Synergistic interaction between leptin and cholecystokinin to reduce short-term food intake in lean mice

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ABSTRACT

Leptin is a circulating protein involved in the long-term regulation of food intake and body weight. Cholecystokinin (CCK) is released postprandially and elicits satiety signals. We investigated the interaction between leptin and CCK in the short-term regulation of food intake induced by 24-hr fasting in lean mice. Leptin, injected intraperitoneally (i.p.) at low doses (4–120 μg/kg), which did not influence feeding behavior for the first 3 hr postinjection, decreased food intake dose dependently by 47–83% during the first hour when co-injected with a subthreshold dose of CCK. Such an interaction was not observed between leptin and bombesin. The food-reducing effect of leptin injected with CCK was not associated with alterations in gastric emptying or locomotor behavior. Leptin–CCK action was blocked by systemic capsaicin at a dose inducing functional ablation of sensory afferent fibers and by devazepide, a CCK-A receptor antagonist but not by the CCK-B receptor antagonist, L-365,260. The decrease in food intake which occurs 5 hr after i.p. injection of leptin alone was also blunted by devazepide. Coinjection of leptin and CCK enhanced the number of Fos-positive cells in the hypothalamic paraventricular nucleus by 60%, whereas leptin or CCK alone did not modify Fos expression. These results indicate the existence of a functional synergistic interaction between leptin and CCK leading to early suppression of food intake which involves CCK-A receptors and capsaicin-sensitive afferent fibers.

Leptin is an adipose tissue-derived circulating protein, originally identified as a key element in the long-term regulation of food intake and body weight homeostasis in ob/ob mice (1–6). Although ob/ob mice are more sensitive to leptin effects, reduction of food intake and weight loss can also be elicited by chronic hyperleptinemia achieved by repeated peripheral injections of leptin or by adenovirus-mediated leptin gene therapy in lean mice and rats (1–3, 5, 7). By contrast, less is known about the short-term alteration of feeding behavior induced by leptin administered peripherally (3, 8, 9). Kinetic studies indicate that upon a single intravenous or intraperitoneal (i.p.) injection, leptin decreases food intake only after several hours in ob/ob or lean mice (3, 8, 9). The delayed onset may be related to the delayed bioavailability of leptin to reach or influence its target sites of action in the brain (9–12). Alternatively, leptin may require the presence of food-related gastric or postgastric signals. Several peptides released postprandially cause reduction in meal size (13). Cholecystokinin (CCK) is secreted from small intestinal cells in response to food ingestion and functions as a postprandial satiety signal (13–16). Numerous studies using CCK receptor agonists and antagonists have extended these findings in several species, including mice and humans (14, 17–19). Convergent reports indicate that one component of the satiety effect of CCK is initiated at the periphery through the activation of capsaicin-sensitive vagal afferent fibers that relays the information to brain sites (20–24). There is also evidence of synergistic interaction between peripheral CCK and other hormones released postprandially such as insulin and glucagon in rats (25, 26). Recently, we observed in an in vitro vagus–stomach preparation that CCK increases responsiveness of vagal afferents to leptin as assessed electrophysiologically (27). Therefore, in the present study we investigate the potential functional synergistic interaction between peripheral leptin and CCK in the short-term modulation of food intake in lean mice.

MATERIALS AND METHODS

Animals. Male lean mice (C57BL/6 +/+ , 6 to 7 weeks old, 20–25 g) (Harlan Laboratories) were maintained ad libitum on standard Purina laboratory chow and tap water. They were housed, five per cage, under conditions of controlled temperature (20 ± 1°C), humidity (30–35%), and lighting (06:00–18:00 hr). All experiments were started between 8:00 and 9:00 a.m. and performed according to protocols approved by the Veterans Administration Animal Care and Use Committee (Animal Component of Research Protocol number 96-89-8).

Reagents. Aliquots of recombinant murine leptin (Amgen Biologics; 1 μg/μl in vehicle: 0.1% fetal bovine serum in 1:10 saline) were stored at −70°C until used. Stock solutions were diluted in saline immediately before each experiment. Bombesin (Clayton Foundation Laboratories, Salk Institute, La Jolla, CA) was freshly dissolved in saline immediately before use. Devazepide and L-365,260 (1 mg/kg, Merck Sharp & Dohme) were dissolved in 50 μl of dimethyl sulfoxide and 50 μl of Tween 80, and further diluted in saline. Capsaicin (8-methyl-N-vanillyl-6-nonenamide; Sigma) was dissolved in 10% ethanol, 10% Tween 80, and 80% saline.

