Lean (Fa/Fa) but not obese (fa/fa) Zucker rats release cholecystokinin at PVN after a gavaged meal

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De Fanti, Brant A., Robert C. Backus, Jock S. Hamilton, Dorothy W. Gietzen, and Barbara A. Horwitz. Lean (Fa/Fa) but not obese (fa/fa) Zucker rats release cholecystokinin at PVN after a gavaged meal. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E1–E5, 1998.—Neuropeptides play an important role in the integration of dietary signals. Cholecystokinin (CCK) has been implicated in regulating ingestive behavior, particularly satiety. The primary objective of this study was to examine whether the hyperphagia characteristic of obese (fa/fa) rats involves impaired neural CCK secretion. Dynamic release of CCK at the hypotalamic paraventricular nucleus (PVN) of age-matched lean (Fa/Fa) and obese Zucker rats was determined using push-pull perfusion. The gavage of a 10.3-kcal (6 ml) liquid diet during light cycles was followed by increased CCK release in lean rats (from 13.6 ± 1.1 to 22.1 ± 1.4 fmol in the 1st postprandial period and 18.4 ± 2.5 fmol in the 2nd postprandial period). An identical meal load resulted in no postprandial increase in CCK release in obese rats, despite the fact that high-K+ artificial cerebrospinal fluid evoked CCK outflow in all animals. Intubation of 6 ml of nonnutritive 1% carboxymethylcellulose had no effect. These results are consistent with the suggestion that hypothalamic CCK plays a physiological role in satiety, and they demonstrate that obese Zucker rats have blunted hypothalamic CCK release in response to dietary cues.

hypothesis; push-pull perfusion; feeding; obesity

REGULATION OF food intake and energy metabolism is based on homeostatic feedback mechanisms that allow the body to monitor and maintain energy balance. The hypothalamus plays a primary role in processing and integrating these nutrient-related feedback signals and coordinating the appropriate autonomic and behavioral responses (4). Obesity involves a failure in one or more of these homeostatic systems.

In investigating the development of obesity, the fatty Zucker rat, homozygous for the fa gene, has often been used as a model. The fa mutation is a point mutation that results in an alteration of one amino acid, causing decreased functional effectiveness of the leptin receptor (24). It appears that this disruption of the leptin receptor pathway prevents critical hypothalamic areas from receiving pertinent cues on energy status. If the hypothalamus does not receive, sense, or properly integrate the nutritional state of the animal, as in the fatty Zucker, a perceived negative energy balance and subsequent enhanced energetic efficiency ensue, resulting in obesity (6).

The regulation of feeding, nutrient storage, and metabolism involves a complex interaction of neurotransmitters and neuromodulators. Neuropeptides, acting as neuromodulators, play an important role in the integration of dietary signals (5). Meal-related cues providing information on the nutritional state of the animal result in altered peptidergic activity within the hypothalamus and a concomitant change in energy balance (27). This study evaluates the proposal that the altered responsiveness of obese Zucker rats to dietary cues that influence ingestive behavior and metabolism involves a failure in hypothalamic neuropeptide signaling. The focus of this study is an endogenous cholecystokinin (CCK) release at the paraventricular nucleus (PVN) and its relationship to feeding (e.g., role as a satiety signal).

CCK, named for its ability to promote gallbladder contraction, is an important peripheral feeding cue via its contributions to the regulation of appetite. Endogenous levels of CCK in both the periphery and the brain change with feeding status (10, 19). Food in the gastrointestinal tract causes the release of CCK from the duodenum, aiding in digestion and in the satisfaction of appetite (31). Endogenous CCK released from the duodenum in response to a meal results in stimulation of vagal afferents, release of CCK in the hypothalamus, increased energy expenditure, and satiety (11, 27, 36). Exogenous CCK injected peripherally has been shown to influence CCK levels in hypothalamic areas associated with ingestive behavior (18). Peripheral (ip) and intracerebral ventricular administration of exogenous CCK decreases ingestive behavior in a dose-dependent manner, whereas CCK antagonists and antibodies to CCK can increase meal size in lean but not obese Zucker rats (9, 14, 17, 33, 34).

