

Daily CCK injection enhances reduction of body weight by chronic intracerebroventricular leptin infusion

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Matson, Claire A., Dana F. Reid, and Robert C. Ritter. Daily CCK injection enhances reduction of body weight by chronic intracerebroventricular leptin infusion. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R1368–R1373, 2002; 10.1152/ajpregu.00080.2001.—In the present study, we tested the hypothesis that a single daily injection of the gut peptide CCK, together with continuous leptin infusion, would produce significantly greater loss of body weight than leptin alone. We found that a single daily intraperitoneal injection of CCK-8 (0.5 µg/kg) significantly enhanced the weight-reducing effects of 0.5 µg/day leptin infused continuously into the lateral ventricle of male Sprague-Dawley rats by osmotic minipump. However, CCK and leptin together did not enhance reduction of daily chow intake. Furthermore, there was no synergistic reduction of 30-min sucrose intake, although a significant main effect of both leptin and CCK was observed on sucrose intake. These results 1) confirm our previous reports of synergy between leptin and CCK on body weight, 2) demonstrate that enhancement of leptin-induced weight loss does not require bolus administration of leptin, and 3) suggest that enhanced body weight loss following leptin and CCK does not require synergistic reduction of food intake by leptin and CCK.

cholecystokinin; osmotic minipump

LEPTIN mRNA IS EXPRESSED BY adipocytes (23), placenta (15, 24, 36), gastric mucosa (2), and muscle tissue (38), and it has been reported in the pituitary (29). In adipocytes, leptin transcripts have been observed to contain a signal sequence that destines the molecule for secretion. Once in the plasma, leptin protein (relative molecular mass 16,000 or M_r 16 K) is found to be unmodified from the intracellular protein, except for removal of the signal sequence and the addition of a disulfide bridge (5).

Plasma leptin levels are well correlated with body fat mass, percent body fat, and body mass index in humans and rodents (reviewed in Ref. 6); however, leptin levels also vary in response to stimuli that do not actually alter body fat stores (reviewed in Ref. 28). For example, although refeeding following a fast may predict body fat changes, the rapid restoration of plasma leptin levels within 4 h of refeeding precedes actual

changes in body adiposity (33). Therefore, leptin expression is controlled by other factors in addition to fat mass. Independent of adiposity, indexes of leptin expression vary with such factors as gender (14, 22, 32), caloric intake or restriction (4, 7, 19), the gut peptide CCK (1, 2), and energy expenditure (40). An obvious commonality among all of these is their involvement in aspects of the regulation of body weight and/or the control of feeding.

Indexes of leptin expression can vary rapidly: the half-life of leptin mRNA is ~2 h (20), whereas the half-life of leptin in the blood is 1.6 h (8). It is therefore apparent that short-term fluctuations in leptin levels can occur and indeed have been observed in response to circadian and feeding rhythms (33, 35, 37). However, as predicted by the lipostatic hypothesis, it is held that for the purposes of body weight regulation, an integration or assimilation of these endogenous fluctuations takes place, such that the overall level of circulating leptin determines the impact on body weight regulatory mechanisms.

In experimental studies, leptin has been given by bolus peripheral injection (27, 39) and by microinjection into the cerebral ventricles (9, 26) or specific hypothalamic nuclei (17, 34). Some of these studies use repeated-daily or twice-daily injections to study “chronic” leptin administration (10, 18, 25). These treatments are effective and dose dependently reduce body weight and feeding behavior. However, it is not known what exactly the body makes of such excessive intermittent fluctuations in leptin levels (13), nor whether these are integrated through the same putative mechanisms that assimilate endogenous variations. The results suggest that there may be some loss of appreciable response with this method, because chronic infusion of leptin by osmotic minipump generally requires much lower leptin doses to produce body weight reductions equivalent to repeated bolus injections (13, 30).

We observed enhancement of leptin’s body weight-reducing effects by the gut peptide CCK (25–27). Furthermore, we showed that CCK-induced enhancement of body weight loss is due to the central nervous system

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(CNS) action of leptin, in combination with the peripheral action of CCK (25). We hypothesize that this relationship may indicate an endogenous capability for meal-related CCK to enhance the effects of leptin to mediate body weight change. However, we previously observed this response only after bolus leptin injections. In the present study, we used continuous infusion of leptin via osmotic minipump, combined with a single daily intraperitoneal injection of CCK, to assess CCK enhancement of leptin-induced body weight loss. We used a range of leptin doses, including doses that have no effect on body weight loss when leptin alone is administered. A single small dose of CCK (0.5 $\mu\text{g}/\text{kg}$), once daily, significantly enhanced the effects of chronic leptin on body weight.