Treatments and Measurement of Food Intake. Mice, deprived of food for 24 hr with free access to water, were injected i.p. (10 μl/kg) with either vehicle, leptin (4, 12, 80, or 120 μg/kg), CCK (3.5 μg/kg), bombesin (5 μg/kg), leptin (4, 12, 80, or 120 μg/kg) plus CCK (3.5 μg/kg) or leptin (120 μg/kg) plus bombesin (5 μg/kg). Thereafter, preweighed Purina chow was given every hour and food intake was determined by measuring the difference between the preweighed standard chow and the weight of chow and spill at the end of each hour for a 7-hr period. Mice were weighed before and after 24-hr fasting and again at the end of the 7-hr feeding period. Similar studies were performed in mice injected i.p. with devazepide (1 mg/kg) or L-365,260 (1 mg/kg) and 10 min after with leptin (120 μg/kg) plus CCK (3.5 μg/kg) or vehicle. In a series of experiments, mice were anesthetized with halothane (3% vapor in O2) and injected with a single subcutaneous injection...
(0.1 ml) of capsaicin (50 mg/kg) or vehicle (10% ethanol/10% Tween 80/80% saline). One week later, the effect of a single i.p. injection of leptin (120 μg/kg) plus CCK (3.5 μg/kg) on food intake was evaluated as described above. Efficiency of capsaicin pretreatment was verified immediately before euthanasia by the corneal chemosensory test which consists of monitoring the wiping reflex to ocular instillation of a drop of 0.1% NH₄OH solution. None of the capsaicin-pretreated mice showed a wiping response, indicating an effective ablation of primary sensory afferents as previously reported in adult mice with a similar treatment (28), whereas wiping reflex was present in vehicle-pretreated mice.

In the last group of experiments, mice, fasted for a 20-hr period, received a single i.p. injection of leptin (120 μg/kg) or vehicle (5 ml/kg) and the access to food was restricted to the 4th to the 7th hr after leptin injection. Ten minutes before food access, mice were injected i.p. with deoxyepine (1 mg/kg) or vehicle (5 ml/kg). Food intake was monitored hourly, as described above, from the fifth to seventh hour after leptin administration.

**Measurement of Gastric Emptying.** Mice fasted for 24 hr had free access to preweighed Purina chow for 3 hr and thereafter received a single i.p. injection of either leptin (120 μg/kg), CCK (3.5 μg/kg), combined treatment (leptin plus CCK), or vehicles (10 ml/kg). Mice were killed by cervical dislocation 4 hr after i.p. treatment. The stomach was exposed by laparotomy, quickly ligated at both the pylorus and cardia, then removed, and the wet content was weighed. Gastric emptying (% in 4 hr) was calculated according to the following formula: gastric emptying (%) = 1 – (wet weight of food recovered from the stomach/weight of food intake)] × 100.

**Locomotor Activity and Stereotyped Behaviors.** Locomotor activity was measured using a similar method to that described previously (1). Mice were placed in individual Plexiglas cages (14 × 15 × 25 cm), with the bottom divided into 15 equal squares of 5 × 5 cm. Locomotor activity (total number of squares crossed or explored by the animals during the observation time), grooming (washing, licking and/or scratching), and stereotypic activities (sniffing) were evaluated for 1 min, every 5 min, during the first hour after a single i.p. injection of either leptin (120 μg/kg) plus CCK (3.5 μg/kg) or combined vehicles.

