CAP rats start from a lower basal body temperature ($p < 0.01 – 0.05$) and CAP animals reach a higher maximum body temperature (Tmax) during MIT compared to VEH controls during the 10% sucrose trials ($p < 0.05$; see also fig. 1A + 1E).

Figure 2 represents the average MIT of each single trial expressed as area under the curve (A) and Tmax during MIT (B) in the different sucrose trials. ANOVA repeated measurements reveals that there is a significant effect of time on MIT ($F_{14,182} = 2.60$, $p < 0.01$). In VEH animals, MIT is constant over time, but MIT is significantly lower in the last 2 trials ($p < 0.05$). In CAP animals, there is a large variation between the different trials ($p < 0.05$). Yet, there is no significant time × group interaction ($F_{14,182} = 1.12$, NS). Also, between group effects do not reach significance ($F_{1,13} = 3.64$, $p = 0.08$). However, there is a trend and there are significant differences between CAP and VEH rats in MIT the third trial of the 10% (first and last set) and 15% sucrose tests ($p < 0.05$). Tmax does not change significantly over time ($F_{14,182} = 1.42$, NS). Yet, in VEH animals, Tmax is constant over time, but decreases in the last two trials of 10% compared to the 40% trials ($p < 0.05$). In CAP animals, there are

no significant differences over time, but the variation within 1 trial set gets smaller over time. Also, there is no significant time × group interactions over time ($F_{14,182}$ = 1.16, NS); but there is a significant difference between CAP and VEH animals ($F_{1,13}$ = 4.43, p = 0.05).

In general, Tmax of CAP animals is higher compared to VEH controls ($p<0.05$). Figure 2 (C) presents the variance of Tmax within animals during the different trials and the variance between different animals. Results show that CAP rats have significant higher variation than VEH controls in Tmax during the MIT of different sucrose trials when the variance is considered within 1 animal ($p < 0.05$). In contrast, within variance and between variance of Tmax does not differ in CAP rats. Also, the variance in Tmax in one CAP animal is significantly higher compared to VEH controls ($p < 0.05$).

3.2 Effect of CCK on MIT

The IP administration of the gut hormone CCK suppresses MIT in VEH rats ($F_{1,13}$ = 3.75, p = 0.05) in a dose-dependent way (fig 3A). This suppression is not the seen in CAP animals ($F_{1,19}$ = 0.009, p = 0.99) (fig 3B) . In figure 3 C sucrose ingestion is presented. CCK significantly decreases food intake compared to saline IP injections in VEH controls ($p < 0.05$) but is ineffective in CAP rats.

