Central leptin modulates behavioral and neural responsivity to CCK

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Emond, Michael, Gary J. Schwartz, Ellen E. Ladenheim, and Timothy H. Moran. Central leptin modulates behavioral and neural responsivity to CCK. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1545–R1549, 1999.—The mechanisms through which leptin, the protein product of the ob gene, affects food intake remain to be determined. To assess whether the actions of leptin depend on modulation of within-meal satiety signals, we measured the effect of third ventricular leptin administration on the satiety actions of CCK. Leptin (10 μg) administered 1 h before 30-min access to a liquid diet had no effect on intake when administered alone, but doses of 3.5 or 10 μg dose dependently increased the suppression of intake produced by 1 nmol/kg CCK. Examination of patterns of c-Fos activation induced by 3.5 μg leptin and 1 nmol/kg CCK revealed that the combination produced significant c-Fos activation within the area postrema and the caudal and medial nucleus of the solitary tract (NST) compared with either leptin or CCK treatments alone. The leptin-CCK combination also resulted in increased c-Fos activation within the paraventricular nucleus of the hypothalamus above that produced by leptin alone. These data suggest that the actions of leptin in food intake are mediated through its ability to modulate responsivity to within-meal satiety signals.

ob gene product; satiety; food intake; ingestion; brain-gut peptide, cholecystokinin

LEPTIN, THE PROTEIN PRODUCT of the ob gene, serves as a feedback signal involved in body weight regulation and energy balance. Leptin is expressed in adipose tissue (6, 35) and secreted into the blood in proportion to the degree of adiposity (15). Leptin is taken into the brain by a specific transport system (2). Leptin receptors exist in a number of isoforms and are found in dense concentrations in the choroid plexus and within the arcuate nucleus of the hypothalamus (32). Central or peripheral administration of leptin reduces food intake (10, 13). The actions of leptin in the regulation of energy balance and adiposity appear to depend on its interaction with the long form of the ob receptor within the arcuate nucleus (26).

Defects in the leptin signaling system result in obesity in rodent models. The obesity in the ob/ob mouse is the result of a non-sense mutation in the ob gene that prevents the production of the leptin protein, and both the db/db mouse and the fa/fa Zucker rat have mutations that result in defective leptin receptors (7). Administration of exogenous leptin to the ob/ob mouse normalizes body weight and food intake (6).

The mechanisms through which leptin affects food intake remain to be determined. Recent work from a number of laboratories examining meal patterns in response to exogenous leptin administration has shown that the effects of leptin in reducing food intake are specific to a selective reduction in meal size with no change in meal frequency (8, 10, 13). These data suggest that leptin may be exerting its actions on food intake through an interaction with meal-related signals involved in the production of satiety. Leptin may increase the efficacy of within-meal satiety signals and thus reduce meal size.

Data demonstrating interactions between leptin and the brain-gut peptide CCK provide preliminary support for such an action for leptin. Doses of CCK that produce maximal suppressions of food intake in short-term tests, combined with peripheral leptin, reduced 24-h food intake to a greater degree than leptin alone (16). A dose of leptin that does not by itself affect food intake until 5 h after administration enhanced the ability of CCK to reduce food intake (3). In both of these experiments, the peptides were administered simultaneously, and intake was only assessed after a 1-h time point. The effects of CCK on feeding are normally only evident for a 30-min period (19). Thus those experiments did not permit the evaluation of the potential interaction of CCK and leptin within the context of a single meal.

In the present experiments, we sought to more directly assess whether centrally administered leptin would alter the potency of peripherally administered CCK within the context of an individual meal. We also investigated, using c-Fos immunohistochemistry, whether we could identify candidate brain sites that might mediate any observed behavioral synergy.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing between 250 and 300 g were housed in individual hanging wire mesh cages and
maintained on a 12:12-h light-dark cycle. Rats were anesthetized with a mixture of ketamine and xylazine (150 and 30 mg/kg, respectively) and placed in a stereotaxic device. Twenty-three-gauge stainless steel cannulas were angled (10°) into the third cerebral ventricle from a skull site 1 mm lateral to midline at a site 1 mm posterior to bregma. The cannula tip was lowered to a point 7 mm ventral to the dura. Cannula placements were confirmed by a drinking response to the administration of 10 ng angiotensin II. Rats that did not drink 5 ml of water in 60 min were not used for central peptide administration. Rats were allowed to recover for a minimum of 2 wk before testing.