**Fos Histochemistry.** Immunohistochemistry for Fos was performed on perfusion-fixed 30-μm-thick frozen brain sections as described (30). Mice, food deprived for 24 hr, were injected i.p. with either leptin (120 μg/kg), CCK (3.5 μg/kg), leptin plus CCK, or combined vehicles, and killed 2 hr later. Animals were deeply anesthetized with sodium pentobarbital (5 mg in 0.1 ml, i.p.; Nembutal, Abbott) and transcardially perfused with 5 ml of 0.9% saline followed by 30 ml of 4% paraformaldehyde solution and 14% saturated picric acid. Brains were postfixed for 5 hr and cryoprotected by immersion in 20% sucrose overnight. An avidin–biotin–peroxidase complex binding method was used with a rabbit antiserum against human Fos protein N terminal at 1:10,000 dilution (Oncogene Science) as primary antibody. The staining was abolished by preabsorbing the antiserum for 24-hr at 4°C with a ratio of antigen/antibody of 100:1. Cell counting of Fos immunoreactive cells in the paraventricular nucleus of the hypothalamus (PVN) was made unilaterally in 8–9 sections containing the biggest volume of parvo- and magnocellular neurons per animal (bregma: −0.70 to 0.94 mm from Franklin and Paxinos’s mouse brain atlas) (31).

**Statistical Analysis.** Data are expressed as mean ± SEM. Statistical comparisons were done using a nonrepeated measures ANOVA followed by a Newman–Keuls multiple comparisons test. For cell counting, data were analyzed by a Kruskal–Wallis nonparametric ANOVA followed by Dunn’s multiple comparisons test. Data were considered statistically significant when P < 0.05. The ED₅₀ for leptin was determined by nonlinear regression.

**RESULTS**

**Effects of Leptin, Leptin–CCK, or Leptin–Bombesin Coinjection on Food Intake.** Leptin (120 μg/kg) injected i.p. did not significantly modify food intake for the first 4 hr after injection, and CCK (3.5 μg/kg) had no effect throughout the 7-hr experimental period in 24-hr fasted lean mice (Fig. 1). Thereafter, leptin induced a significant reduction of food intake from the 5th to 7th hr postinjection (Fig. 1). By contrast, coinjection of leptin with CCK reduced food intake during the first 3 hr postinjection (Fig. 1). The decrease reached 82.5 ± 6.2%, 82.8 ± 5.8%, and 75.3 ± 8.7% during the first hour compared with vehicle-, leptin-, or CCK-treated groups, respectively (F(27,203) = 10.899, P < 0.0001) (Fig. 1, Table 1).

During the fifth to seventh hour after treatments, the hourly reduction in food intake was no longer different between groups treated with leptin plus CCK (77.2 ± 11.6%, 55.5 ± 11.1%, and 61.1 ± 7.3%, respectively) and leptin alone (88.1 ± 8.4%, 48.0 ± 24.1%, and 55.6 ± 13.0%, respectively).

In the presence of the subthreshold dose of CCK (3.5 μg/kg), leptin (4–120 μg/kg)-induced early suppression of food intake was dose-related in terms of the magnitude (28–80% reduction/2 hr) (Fig. 2) and duration of the response (1–3 hr) (Table 1). The ED₅₀, defined as the dose of leptin that inhibited by 50% the 2-hr cumulative food intake immediately after coinjection with CCK, was 15.6 μg/kg (r² = 0.988, 95% interval of confidence: 9.0–26.0 μg/kg). By contrast, the maximal effective dose of leptin (120 μg/kg) coinjected with bombesin (5 μg/kg) at a satiating subthreshold dose, equimolar to that of CCK, failed to inhibit the cumulative food intake throughout the first 2-hr period after injection (Fig. 2).

Food deprivation for 24 hr induced a 4.3 ± 0.1 g body weight loss. After a 7-hr refeeding period, the body weight gain in leptin plus CCK treated mice was significantly lower (0.7 ± 0.3 g) to that gained after vehicle, CCK, or leptin treatment (1.7 ± 0.2, 1.8 ± 0.2, and 1.5 ± 0.2 g, respectively). There was a good correlation between the body weight gain and the food intake after leptin coinjection (vehicle + leptin, 88.1 ± 8.4%; vehicle + CCK, 88.1 ± 8.4%; vehicle + leptin plus CCK, 88.1 ± 8.4%).

![Fig. 1. Early inhibition of cumulative food intake induced by i.p. injection of leptin plus CCK in fasted lean mice (+/-). Animals were exposed to food immediately after a single i.p. injection of control vehicle (10 ml/kg, n = 8), vehicle plus leptin (120 μg/kg, n = 8), vehicle plus CCK (3.5 μg/kg, n = 7) or leptin plus CCK (n = 8). Data are expressed as mean ± SEM. * P < 0.05 vs. the group treated with combined vehicles [ANOVA, F(27,203) = 10.899].](image-url)
amount of food ingested among the different experimental groups ($r^2 = 0.768$).