Although the defective leptin signaling of the fatty Zucker rat has been associated with altered long-term dietary cues (notably elevated neuropeptide Y), the downstream consequences of this defect on short-term cues remain unclear (12). Recent studies observed a synergistic interaction between leptin and CCK in the regulation of food intake (2, 16). Thus, although the genetic defect of the obese Zucker rat is in the leptin pathway, the characteristic hyperphagia of the obese rat may also involve impaired sensitivity to short-term satiety factors, such as CCK. One possibility is that without effective leptin signaling, release of CCK from the PVN is blunted, thus contributing to the increased meal size of these mutants.

In the present study, we measured endogenous CCK release at the PVN in conscious, unrestrained rats by using push-pull perfusion to test the hypothesis that fa/fa rats would release less CCK at the PVN after a...
meal than would FA controls. Because the PVN forms the major interconnection between the autonomic, neuroendocrine, and limbic systems (15), this approach allows us to correlate feeding with neuropeptide activity and offers insight into the homeostatic control mechanisms regulating body nutrient status.

METHODS

Animals. Specific pathogen-free male homozygous lean (262.8 ± 5.9 g) and obese (341.7 ± 12.2 g) Zucker rats were obtained from the Clinical Nutrition Research Unit's Animal Models Core at the University of California at Davis. The 10-wk-old age-matched animals were individually housed in hanging wire cages at 25°C on a 12:12-h light-dark cycle (lights on at 0130) at the Food Intake Laboratory. Animals were provided with ad libitum water and balanced, semipurified powdered diet (% kcal/kg: 21 protein, 68 carbohydrate, 11% fat; g/kg: 210 vitamin-free casein (US Biochemicals, Cleveland, OH), 3 l-methionine, 10 vitamin premix (US Biochemicals), 50 salt mix (AIN 76; ICN Biomedical, Aurora, OH), 452 cornstarch, 224 sucrose, 50 corn oil, and 1 choline chloride).

Surgery. Rats were anesthetized with a ketamine-xylazine-acepromazine cocktail (30:6:1, 0.1 ml/100 g body wt) and placed in a stereotaxic apparatus. A 21-gauge stainless steel guide cannula (Plastics, One, Roanoke, VA) was aimed at the PVN of the hypothalamus according to the atlas of Paxinos and Watson (23). Coordinates for the lean rats were 1.6 mm posterior to bregma, 0.4 mm lateral to midline, and 7.8 mm ventral to skull surface; those for the obese rats were 1.4 mm, 0.4 mm, and 7.7 mm, respectively. Cannulas were kept in place with dental cement and three stainless steel screws anchored in the skull. A stainless steel stylet kept the guide cannula clear, and the animals were allowed a 1-wk recovery period after surgery. Recovery was assessed by the return of the animals to presurgery food intake and body weight.

Experimental protocol. On the day of the experiment, the stylet was replaced with a 28-gauge push cannula (Plastics, One). This push-pull assembly was connected to an Ismatech peristaltic pump via polyethylene tubing (PE-20). To prevent the tangling of the PE-20 as the animal moved around the chamber, a dual-channel liquid swivel (Instech Labs) was used. At 60 min after the animals had ad libitum access to water throughout the procedure, artificial cerebrospinal fluid (aCSF) contained (in mmol/l) 128 NaCl, 2.5 KCl, 1.4 CaCl2, 1.0 MgCl2 ·6H2O, and 1.2 Na2HPO4. This aCSF, with added 0.1% BSA and 0.03% bacitracin, was perfused at a rate of 10 µl/min. After a perfusion equilibration time of 1.5 h, during which no fractions were collected, the animals were perfused with an additional 4-h period. The first hour was in the light and was followed by 3 h of perfusion in the dark. During this 4-h period, perfusate samples were collected every 20 min, acidified with acetic acid (3% final volume), and placed in liquid nitrogen until storage at −70°C. In an attempt to mimic their natural rhythm and meal pattern (e.g., feeding primarily during the dark), we gave rats the meal after the lights went off. That is, after 3 baseline perfusion samples were collected, the rats were perfused with a high-K+ (100 mM) aCSF, with the isosmolarity of the solution being maintained by increasing the NaCl concentration. Three more 20-min samples of perfusate were collected. After the last collection, rats were killed and brains were removed and stored in Formalin fixative for histology. Before CCK analysis with RIA, all samples were lyophilized via vacuum centrifugation (SpeedVac Concentrator SVC200H; Savant Instruments, Farmingdale, NY).