During this postoperative period, rats were adapted to a schedule of access to chow from 5:00 PM to 9:00 AM. After a 6-h daytime deprivation, rats had 30-min access to nutritionally complete liquid Ensure diet (Ross Laboratories; 1.06 kcal/ml). When daily Ensure intake had stabilized, the feeding responses of the rats to a number of treatment conditions were assessed. For these tests, leptin or vehicle was administered into the third ventricle 60 min before Ensure access. Human recombinant leptin (Peprotech), dissolved in 0.9% sterile saline, was administered in doses of 3.5 µg in 3.5 µl and 10 µg in 5 µl over 30 s to 1 min. Immediately preceding Ensure access, rats received injections (1 ml/kg body wt ip) of 1 nmol/kg CCK (CCK-8, Bachem) or 0.9% sterile saline vehicle. In the initial experiment four conditions were run with the 10-µg leptin dose: vehicle-vehicle, vehicle-CCK, leptin-vehicle, and leptin-CCK. Feeding response to an additional condition of 3.5 µg leptin plus 1 nmol CCK was also assessed. Ensure intake was recorded at the end of the 30-min access period. Each animal (n = 18) received all treatment conditions. The sequence of testing was vehicle-vehicle, CCK alone, 3.5 µg leptin-CCK, 10 µg leptin-CCK, and 10 µg leptin alone. Two or three days separated each peptide test day. The effects of 10 µg leptin and 1 nmol/kg CCK on volume of Ensure consumed were analyzed by a 2 × 2 repeated-measures ANOVA with planned t comparisons to determine differences among the treatment conditions. Data assessing the ability of 3.5- and 10-µg leptin doses to affect the percent inhibition of intake by 1 nmol/kg CCK were analyzed by repeated-measures ANOVA.

After feeding tests, 16 of these and three additional rats were divided into four experimental groups for analyses of c-Fos immunoreactivity patterns elicited by each of the treatment conditions. The sequence of testing was vehicle-vehicle, vehicle-CCK, leptin-vehicle, and leptin-CCK. Feeding response to an additional condition of 3.5 µg leptin plus 1 nmol CCK was also assessed. Ensure intake was recorded at the end of the 30-min access period. Each animal (n = 18) received all treatment conditions. The sequence of testing was vehicle-vehicle, CCK alone, 3.5 µg leptin-CCK, 10 µg leptin-CCK, and 10 µg leptin alone. Two or three days separated each peptide test day. The effects of 10 µg leptin and 1 nmol/kg CCK on volume of Ensure consumed were analyzed by a 2 × 2 repeated-measures ANOVA with planned t comparisons to determine differences among the treatment conditions. Data assessing the ability of 3.5- and 10-µg leptin doses to affect the percent inhibition of intake by 1 nmol/kg CCK were analyzed by repeated-measures ANOVA.

After feeding tests, 16 of these and three additional rats were divided into four experimental groups for analyses of c-Fos immunoreactivity patterns elicited by each of the experimental treatments examined in the previous study. The four groups were vehicle-vehicle (n = 3), vehicle-CCK (n = 6), leptin-vehicle (n = 5), and leptin-CCK (n = 5). In this experiment, the leptin dose was 3.5 µg and the CCK dose was 1 nmol/kg. Again, leptin was administered 1 h before CCK administration. Seventy-five minutes after intraperitoneal CCK or saline vehicle injection, rats were anesthetized with a mixture of ketamine and xylazine or Euthanol (pentobarbital sodium and phenytoin; Delmarva Laboratories) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in phosphate buffer. Brains were removed and placed in 25% sucrose-4% paraformaldehyde overnight. Forty-micrometer coronal sections were cut on a cryostat at the level of the hypothalamus and through the rostrocaudal extent of the nucleus of the solitary tract (NST). Sections were incubated and exposed to c-Fos antibody as previously described (9). Sections were mounted on gelatin-coated slides and placed under coverslips. Images of sections were captured by video camera, and the NTS was outlined based on cellular morphology for analysis of the number of c-Fos-positive cells using the IPLab imaging system (Scanalytics). c-Fos-positive cells were automatically counted by the imaging program by setting minimum and maximum optical density levels. Cell counts of c-Fos immunoreactivity were made at four standardized rostrocaudal levels of the caudal brain stem that spanned the entire NST: the most caudal sections began 0.3 mm caudal to the obex, medial sections were taken 0.2–0.3 mm rostral to the caudal border of the obex, rostral sections were taken at the maximal mediolateral extent of the area postrema (AP), and the most rostral regions were sectioned beginning 0.2 mm anterior to the rostral border of the rostralmost position of the AP. In addition, c-Fos immunoreactivity was assessed at the level of the hypothalamic paraventricular nucleus (PVN). The duration from the angiotensin tests to death for examinations of c-Fos expression was a maximum of 2 wk. Data were analyzed by separate ANOVA at each sampling site across the four experimental conditions.

