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Fasting hypometabolism and refeeding hyperphagia in rats: Effects of capsaicin desensitization of the abdominal vagus

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ABSTRACT

Capsaicin-sensitive abdominal vagal fibers contribute to postprandial satiety and hypermetabolism. We hypothesized that the hypometabolic adaptation to fasting involves similar mechanisms and that blockade of such signals might enhance loss of body weight upon fasting. A low dosage of capsaicin (5 mg/kg) administered intraperitoneally desensitizes the local afferent vagal nerve endings for approximately three weeks without causing systemic desensitization or damaging the efferent fibers. Following such desensitization, male Wistar rats deprived of food for 120 h lost significantly ($18.9 \pm 0.4\%$ *vs.* $15.8 \pm 1.0\%$), *i.e.* 20% more weight than the controls. Based on the present results, this can only be explained by the demonstrated defective hypometabolic adaptation in desensitized animals. Other mechanisms do not seem to make up for this defective function. Upon refeeding following a period of fasting, in the first 0.5–3 h the food intake was significantly greater in capsaicin pretreated compared to the control group, demonstrating blockade of satiety as a sign of desensitization. The delayed gastrointestinal passage supported that vagal afferent nerve endings were in a desensitized state in these rats. In conclusion, local desensitization of the abdominal capsaicin-sensitive fibers attenuates the hypometabolic adaptation to food deprivation and the lack of fasting-induced activation of these fibers cannot be substituted by other fasting-dependent mechanisms. It is suggested that reports of low body weight in mice lacking the transient receptor potential vanilloid-1 channel and in rats with systemic capsaicin desensitization might be explained by a lasting absence of similar (vagus-mediated) hypometabolic processes, preventing weight gain or obesity.

1. Introduction

]In nature, animals often survive long periods of fasting. Fasting induces adaptive hypometabolism/hypothermia (Pétervári et al., 2002) to attenuate the fall in energy stores and body weight and also alters the utilization of nutrients (Cherel and Le Maho, 1991; Bertile and Raclot, 2006). Regulatory peptides may participate in these changes, *e.g.* leptin/insulin levels decrease, expression of orexigenic neuropeptides in the hypothalamus, brainstem and vagus (Burdyga et al., 2006; Palou et al., 2009) increases, while that of the anorexigenic ones (Perello et al., 2007) decreases (Schwartz et al., 1995; Székely and Szelényi, 2005). One information route eliciting central regulatory changes is the afferent vagus (Bray, 2000; Williams et al., 2000), which conveys feeding/nutritional state-related signals primarily to the brainstem. Capsaicin-sensitive fibers of the abdominal vagus contribute to satiety (South and Ritter, 1988; Havel, 2001) and postprandial hypermetabolism (Pétervári et al., 2005). We hypothesized a principal role for such fibers also in the hypometabolic adaptation to fasting.

Administering small doses of capsaicin or resiniferatoxin intraperitoneally (i.p.) is used to cause transient (about 3 weeks duration) local desensitization of abdominal vagal afferent fibers without affecting the efferent ones (Székely and Romanovsky, 1997; Székely et al., 2000; Dogan et al., 2004; Steiner et al., 2007). Other approaches for studying the role of the vagus and capsaicin desensitization seem inappropriate. Abdominal vagotomy destroys both afferent and efferent fibers (Joyner et al., 1993; Schwartz et al., 1993), interferes with energy balance (Andrews et al., 1985; Romanovsky et al., 1997; Székely et al., 2000), and causes severe (often lethal) anorexia. In contrast, animals desensitized systemically by large doses of capsaicin (Raybould and Taché, 1988; South and Ritter, 1988) cannot easily increase heat dissipation and have a tendency for hyperthermia (Szolcsányi, 1982), abnormalities in thermoregulation (Székely and Romanovsky, 1997) and brown fat (Cui and Himms-Hagen, 1992). High body (De Vries et al., 1993; Himms-Hagen, 1995) or environmental temperatures (Harikai et al., 2003) have profound effects on food intake and metabolic rate, possibly interfering with any effect induced by the afferent vagus. Thus, caution is needed when assessing ingestive behavior in vagotomized or systemically desensitized animals. Local desensitization with capsaicin or resiniferatoxin inhibits the satiating effect of i.p. cholecystokinin without systemic desensitization: it inhibits afferent (not efferent) nerve functions, but it is devoid of direct thermoregulatory actions (Székely and Romanovsky, 1997; Dogan et al.,

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2004). Perineural (local) capsaicin treatment of the vagal trunk causes much greater stress to the animals, but its effects on postprandial changes are similar to those of i.p. desensitization (Pétervári et al., 2005). In our study we applied i.p. desensitization by small doses of capsaicin.

In the present experiments we analyzed whether such small-dose, transient i.p. capsaicin desensitization has any significant influence on i) the rate of fasting-induced weight loss (related to the adaptive hypometabolism), ii) the refeeding hyperphagia after fasting, iii) the spontaneous daily food intake and rate of weight gain and iv) the fasting-related changes in O_2 consumption (hypometabolism) and body temperature, which might explain differences in weight loss.

2. Materials and methods

2.1. Animals

Seven to nine week-old male Wistar rats weighing 160–180 g were brought from the animal house of the University of Pécs to an animal room of our department and kept at a room temperature of 22–25 °C, with lights on between 06.00 h and 18.00 h. The rats were placed individually in plastic cages, where standard laboratory chow and tap water were available *ad libitum*. A group of the animals was food deprived for 48 h, some other rats for 120 h, in order to study the characteristics of various phases of fasting [including the late P3-phase with pronounced hypometabolism (Cherel and Le Maho, 1991; Bertile and Raclot, 2006)] and to analyze the process of refeeding. In another group, rats were kept individually for two weeks prior to and two weeks following capsaicin (or vehicle) administration in 35×30×25 cm cages with a metal grid floor in which daily food intake and body weight were measured.