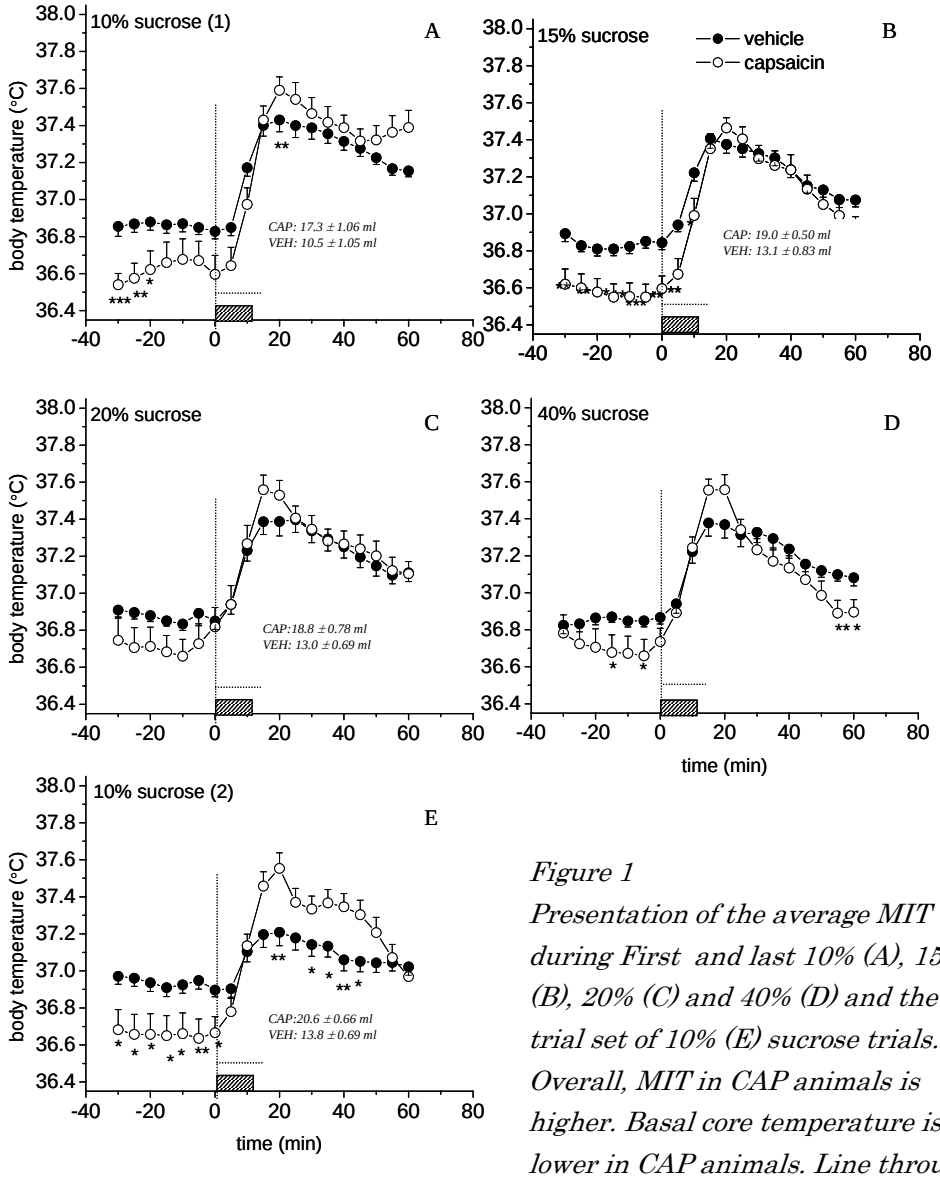
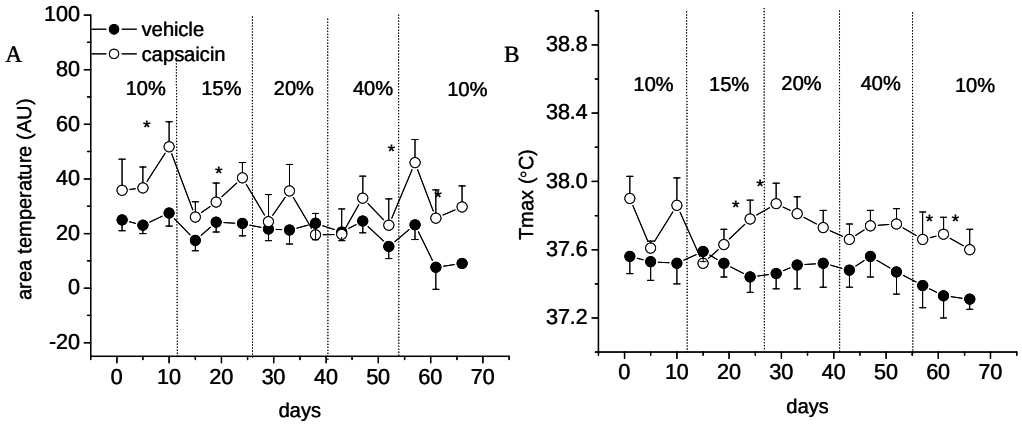


Figure 1
*Presentation of the average MIT during First and last 10% (A), 15% (B), 20% (C) and 40% (D) and the last trial set of 10% (E) sucrose trials. Overall, MIT in CAP animals is higher. Basal core temperature is lower in CAP animals. Line through zero represents the start of the meal meal. Bar and dashed line represent average meal duration of VEH and CAP rats resp. Experiments were performed at ZT 2. *, **, ***; p < 0.05, 0.01, 0.001 resp.*