RESULTS

The effects of CCK and leptin on Ensure intake are demonstrated in Fig. 1A. There was a significant effect

![Fig. 1. Effects of leptin and CCK on 30-min Ensure intake. A: mean ± SE intake in response to individual or combined leptin (10 µg icv) and CCK (1 nmol/kg ip) administration compared with control. B: mean ± SE percent intake suppression in response to 1 nmol/kg peripheral CCK with 0, 3.5, or 10 µg leptin administered into 3rd cerebral ventricle. *P < 0.05 vs. vehicle-vehicle baseline condition. §P < 0.05 vs. CCK/VEH condition.](http://ajpregu.physiology.org/ Downloaded from)
of CCK \[F(1,17) = 88.924, P < 0.0001\] and a significant CCK-by-leptin interaction \[F(1,17) = 19.984, P < 0.0003\]. CCK at a dose of 1 nmol/kg caused a significant reduction of Ensure consumption \((P < 0.01)\). Combinations of leptin and CCK significantly reduced intake beyond that produced by CCK alone \((P < 0.01)\). Figure 1B shows the percent suppression of Ensure intake from baseline levels when 1 nmol/kg CCK is administered intraperitoneally in combination with intracerebroventricular saline vehicle and with each of the two central leptin doses. Central leptin administration in combination with peripheral CCK resulted in a dose-related increase in the magnitude of suppression of Ensure intake \([F(2,34) = 10.417, P < 0.001]\) where the suppression produced by the 10-µg dose was significantly greater than that elicited by the 3.5-µg dose \((P < 0.05)\).

The results of the quantification of patterns of c-Fos expression in response to the CCK and leptin treatments are shown in Fig. 2. A 3.5-µg dose of leptin injected alone resulted in a significant increase in c-Fos-positive cells in the PVN \((P < 0.026)\). Leptin alone \((3.5 \mu g)\) did not elicit a significant c-Fos response at any level of the NST compared with baseline vehicle control values \((P > 0.7)\). CCK alone \((1 \text{ nmol/kg})\) did not produce a significant c-Fos response in either the PVN or at any level of the NST \((P > 0.6)\). The combination of peripheral CCK and central leptin produced significantly elevated levels of c-Fos-positive cells within the PVN compared with that produced by leptin alone \((P < 0.01)\) and resulted in significant c-Fos induction in both the caudal \((P < 0.05)\) and medial \((P < 0.01)\) regions of the NST as well as the AP \((P < 0.01)\).

**DISCUSSION**

These data demonstrate that leptin administered 1 h before food access has the ability to significantly increase the magnitude of suppression produced by peripheral administration of CCK. The c-Fos data suggest two potential sites of action for this result. The leptin-CCK combination produced elevated levels of c-Fos expression within the PVN, a hypothalamic site where leptin alone resulted in c-Fos activation, as has previously been demonstrated \((33)\). Leptin's actions on food intake appear to depend on its interaction with the long form of the leptin receptor within the arcuate nucleus \((26)\). The arcuate nucleus projects to the PVN, and roles for both neuropeptide Y and melanocortin in mediating the actions of leptin through this pathway have been proposed \((27, 28, 31)\). Ascending signals related to the peripheral site of action and vagal mediation of CCK may project to cells within the PVN that are activated by leptin.