Histology. To verify cannula placement, the fixed brains were sectioned on a cryostat and stained with cresyl violet. Only those brains in which the cannula was in the PVN (Fig. 1) were used for data analysis.

CCK radioimmunoassay. A modification of the method described by Backus et al. (1) was used with the antiserum DINO (provided by Dr. John Calam, Royal Postgraduate Medical School, London, UK). For each assay, 3 × 10^6 titer antiserum and 2,000 counts/min of Bolton-Hunter 125I-labeled sulfated cholecystokinin octapeptide (CCK-8; Amersham, Arlington Heights, IL) were added to polystyrene tubes containing dried perfusate and a pH 7.4 buffer of 0.05 mol/l NaPO4, 0.01 mol/l EDTA, 0.25% gelatin, and 0.02% NaN3. The 1-ml mixture was incubated for 3 days at 4°C. Free and bound radiolabel portions were separated by addition of 0.2 ml of charcoal (30 g/l, Norit P5; BDH Lab Supplies, Poole, UK) suspended in assay buffer containing dextran (1.5 g/l, average mol wt 87,000; Sigma). Relative to sulfated CCK-8, cross-reactivities of the RIA antiserum were 5, 120, 0.05, and 0.2% for nonsulfated CCK-8, CCK-33, gastrin-17, and sulfated gastrin-17, respectively. The dose giving 50% inhibition at maximum antiserum binding of 35% was 6 pM, and the previously determined intra-assay and interassay coefficient of variation values were 6.7 and 9.1%, respectively.

Statistical analysis. Comparison between lean and obese rats was accomplished using the Student’s t-test. Pre- and postprandial perfusate CCK levels were compared in lean rats using ANOVA. A value of P ≤ 0.05 was considered significant.

RESULTS

Figure 1 is a schematic diagram showing the location of the perfusion site in rat PVN at which CCK release was recorded. The oval represents the area that encou-
passes the tips from all push-pull cannulas used in the data analysis.

Endogenous CCK release before and after gavage is summarized in Fig. 2. Pregavage CCK levels of lean and obese rats were not significantly different. In the lean rats given the meal load, there was a significant increase in postprandial CCK release (a rise of 57%, $P < 0.05$), but no change was seen after the nonnutritive solution was gavaged. In the first 20-min fraction postmeal, CCK release averaged 22.1 ± 1.4 fmol (an increase of 8.0 ± 1.0 fmol from baseline) and remained elevated at 18.4 ± 2.5 fmol in the next 20-min fraction. By 60 min postgavage, the values had returned to baseline levels.

Perfusion with 100 mM KCl aCSF resulted in an additional release of CCK (4.6 ± 2.8 fmol/20-min sample) in the lean rats previously gavaged with a meal, whereas the KCl induced a much larger release of CCK (21.7 ± 1.4 fmol/20-min sample) in the lean rats intubated with the nonnutritive carboxymethylcellulose (Fig. 2). There was no change in postprandial CCK release in obese rats, but neuronal depolarization due to perfusion with 100 mM KCl aCSF resulted in an increase in CCK release (4.6 ± 1.3 fmol/20-min sample) that was comparable to that elicited by KCl in the meal-gavaged lean rats. Because the obese rats did not respond to the meal gavage, we did not intubate them with 1% carboxymethylcellulose.

**DISCUSSION**

Dietary cues indicate overall body energy status as well as the more immediate sensations such as appetite or the satisfaction of appetite after a meal. Satiety is mediated in part by gastrointestinal peptide hormones that are released in response to ingested food (29). The diet-induced signals are relayed to brain areas where they orchestrate appropriate alterations in food intake and regulatory energy expenditure (22).

Our results support a physiological role for CCK in the PVN as a cue to signal or induce satiety. We demonstrated that, concomitant with physiological stimuli associated with satiety, endogenous CCK is released at the PVN, an area where exogenous CCK administration suppresses ingestive behavior. That is, endogenous, extracellular CCK within the PVN increased immediately after the intubated meal to Fa/Fa rats, implicating this area as an important site mediating CCK-induced termination of feeding during normal meal intake. The fact that the intubated meal elicited no such increase in CCK in the fa/fa rats further supports the view that the fatty mutation (i.e., a dysfunctional leptin receptor) renders the obese Zucker rat less sensitive to dietary cues (32). Moreover, it also provides evidence that CCK release at the PVN is part of the leptin-regulated pathway.