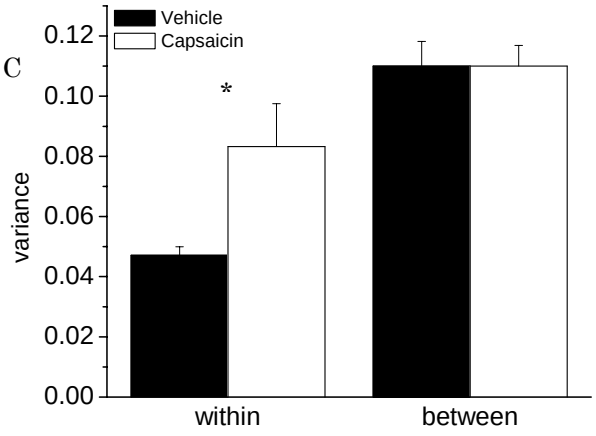
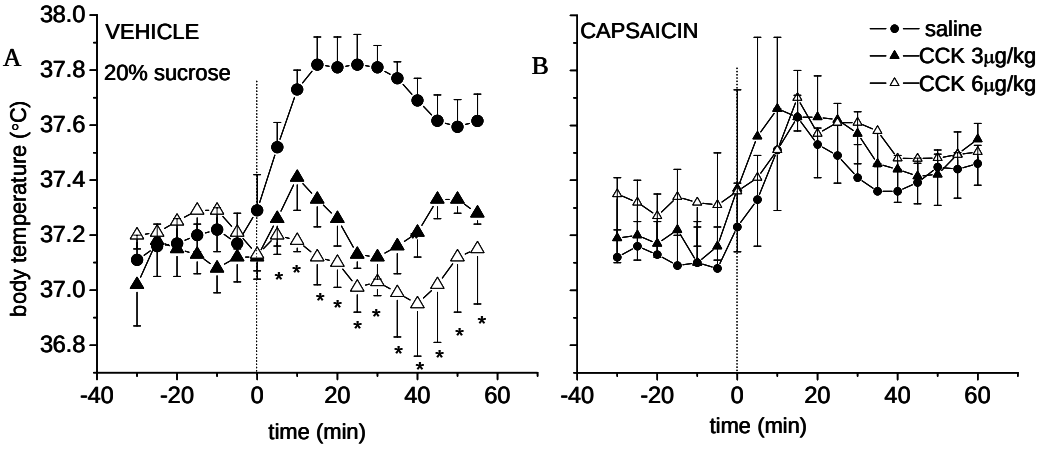


Figure 2
A) Average MIT during the Different sucrose trials is shown and expressed as area under the curve (0-60 min). VEH rats are relatively constant over time while CAP rats show a large variation between the different trials.
*B) T max during the MIT in different sucrose trials. VEH rats are relatively constant over time, but large variation in Tmax in the first trials. Yet, as experience increases; the variation in Tmax between different sucrose trials decreases. C) Shows the average within variance and between variance of Tmax in VEH rats and CAP rats. The within variance is significantly higher in CAP rats compared to VEH rats. The between variance does not vary between both groups; *, significantly different from VEH animals, $p < 0.05$.*



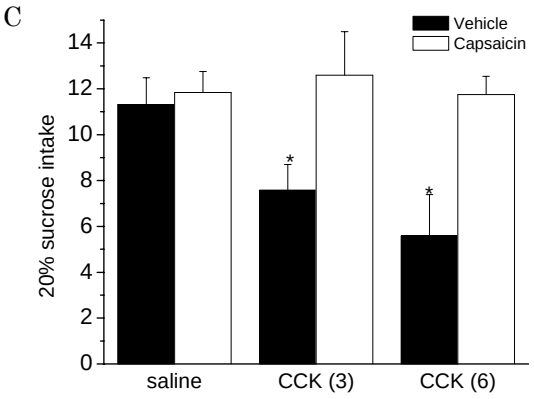


Figure 3
*MIT during 20% sucrose intake in VEH (A) and CAP (B) animals. CCK significantly suppresses MIT in VEH animals ($p < 0.05$), but not in CAP rats. CCK significantly suppresses sucrose intake (C) in VEH rats, but not in CAP rats. *; $p < 0.05$.*