**Effects of CCK Receptor Antagonists and Systemic Capsaicin on Leptin–CCK Interaction.** Pretreatment with the specific CCK-A receptor antagonist devazepide completely prevented the early inhibition of food intake induced by leptin (120 mg/kg) plus CCK (3.5 mg/kg) observed during the 2-hr period posttreatment, whereas the specific CCK-B receptor antagonist L-365,260 had no effect (Fig. 3). Capsaicin pretreatment 1 week before the experiments also abolished leptin (120 mg/kg) plus CCK (3.5 mg/kg)-induced reduction of food intake during the 2-hr postpeptide administration period (Fig. 3). Although there was slight food intake increase in mice treated with devazepide or capsaicin alone during the same 2-hr period, the difference did not reach statistical significance (Fig. 3).

**Effect of Devazepide on Leptin-Induced Reduction of Food Intake.** When mice fasted for 20 hr were injected i.p. with leptin (120 mg/kg) and the food access was withheld up to the fourth hour after leptin administration, the decrease in food consumption compared with the vehicle group was not significantly different during the first hour of food access, corresponding to the fifth hour after leptin injection (Fig. 4). Thereafter, leptin significantly reduced hourly food intake by

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Table 1. Dose-related inhibition of hourly food intake induced by leptin coinjected with CCK (3.5 mg/kg) during the first 3 hr postinjection

<table>
<thead>
<tr>
<th>Hour postinjection</th>
<th>Leptin dose, mg/kg, i.p.</th>
<th>Inhibition of food intake per hour,* %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>47.4 ± 6.8†</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>62.4 ± 9.7†</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>84.9 ± 3.4†</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>82.5 ± 6.2†</td>
</tr>
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</table>

*Mean ± SEM of 6–12 animals per group; the percentage of food intake inhibition was calculated taking values of combined vehicles-treated mice as 0% inhibition.

†$P < 0.05$ compared with combined vehicles-treated group.
72.2 ± 13.9% and 75.3 ± 9.8% at the sixth and seventh hour, respectively (Fig. 4). Reduction of food intake elicited by leptin during the sixth and seventh hour postadministration was completely prevented by pretreatment with devazepide, administered 10 min before food access (Fig. 4).

**Fos Immunoreactivity in the PVN after Leptin–CCK Coinjection.** In mice deprived of food for 24 hr, Fos immunoreactivity in the PVN showed a similar number of Fos-positive cells in untreated animals or 2 hr after i.p. injection of vehicle, CCK (3.5 μg/kg), or leptin (120 μg/kg) (Fig. 5A–C, E, and F). Cells

![Fig. 5](image_url)

**Fig. 5.** Fos immunoreactivity in the PVN after leptin + CCK coinjected in lean mice (+/ +). Representative microphotographs show Fos protein immunoreactivity in the PVN 2 hr after a single i.p. injection of (A) combined vehicles (10 ml/kg), (B) CCK (3.5 μg/kg), (C) leptin (120 μg/kg), or (D) leptin plus CCK, and (E) in fasted untreated animals. Marked increase in Fos immunoreactivity, resulting in dark and well-shaped nuclei, was observed in the parvo- and magnocellular divisions of the PVN after leptin plus CCK treatment, whereas other groups exhibited only scattered and lightly labeled cells. (Bar = 100 μm.) (F) The number of cells per section (unilateral). Data are mean ± SEM of four animals per group. *, P < 0.05 vs. all other groups (Kruskal–Wallis ANOVA, KW = −1308.4).
were found mainly in the magnocellular division and in lower proportion in the ventral and parvocellular divisions. In contrast, CCK plus leptin-treated mice exhibited a 60% significant increase in the number of Fos-positive cells in the PVN compared with the other experimental groups (KW = −1308.4, P < 0.0001) (Fig. 5 D and F).

**Effect of Leptin–CCK Coinjection on Gastric Emptying.** The 4-hr rate of gastric emptying of a nutrient solid meal was not modified by leptin (120 μg/kg) coinjected with CCK (3.5 μg/kg) (87.2 ± 3.5%, n = 7), compared with either vehicles (92.5 ± 0.9%, n = 6), leptin (91.5 ± 0.6%, n = 7), or CCK (91.6 ± 1.3%, n = 7) alone.