The leptin-CCK combination, but neither treatment alone, also resulted in high levels of c-Fos activation within the NST. The NST is the site of vagal afferent terminations, and CCK-induced activation of single gut vagal afferents and neurons within the NST have been demonstrated \((22, 25)\). CCK has been shown to induce Fos responses in the NST in numerous prior studies \((11, 20, 23)\). However, these have routinely used much higher CCK doses \((10–100 \mu g/kg)\) than we employed, and it appears that the 1 nmol/kg dose was subthreshold for activating an NST Fos response. The present results suggest that a leptin-activated descending pathway from the hypothalamus may alter the excitability of NST cells responsive to peripheral CCK. The medial and caudal regions of the NST where the leptin-CCK combination resulted in significant c-Fos activation communicate with vagal fibers arising from subdiaphragmatic gastrointestinal sites \((21)\). An increase in c-Fos induction in the PVN by combined leptin-CCK administration has previously been reported by Wang et al. \((34)\). The induction of c-Fos in the NST by the CCK-leptin combination is a new finding.

Exogenous peripheral administration of CCK reduces food intake \((30)\), and a critical action for CCK in the production of satiety is demonstrated by data...
showing that CCK antagonists increase food intake by increasing meal size (17). Also, we have recently shown that rats lacking the CCK-A receptor, which mediates the feeding-suppressive actions of CCK, are hyperphagic and obese, and their hyperphagia is characterized by a specific increase in meal size (18).

Variable potency of CCK has been demonstrated in response to food deprivation (5), at different developmental periods (24), across differences in metabolic status (4), and across the estrous cycle in female rats (12). In some cases, this variation in potency correlates with variations in levels of circulating leptin. For example, leptin levels are low after food deprivation (15), a time of decreased CCK potency (5). Also, leptin levels increase around the time of puberty (1), coinciding with an increase in CCK potency (14). Different background levels of leptin secretion may underlie some of the overall variation in CCK potency.

Results from the present experiments further elucidate the potential interactions between leptin and CCK in the production of satiety. Although leptin-CCK interactions in food intake have been demonstrated (3, 16), the two peptides were administered simultaneously, and potential interactions in the controls of individual meals were not demonstrated. Leptin appears to be a long-term signal related to the level of adiposity, whereas CCK secretion is meal related and plays a role in terminating an individual meal. Our design of giving the leptin 1 h preceding the meal represents an attempt at allowing the effect of CCK to be expressed at differing background levels of leptin. We do not know if acute and chronic leptin would have the same effects, and we do not know whether leptin reaches all its target sites in the brain within this 1-h period. However, increasing the background level of leptin in this way increased the potency of CCK at inhibiting intake within a brief access test, and the relative timing of the leptin and CCK administration may have allowed the increased c-Fos in the NST to be expressed. Leptin-induced modulation of the responsivity of cells within the medial and caudal NST may have increased the efficacy of CCK in the production of cellular activation. In the prior Wang et al. experiment (34) in which CCK and leptin were given simultaneously, no effect for combined administration was found at this dorsal hindbrain site.

Perspectives

Smith (29) has proposed that signals that modify food intake through alterations in meal size should be viewed as having direct or indirect actions. Direct signals are those that arise from nutrient contact with the oropharynx, stomach, and intestine, whereas indirect signals are those that arise from other sources. According to this schema, indirect signals affect food intake by modifying the efficacy or potency of direct signals. The present results provide support for this view. CCK is a direct signal arising from nutrient contact with the intestinal lumen. It plays a negative feedback role, inhibiting intake and producing satiety. Leptin is an indirect signal arising from adipose tissue and secreted in direct proportion to the degree of adiposity. Our data demonstrate that increasing available leptin at brain sites that mediate its actions on food intake amplifies the ability of a single dose of CCK to suppress ingestion within a brief access feeding test. Leptin may modulate the efficacy of CCK at a hypothalamic site and/or at the NST, where CCK-related signals first enter the brain. The fact that a positive synergy was evident at the level of the first central synapse for vagal afferent fibers suggests to us that leptin administration may produce descending hypothalamic signals that modulate the dorsal hindbrain neural processing of meal-related gut vagal afferent input.

Our results do not imply that modulation of responsivity to CCK is the only mechanism through which the actions of leptin in food intake are mediated. The gut produces a variety of feedback signals that may depend on CCK-independent pathways and other signaling peptides. However, the current experimental design of assessing changes in efficacy of direct signals against differing levels of an indirect signal should allow the identification of other pathways and signals through which the feeding-inhibitory actions of leptin are mediated.

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