4 DISCUSSION

Results showed that the temperature response to a sucrose meal does not differ significantly between CAP and VEH when the average response per trial set is considered (this is 3 trials of the same concentration). Yet, during the 10% trials, CAP rats reach a higher body temperature compared to VEH

controls. A possible explanation for this phenomenon is that CAP animals also have the highest volume intake during the 10% trials. Typically, the meal ended between 10 and 15 minutes; CAP rats ate longer because their meal intake was larger. Both CAP and VEH rats have their maximum temperature (Tmax) during MIT 20 minutes after the start of food intake. Thus, Tmax is reached after meal termination. This longer temperature rise has been previously reported by de Vries et al. (7), who measured liver temperature and skin temperature. They found that the rise of skin temperature followed the rise of liver temperature; also, skin temperature was longer elevated compared to the liver temperature. This indicates that there is a heat flux from the core of the body to the periphery.

In general, it is obvious that the MIT response shows a wider fluctuation in CAP rats compared to their VEH controls. This suggests that CAP animals have less homeostatic temperature regulation compared to VEH controls. CAP animals that start from a lower basal temperature have a larger temperature increase compared to VEH controls. A more detailed analysis of MIT revealed that total MIT expressed as area under the curve shows a very large variation between the different trials in CAP animals, while in VEH controls this is relatively constant over time. Also, the total MIT response tends to be higher in CAP animals. The reason for this may be that CAP rats do also drink more sucrose water and drink longer compared to their VEH controls. Therefore, it may be that CAP animals spend more energy to digest, absorb and subsequently dispose the ingested food. These data suggest that in intact animals, there is no clear relationship between the amount of sucrose intake and MIT response. Yet, it has to be mentioned that 1) experiments are done during the light phase; and 2) the ingestion of sucrose water does probably not reflect the ingestion of a solid meal.

Overall, volume intake in VEH controls does not vary significantly between the trials. Animals do consume different sucrose concentrations during the various trial sets; therefore, animals ingest a different amount of calories during the trials. Yet, MIT is not correlated to these changes in calorie intake. Rather, it seems that in intact animals MIT during sucrose ingestion is remains constant. This indicates that during food intake the same amount of energy is spent to deal with the arrival of calories. Interestingly, MIT response

decreases during the last trials of 10% sucrose water in VEH controls. This suggests that intact animals learn to handle the lower sucrose concentration more efficiently after repeated exposure to different concentrations of sucrose water. Apparently, they learned to deal with their energy more efficiently after repeated metabolic experience. Therefore, we could say that capsaicin-sensitive vagal afferents may be important to influence and/or regulate MIT and probably also energy expenditure during a liquid sucrose meal. Also, the involvement of vagal hepatic fibers in the thermostatic control of food intake has been demonstrated before by di Bella et al. (8) and Adachi (1).

The thermostatic theory includes that temperature is involved in meal termination. De Vries et al. (7) nicely demonstrated that at the end of a meal liver temperature reaches the exact temperature point just above 39 ºC. This suggests that meal termination and Tmax are closely correlated. We did not measure liver temperature in our studies. However, the transmitters are located in the abdominal cavity and presumably also detect heat production of the liver. When the Tmax of the sucrose trials are considered, it is striking that VEH controls are remarkably constant over time. Only during the last trials of 10% sucrose ingestion, Tmax during MIT is lower compared to the other trials. This reflects also the reduced MIT response during these trials in VEH controls and is in line with the explanation that energy expenditure during sucrose intakes decreases after repeated exposure.