**Behavioral Effects of Leptin–CCK Coinjection.** Locomotor activity during the first hour after i.p. injection of leptin (120 μg/kg) plus CCK (3.5 μg/kg) resulted in 96.3 ± 25.5 squares crossed or explored (n = 4). This value was not significantly different from that observed in mice injected with combined vehicles (119.3 ± 48.4 squares, n = 4, P = 0.689). Similarly, no difference in the total numbers of stereotypic and grooming activities was observed between the two experimental groups (leptin plus CCK: 54.5 ± 6.1 events; combined vehicles: 50.0 ± 10.0 events, P = 0.715).

**DISCUSSION**

Leptin did not significantly modify food intake for the first 4 hr after a single i.p injection at 120 μg/kg in 24-hr fasted lean mice exposed to food. These results are consistent with previous time course studies using similar or higher doses of leptin injected i.p. (8, 9). By contrast, leptin injected simultaneously with CCK results in a dose-dependent reduction of food intake as it relates to the magnitude and duration of the response. A dose as low as 12 μg/kg of leptin in the presence of CCK decreased hourly food intake by 62% and 46% during the first and second hour postinjection, respectively. Such a response is unlikely to reflect the anorectic effect of CCK. The peptide was injected at a dose of 3.5 μg/kg, subthreshold to induce a significant decrease in hourly food intake in lean mice, as reported previously (18, 19, 32). These data provide clear evidence for a synergistic interaction between leptin and CCK leading to short-term reduction in food intake upon a single peripheral injection in lean mice. Such a potentiating interaction is specific for peripheral administration of CCK as bombesin, injected i.p. at a subthreshold dose, failed to influence the pattern of leptin-induced change in food intake. Although bombesin is released postprandially and reduces food ingestion in a variety of species including mice, its action is mediated through different mechanisms than CCK, which may account for the difference observed (18, 33–35).

The reduction of food consumption can be secondary to the nonspecific behavioral suppressing effects of treatments under study. However, leptin injected i.p. at doses up to 10 mg/day did not affect total locomotor or grooming activity in lean mice (1). Likewise, in the present study, leptin plus CCK-treated mice were alert and no changes in exploratory locomotion or induction of any form of stereotyped behaviors were observed during the first hour, when food intake was reduced by 83%. Leptin–CCK action is also unlikely to reflect an aversive effect of treatments. Consistent reports established that the food-reducing effect of i.p. injection of CCK is unrelated to taste aversion and CCK doses over 300-fold higher than used in the present study are required to elicit a mild aversive effect in rats (14, 20). There is also evidence that leptin, injected into the lateral brain ventricle at a dose reducing short-term food intake, did not cause aversive side effects as monitored by the lack of conditioned taste aversion to saccharine (36). Therefore it may be inferred that leptin–CCK action most likely reflects a specific effect on ingestive behavior.

Gastric satiety signals resulting from faster or slower clearance of nutrients from the stomach and/or stimulation of gastric stretch receptors by gastric distention may play a role in the short-term regulation of food intake (37–40). In the present study, leptin injected i.p. at a dose resulting in maximal suppression of food intake in presence of CCK did not significantly modify the 4-hr rate of gastric emptying of a nutrient solid meal ingested before the treatment. Peripheral injection of leptin alone, as reported previously, did not influence gastric emptying of a solid nutrient meal in mice (8). CCK at the dose used in the present study was also subthreshold to inhibit gastric emptying, which occurs at peptide doses higher than 10 μg/kg in mice (41). These data, added to the rapid onset of food intake suppression induced by leptin-CCK injection, support the view that gastric satiety signals linked with alterations of gastric emptying are unlikely to play a role in the food intake reducing effect of leptin–CCK interaction in normal lean mice. The lack of change in gastric emptying in response to leptin–CCK treatment at doses inducing maximal food intake reducing effect also provides functional evidence that leptin–CCK action does not result from a nonspecific stress-related response. Convergent reports showed that delayed gastric emptying is a reliable autonomic response to activation of brain corticotrophin-releasing factor receptors induced by stress (42).