All experiments and interventions were undertaken according to the general rules and special approval of the University of Pécs Ethical Committee for the Protection of Animals in Research (BA 02/2000-13/2006), in accordance with the directives of the National Ethical Council for Animal Research and those of the European Communities Council (86/609/EEC).

2.2. Substance administration

In the capsaicin-treated group 5 mg/kg capsaicin (2 mg/kg at 09.00 h, and 3 mg/kg at 15.00 h) was administered i.p., as described in earlier studies (Székely and Romanovsky, 1997; Dogan et al., 2004). Capsaicin (Sigma) was dissolved in 96% alcohol. Immediately preceding injections, fresh dilutions were made from this stock with addition of a drop of Tween-80 plus 0.9% NaCl, to reach a final concentration of 2 mg/ml capsaicin in 5% alcohol. Control rats received complete vehicle without capsaicin, in similar volumes. A variety of tests (checking spontaneous daily food intake and weight gain rates, checking changes of body weight after fasting, measuring O_2 consumption and core temperature, observing food intake and body weight changes upon refeeding, testing gastric emptying and gastrointestinal passage, etc.) were performed 5 to 15 days after i.p. capsaicin pretreatment.

2.3. Assessment of food intake and body weight

Rats placed in cages with metal grid floor were used for measuring the 24 h spontaneous food intake and rate of spontaneous body weight gain. At 09.00 h the weight of chow in the food container was measured. The following day the consumed amount of food was calculated based on the weight of the remaining chow and spillage. Body weight was also measured. This procedure was repeated daily for two weeks prior to and for other two weeks following treatment with capsaicin or the vehicle.

In food deprivation studies, the rats remained in their plastic boxes. Fasting began at 09.00 h for a duration of 48 h, while water remained available *ad libitum*. At the end of these fasting periods the usual chow was returned (again at 09.00 h) and body weight changes were measured in 30 min intervals for a total of three hours. The 30 min fractional and the cumulative weight changes (expressed as % of initial body weight before refeeding) were analyzed, along with the cumulative food intake for the 3 h period. The cumulative food intake in the following 21 h period and body weight at 09.00 h on the following day were also measured. It has been demonstrated that the 3 h body weight changes correlate well with chow consumption during the early part of refeeding (Uzsoki et al., 2001), thus weight changes in this period were taken as indicators of the dynamics of food intake. Some rats fasted for 120 h and were refed similarly, with daily measurements of body weights.

2.4. Assessment of metabolic rate and body temperature during fasting

One control and one capsaicin-desensitized groups of freely moving rats (initial body weight 273±11 g) were placed singly into ventilated 30×25×20 cm Plexiglas metabolic chambers, with food and water available *ad libitum*. For better assessment of the expected hypometabolism, a constant, but slightly sub-thermoneutral (20 °C) chamber temperature was applied. For measuring O_2 consumption (representing metabolic rate) an Oxymax system (Columbus, OH) was used: from air perfusing the chamber the O_2 consumption (ml/kg/min), CO_2-production (ml/kg/min) and respiratory quotient (RQ) were measured in 10 min intervals for 3 days prior to fasting and during 5 days of food deprivation (except for two 10 min periods/day when the chamber was opened for cleaning in the morning and the evening). The results were collected on a computer and the data were averaged for 12 h periods. After the circadian rhythm was established, the food was removed for a 120 h period, but the measurements went on, including the first day of refeeding.

In other tests the circadian changes of core temperature (T_c) and spontaneous activity (ACT) of freely moving control and desensitized rats (initial body weight 284±12 g) were continuously recorded by biotelemetric methods (MiniMitter, Sunriver, OR) for 3 days prior to food deprivation (circadian rhythm of Ta established), for 5 days during fasting plus one more day of refeeding. The emitters were previously implanted under surgical anesthesia (Székely et al., 2005) into the peritoneal cavity. The signals were converted to °C and ACT in arbitrary units; the data were sampled every 5 min and they were averaged for 12 h (light vs. dark) periods by a computer system (VitalView software). In this system food and water were freely available, while the 20 °C ambient temperature and lights were similar to those in the other metabolic tests.

2.5. Postmortem investigations

At the end of the experiments (5–6 days after refeeding) the rats were euthanized by a narcotic (urethane) overdose at 09.00 h. Half of them had a 24 h food deprivation prior to this. Gastric weights and weights of gastric contents were measured both in control and in desensitized groups, in order to observe any differences of gastric emptying: similarly to vagotomy, afferent fiber damage can influence gastric emptying (Raybould and Taché, 1988; South and Ritter 1988). Any difference in gastric emptying may suggest vagal damage.

2.6. Statistics

ANOVA repeated measures or one-way ANOVA with Scheffe's *post hoc* tests was used for statistical analyses, and Student's *t*-test for the comparison of gastric contents. Differences were regarded statistically significant at the level of $P<0.05$ ($^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$). All data were expressed as mean values±SEM, and the number of animals is also indicated for each group.

3. Results

3.1. Spontaneous food intake and gain of body weight

The transient capsaicin-desensitized state, *per se*, did not result either in lasting enhancement or in lasting suppression of daily food