In contrast, CAP rats show a large variation in Tmax during MIT in the first trials. However, when experience increases, the variation in Tmax decreases and in the last 6 trials the Tmax during MIT is relatively constant. It is remarkable that CAP animals do never exceed the body temperature of 37.9 ºC. If core temperature reaches that point during a trial; subsequently, Tmax decreases in the next trial. This suggests that somehow, CAP animals are still able to sense critical body temperature and are therefore able to avoid hyperthermia. An interesting observation is that the variance of Tmax within animals in VEH controls is lower then the variance within animals in CAP rats. Moreover, the between variance and the within variance of Tmax does not differ in CAP rats. This result also suggests a less precise regulation of Tmax control in CAP animals compared to intact rats. Apparently, since peripheral afferent information is blocked by capsaicin treatment; the sensor modality for

Tmax occurs not primarily or not only in the periphery but in the brain as well, most likely in the hypothalamus. Thus, capsaicin-sensitive nerves appear to have a temperature sensor modality as suggested by the large variation in Tmax in the first trials. Indeed, the sensor modality for temperature of the vagus (8, 10) and the nodose ganglion (27) has been proposed before by different scientists. Yet, to our knowledge this is the first study that demonstrates that capsaicin-sensitive nerves are not crucial to control Tmax during MIT during sucrose consumption in the light phase. However, these afferents appear to be involved in the regulation of Tmax control. We may hypothesize that CAP rats will have serious problems to cope with changes in their environment which are challenging for internal temperature homeostasis.

Another suggestion from our results is that pathways controlling sucrose intake and pathways for the control of Tmax are dissociated in CAP rats. It may be that in intact animals the vagus has a role to integrate information about temperature and food intake. Likewise, by the lack of afferent information transmitted via capsaicin-sensitive afferents; adequate efferent responses to challenges of homeostasis may be disturbed as well. This could be a possible explanation for the large variation in total MIT. There are several reports that that capsaicin denervation leads to problems with integrative control of body temperature (15, 18). Also, CAP animals had impairment of thermoregulation at both high and low environmental temperatures (26). Thus, it seems that for the integration of the information about heat production and heat loss capsaicin-sensitive afferents are required. In agreement with this, CAP animals have a stronger hypothermia and recover slower from an 8-OHDPAT induced hypothermia (own observation, results not shown here). Therefore, we could say that the thermoregulation in CAP rats appears to be less efficient. However, although wider fluctuations occur CAP rats are still able to regulate their core temperature.

In the second experiment, CCK was injected (IP) and food intake and MIT during 20% sucrose ingestion were measured. CCK decreased sucrose intake in VEH. This effect was completely abolished in CAP rats. This is in agreement with previous studies (16, 25) and indicates that denervation with capsaicin has been successful. CCK suppressed MIT completely in intact rats, but not in CAP animals. Thus, results demonstrated that capsaicin-sensitive

nerves are required for this suppression. There is some evidence in the literature that CCK has lowering effects on body temperature, but this effect was abolished after capsaicin treatment (24). Yet, an earlier study done by Kapas et al. (14) reports that capsaicin-sensitive nerves are not required for CCK-induced hypothermia. One critical remark that may explain this difference is that in the last study the authors used very high doses of CCK while South et al. (24) used a dose that is comparable to ours. This is the first study that investigates the effect of CCK on MIT and we also demonstrated that CCK-induced suppression of MIT occurs via capsaicin-sensitive vagal afferent C-fibers. What we also can say from these findings is that CCK's suppressive effect on sucrose ingestion is not caused by high body temperature. It has to be mentioned though that this amount of CCK is supraphysiological and therefore not reflecting physiological processes.

Concluding we can say that the results of the current study suggest that capsaicin-sensitive fibers are involved in the regulation and/or damping of fluctuation of MIT. Capsaicin-sensitive nerves appear to be required for Tmax control during MIT response. Also, we found that CCK-induced suppression of MIT occurs via capsaicin-sensitive fibers. Yet, this hormone also induces satiety. Therefore, the inhibition of food intake by CCK is not induced by high body temperature. Thus, it appears that CAP treated animals have disturbances in temperature homeostasis and hence capsaicin-sensitive nerves are involved in homeostatic control of thermogenesis during food intake.

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