The biological actions of CCK are exerted through interaction with two subtypes of CCK receptors, CCK-A and CCK-B, which are selectively antagonized by devazepide and L-365,260 respectively (42). Leptin coinjected with CCK induced short-term suppression of food intake was completely prevented by an i.p. injection of devazepide whereas L-365,260 had no effect. The reversal by the CCK-A receptor antagonist was observed under conditions that did not influence basal food intake as reported previously (18, 32). It is also unlikely to represent the anxiolytic-like effect of devazepide because L-365,260, which has established anxiolytic-like properties in mice (43, 44), did not influence the food-suppressing effect of leptin–CCK. These results indicate that the CCK-A receptor subtype is critical for leptin–CCK-induced food intake suppression as established previously for the satiating effect of peripherally administered CCK alone (14, 18, 45, 46).

CCK-A receptors are present in vagal afferent fibers and systemic administration of CCK stimulates gastric vagal afferent discharge in rats (22, 47–51). Convergent findings indicate that afferent fibers in the vagus nerve represent a major initial target in peripheral CCK-induced satiety (20, 24, 52). Systemic capsaicin pretreatment in mice at a dose that causes selective degeneration of small diameter unmyelinated sensory neurons (28) prevented the decrease in food intake for the 2-hr period after i.p. injection of leptin and CCK. These observations are consistent with leptin plus CCK interaction involving activation of CCK-A receptors located in visceral capsaicin-sensitive afferents, most likely of vagal origin. This is further supported by recent electrophysiological recordings in an isolated vagus–stomach preparation in rats showing an increased responsiveness of gastric vagal afferents to leptin by pretreatment with CCK (27).

Afferent sensory fibers are the primary neuroanatomical link between nutrient-related events in the gut and the central neural substrates that mediate the control of food intake (53–55). Among them, the PVN is a target site at which visceral signals are interpreted by limbic structures to regulate homeostatic functions, including ingestive behavior and the satiety effect of peripheral injection of CCK (56–58). In addition, Fos expression was observed in the PVN in response to a single i.p. injection of leptin (1 mg/kg) in ob/ob mice, but not in lean mice (59) or rats (60). Leptin coinjected with CCK enhanced the number of Fos-positive cells in the PVN by 60%. Preliminary studies on the immunohistochemical mapping of oxytocin and vasopressin neurons in the PVN in mice suggest that neurons in both the parvo- and magnocellular parts of the PVN are activated (unpublished data). By contrast, leptin or CCK alone did not modify Fos expression above the basal level.
which normally occurs in lean mice after a 24-hr fast (61). The activation of PYN neurons in response to coinjection of leptin and CCK may be part of the central neural pathways underlying the mechanism of action of leptin–CCK interaction. The biochemical coding and exact mapping of activated neurons in the PYN needs to be further characterized. Previous studies in rats showed that peripheral CCK activates oxytocinergic neurons in the PVN (62, 63).

The synergistic interaction between CCK and leptin may have physiological relevance. CCK is known to be released after a meal and to produce postprandial satiety by nonendocrine mechanisms (14, 16, 64). In the present study, the onset time required to reduce food intake after an i.p. injection of leptin alone was further delayed when the food was withheld during the 4-hr period after leptin injection suggesting an interaction with a food-related signal. In addition, the CCK-A receptor antagonist devazepide prevented the reduction of food intake occurring 5–7 hr after i.p. injection of leptin alone. The interaction between leptin and endogenous CCK was observed at 5–7 hr after an i.p. injection of leptin, at a time when circulating leptin levels may have returned to physiological ranges as shown by kinetic studies in lean fasted or fed mice injected i.p. with 40 μg/kg of leptin (65).

Leptin is exclusively produced in the periphery by adipocytes and has been proposed to be transported from the circulation to the brain where hypothalamic cells are the target of chronic leptin treatment (1, 10, 66, 67). In addition to this long-term action, the present data reveal the existence of a peripheral neuronal mediated synergistic interaction between leptin and CCK released postprandially to induce short-term modulation of food intake. Results obtained also show that this process depends upon CCK-A receptors and activation of capsicin-sensitive afferent neurons sending signals to the PVN. Abnormalities in the release or sensitivity to either factor may be involved in alterations of food intake. In addition, the potentiation of peripheral leptin action by a subthreshold dose of CCK may provide a useful concept for the understanding of multifactorial control of ingestive behavior and open new avenues for the treatment of obesity and eating disorders.

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