The anorectic effect of peripheral CCK involves a sensory relay to regions of the central nervous system involved in regulating feeding behavior. A peptide-encoded neuronal cascade, beginning with vagal sensory fibers, carries afferent input from the gastrointestinal tract, which terminates primarily in the nucleus of the solitary tract (NTS) (25). Ascending projections of the NTS connect with the parabrachial nucleus and make synaptic relay at the PVN, ventromedial hypothalamus, and lateral hypothalamus (LH) (13, 26, 37). Selective gastric vagotomy markedly reduces peripheral CCK-induced satiety, suggesting that intact vagal input to brain stem areas is required (30). Peripheral administration of CCK also activates c-fos expression in the NTS and PVN via vagal afferents (21). Lesions of the NTS and/or the PVN effectively abolish the ability of CCK to inhibit feeding, which is consistent with the idea that the PVN is a site where integration of neuroendocrine, metabolic, and limbic activity is organized (8).

The LH has also been implicated in the ability of CCK to suppress feeding behavior (27). Previous studies demonstrated that endogenous CCK can be released from the LH after a gastric meal load as well as a water load, this release being ascribed to gastric distension (28). We intubated with a nonnutritive solution (1% carboxymethylcellulose) with similar viscosity as the liquid meal, thereby approximating the distension due to the meal (3), and found no CCK release in the PVN. On the basis of a total daily intake of ~80 kcal, the energy content of the intubated liquid meal was designed to provide a caloric stimulus approximately equivalent to two meals (7). This somewhat large meal size was intended to provide a sufficient caloric load to the obviously larger obese rat and yet not cause malaise to either group of rats. Therefore, although stomach distension may contribute to the inhibition of feeding at the level of the LH, our data indicate that this is not the case in the PVN, where the primary signal for CCK release appears to be nutrient/caloric composition of the meal.

Fig. 2. Endogenous CCK release at PVN in lean and obese Zucker rats in response to a meal or nonnutritive gavage. Rats were perfused with artificial cerebrospinal fluid at a flow rate of 10 µl/min, and 20-min fractions were collected and analyzed for CCK. The 10.3-kcal test meal was delivered via gavage at 80 min (arrow) to 9 lean (○) and 9 obese (△) rats. Nonnutritive 1% carboxymethylcellulose was also gavaged at 80 min to 7 lean rats (○). The 100 mM KCl, which induces neuronal depolarization, was administered for 15 min (horizontal bar) to all 3 groups of rats. Values are expressed as means ± SE. *Significantly different from obese (P < 0.05). **Significantly different from baseline (P < 0.01).
Summary. Endogenous CCK release at the PVN significantly increased in homozygous lean (Fa/Fa) Zucker rats in response to intragastric administration of a 10.3-kcal liquid meal. That is, CCK release at the PVN changed in relation to the ingestive state of the rats. In contrast, obese (fa/fa) Zucker rats demonstrated no such change, indicating an unresponsiveness to the physiological stimulus. As evidenced by their response to 100 mM KCl, the obese rats may also have less CCK available for release at the PVN. This suggests that the obesity resulting from the act synergistically in regulating ingestive behavior.

CCK release would be required for the same response in the caloric load were a factor in CCK release (e.g., a larger satiety) cues. This would be true even if meal size/metabolism. This implies that the absence of hypothalamic CCK plays a physiological role in satiety and that release at the PVN reflects the nutrient composition of the meal rather than gastric distension. This implies that the abundance of CCK release in the meal-gavaged obese Zucker rats reflects their deficient responsiveness to nutrient (e.g., satiety) cues. This would be true even if meal size/caloric load were a factor in CCK release (e.g., a larger load would be required for the same response in the obese vs. lean Zucker rats).

As previously mentioned, CCK and leptin appear to act synergistically in regulating ingestive behavior. This suggests that the obesity resulting from the disruption of the leptin-regulated pathway in the Zucker rat may be associated with a failure of both short- and long-term dietary cues.

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