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Neonatal capsaicin causes compensatory adjustments to energy homeostasis in rats

E.H.E.M. van de Wall, P.Y. Wielinga, J.H. Strubbe, G. van Dijk *

Department of Animal Physiology, Unit Neuroendocrinology, University of Groningen, PO box 14, 9750 AA Haren, The Netherlands

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Abstract

Several mechanisms involved in ingestive behavior and neuroendocrine activity rely on vagal afferent neuronal signaling. Seemingly contradictory to this idea are observations that vagal afferent neuronal ablation by neonatal capsaicin (CAP) treatment has relatively small effects on glucose homeostasis and long-term regulation of energy balance. It may be proposed that humoral endocrine factors and/or their sensitivities compensate for the loss of vagal afferent information, particularly when subjects face disturbances in ambient fuel levels. Therefore, male adult rats neonatally treated with CAP or with the vehicle (VEH) underwent intravenous glucose tolerance tests (IVGTTs) during which blood fuel levels, and circulating adipose, pancreatic, and adrenal hormones were assessed. CAP rats displayed similar hyperglycemia as VEH rats, but with markedly reduced plasma insulin and corticosterone responses. These results indicate that CAP rats have increased insulin sensitivity during hyperglycemic episodes, and lower plasma levels of corticosterone in CAP rats relative to VEH rats could underlie this effect. After the IVGTT, CAP rats had increased plasma adiponectin and reduced plasma resistin levels, and these alterations in adipose hormones might be relevant for post-ingestive metabolic processes. In a second experiment, anorexigenic efficacies of cholecystokinin and leptin were assessed. While VEH rats, but not CAP rats, responded with reduced food intake to i.p. injected cholecystokinin, only CAP rats responded to i.v. infused leptin with a reduction in food intake. It is concluded that reduced HPA axis activity and/or increased leptin signaling could underlie compensations in fuel handling and energy balance following CAP treatment.

Keywords: IVGTT; leptin; adiponectin; corticosterone; FFA; melanocortin; CCK; vagal afferents

1. Introduction

Energy homeostasis is maintained by an array of biochemical and physiological mechanisms that help to ensure the constancy of the internal environment under varying nutritional conditions and energy demands. An important component of the underlying regulatory processes consists of peripheral information regarding the energetic status which is conveyed via vagal neuronal afferents to the CNS. In turn, these signals are relayed in CNS neuronal networks where they play a role in the sensation of hunger and satiety as well as in the regulation of neuroendocrine control of energy homeostasis [1].

With the advent of capsaicin (CAP)–a pungent ingredient of red peppers which selectively destroys primary C-afferents and small myelinated Aδ-afferents (for review see [2,3])–a pharmacological tool became available to study the effect of ablation of vagal primary afferents on regulation of energy balance. CAP-treated animals have disturbances in short-term satiety signaling and do not respond to cholecystokinin (CCK) with reduced food intake [4–6]. However, CAP-treated rats have similar daily food intake [7], similar or even lower body weight [8] and improved glucose homeostatic control [17] compared to controls. Furthermore, deafferentated animals have a long-term decrease in white adipose tissue mass [9] and are more resistant to ageing-associated obesity [10]. Finally, CAP treatment results in increased whole body insulin sensitivity [11] and a lower degree of ageing-associated insulin resistance [10]. These observations indicate that CAP-treated animals are able, or even have improved capability, to maintain body weight and energy homeostasis, despite the fact that they lack seemingly important information transmitted via vagal afferents to the CNS. To date, the underlying mechanisms are poorly understood.

* Corresponding author. Tel.: +31 50 3632116; fax: +31 50 3632331.
E-mail address: gertjan.van.dijk@rug.nl (G. van Dijk).

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Another class of peripheral factors highly relevant to the regulation of ingestive behavior, energy homeostasis, and body weight maintenance consists of endocrine/hormonal factors which are released into the blood stream and affect enzymatic/endocrine processes and metabolic fluxes in various peripheral organs and tissues [12]. In addition, most of these factors can enter the CNS where they alter the activity of neuronal circuitry involved in ingestive behavior, neuroendocrine outflow and metabolism [12]. One hypothesis pertinent to the observations that CAP-treated rats are able to maintain body and energy homeostasis might be that vagal afferent ablation is compensated by these redundant endocrine factors involved in the regulation of energy homeostasis and ingestive behavior. To investigate this hypothesis, the concentration of blood fuels (i.e., plasma glucose and free fatty acids) and circulating hormones involved in blood glucose regulation and ingestive behavior (i.e., insulin, leptin, adiponectin, resistin and corticosterone) were investigated in overnight fasted rats that were neonatally treated with CAP or with the vehicle (VEH). In addition, the changes in these blood parameters were assessed during and after an intravenous glucose tolerance test (IVGTT). In a second experiment, anorexigenic efficacies of CCK, leptin, and the synthetic melanocortin (MC) receptor-agonist, melanotan II [13], were assessed in CAP and VEH rats. The latter study was performed since the MC4 receptor is implicated in the leptin signaling cascade [14].

2. Materials and methods

2.1. Animals and housing

Twenty-eight male Wistar rats from the breeding facility of our university were used and housed in climate-controlled rooms (22±2 °C) under a 12-h:12-h light–dark cycle (lights on at 8:00 a.m.). Food and water were ad libitum available, unless mentioned otherwise. All experiments were checked and approved by the Local Ethics Committee of our university.

2.2. Capsaicin treatment

Rats were treated neonatally with CAP (8-methyl-*N*-vallinyl-6-nonenamide, 50 mg/kg; Sigma Chemical, The Netherlands) at the age of day 2 ($n=14$) by subcutaneous (s.c.) injection. This was done under 100% O_2 conditions to avoid hypoxia. CAP was dissolved in vehicle consisting of 10% ethanol (10%) and 5% cremophore–0.9% sodium chloride solution (90%). As a control, VEH solution was injected s.c. ($n=14$). Each animal was given the same volume of 50 μl based on an average weight of the pups of 8 g. At injection, both groups did not differ significantly in body weight (CAP 8.84±0.20 g; VEH 8.32±0.23 g). CAP-treated and VEH-treated pups grew up separately–to avoid selective mother care–in litters of 5–9 pups, in the proportion of 5–7 male on 2 females (untreated). After weaning at the age of 23 days, rats were individually housed in clear Plexiglas cages (25×25×30 cm) with a bedding of sawdust. Following treatments, body weights were assessed at days 34, 58, and thereafter, every 10 days until experiments. An eye-wipe response (0.1% capsaicin solution) was done at the age of 3 months in order to test the effectiveness of the CAP treatment. As opposed to the VEH controls, none of the neonatally CAP-treated animals responded to the test and all animals were therefore included in the experiment.

2.3. Surgery

After the eye-wipe test, 16 animals were implanted with double heart catheters in the left and right jugular veins according to techniques described by Steffens [15]. An additional 12 animals were provided with heart catheters only in the right jugular vein according to the same techniques. Surgery was performed under anaesthesia with isoflurane/N_2O/O_2. Fynadine (0.01 ml/100 g body weight) was given s.c. as post-surgical analgesia. Animals had at least 2 weeks of recovery before the start of experiments.

2.4. Intravenous glucose tolerance test (IVGTT)

Body weights did not differ significantly between both groups (CAP: 403±7.7; VEH: 410±10.9). Overnight food-deprived CAP ($n=8$) and VEH-treated rats were subjected to an IVGTT, which was performed in the light period between 12:00 a.m. and 1:00 p.m. At least half an hour before the start of the IVGTT, rats were connected with their indwelling cannulae to blood sampling (right jugular catheter) and infusion (left jugular catheter) tubing. These tubes extended out of the rats' cages, which allowed stress-free blood sampling and/or intravenous infusion. After taking two basal blood samples at $t=-11$ and $t=-1$ min, a glucose solution (15% dissolved in sterile demineralized water) was infused over a 30-min period at a rate of 15 mg/min (450 mg total). Additional samples were taken at $t=1$, 3, 5, 10, 15, 20, 25, 30, 40, 50 min in order to assess blood glucose and plasma insulin. In general, samples consisted of 0.2 ml whole blood for assessment of blood glucose (50 μl) and plasma insulin (50 μl) levels. At $t=-11$, $t=30$, and $t=50$, an additional 0.2 ml of blood was taken for determination of plasma levels of adiponectin (3 μl), leptin (30 μl), resistin (30 μl), corticosterone (10 μl), and free fatty acids (FFAs, 10 μl). Blood and plasma samples were stored at −20 °C until analysis. Blood glucose levels were measured by the ferricyanide method of Hoffman; plasma levels of insulin, adiponectin, leptin, resistin and corticosterone were measured by commercial radioimmunoassay kits (Linco Research, Nucli lab, The Netherlands), and plasma levels of FFAs were assessed with a NEFA C enzymatic kit (WAKO Chemicals GmbH, Germany).

2.5. Anorexigenic efficacies of CCK, leptin and melanotan-II

In another group of CAP- ($n=4$–6) and VEH-($n=4$–6)-treated rats, the anorexigenic efficacies of CCK, leptin, and the synthetic melanocortin 3/4 receptor agonist, melanotan-II were assessed. Therefore, rats' food hoppers were removed from their home cages 2 h before lights off. In a counterbalanced design, and with 5 days elapsing between successive experiments, rats were i.v. infused between 30 and 15 min before lights off solutions containing leptin (70 μg/250 μl saline, Calbiochem,