METHODS

Male Sprague-Dawley rats (300–350 g; Simonsen Laboratories, Gilroy, CA) were housed individually in hanging wire cages on a 12:12-h light-dark cycle with lights on at 7:30 AM. Rats were adapted to the following daily schedule. Pelleted rodent chow (Harlan Teklad Diet 8664, 3.3 kcal/g) was removed each day at 9 AM, and the rats were weighed. Before surgery, they were accustomed to 30-min access to 15% sucrose (0.6 kcal/ml) every day beginning at 12 PM for 10 days until a stable daily intake was observed.

For intracerebroventricular cannula placement, rats were deeply anesthetized by intraperitoneal injection of chloropentobarbitol. Unilateral 23-gauge stainless steel guide cannulas (8 mm) were implanted stereotaxically into the lateral ventricle at the coordinates: -1.0 mm from bregma, ± 1.5 mm from midline, and -3.9 mm from dura. The surgeon alternated right or left lateral ventricle cannulation with every rat, resulting in 50% of rats cannulated on either side. After cannulation, rats were allowed to recover for 1 wk while baseline body weight and food intake data were collected according to the daily schedule described above. Before implantation of osmotic minipumps, rats were divided into eight weight-matched groups. The rats were lightly anesthetized with Halothane while osmotic minipumps (model 2002, 14-day infusion; Alza, Palo Alto, CA) were placed subcutaneously between the scapulae. The pump was connected by several centimeters of medical grade tubing (Scientific Commodities, Lake Havasu City, AZ) running beneath the skin to an 8.5-mm L-shaped infusion cannula. After removal of the obturator, the infusion cannula was placed within the guide and secured in place with dental acrylic cement. The entire procedure took <10 min per rat. Subsequent to minipump implantation and connection, the animals were returned to their home cages and chow was returned later in the afternoon. Treatment with CCK or saline began on the following day (*day 1*). Rats were not allowed access to sucrose on the day of surgery.

Animals that showed any sign of illness throughout the experiment were excluded from further treatment, and previous data from these animals were not included in analysis. Immediately before the beginning of these experiments, we were alerted that a previous shipment of animals from our supplier had tested positive for pastorella. Although subclinical in immune-competent adult rats, recovery from surgery in some subjects appeared to be compromised by pastorella-related clinical signs, such as diarrhea and hypothermia, in several cases resulting in death. As a result, a detailed health-rating scale was devised, and each animal was rated daily at the time of weighing. All animals included in the

present analysis were recorded as in good health throughout the study, although probably carriers of pastorella. In total, eight animals were excluded for illness shortly after surgery. In addition, five animals died during the course of the two surgical procedures, and in two animals the tubing connecting the pump to the L-shaped cannula was found disconnected at the termination of the study and the animals were therefore excluded.

The pumps infused leptin (0.1, 0.5, or 1 $\mu\text{g}/\text{day}$) or saline at a constant rate of 0.5 $\mu\text{l}/\text{h}$. On each of the 10 consecutive days of the treatment period (*days 1–10*), each rat received a single intraperitoneal injection of either 0.9% sterile saline or 0.5 $\mu\text{g}/\text{kg}$ CCK-8 immediately before sucrose presentation at 12:30 PM. Chow was returned each day after the sucrose test (1:30 PM).

The total number of animals used for analysis was 49: saline (sal)/sal $n = 6$; sal/CCK $n = 5$; 0.1 $\mu\text{g}/\text{day}$ leptin (lep)/sal $n = 8$; 0.1 lep/CCK $n = 5$; 0.5 $\mu\text{g}/\text{day}$ lep/sal $n = 6$; 0.5 lep/CCK $n = 5$; 1.0 $\mu\text{g}/\text{day}$ lep/sal $n = 7$; and 1.0 lep/CCK $n = 7$. Data were analyzed separately for each dose of leptin as follows: a 2×2 factorial ANOVA was calculated for each day for four groups (sal/sal, sal/CCK, lep/sal, lep/CCK), with α -level set at $P < 0.05$.

RESULTS

In the present study, a low dose of the satiety peptide CCK, given once daily, significantly enhanced the effect of chronic leptin infusion on body weight. There was a significant leptin-by-CCK interaction at 0.5 $\mu\text{g}/\text{day}$ leptin on *days 3–10*. Daily CCK prevented the body weight gain of rats treated with a subthreshold dose of leptin (0.5 $\mu\text{g}/\text{day}$) (Fig. 1C). These observations indicate that CCK may enhance the effects of leptin over a wide range of leptin doses, including doses that induce no loss of body weight on their own. However, at the highest dose, 1.0 $\mu\text{g}/\text{day}$, a significant main effect of leptin treatment was observed, but the interaction between leptin and CCK was significant only on *day 7* (although both *days 5* and *6* approached significance, $P < 0.10$). At this dose, the effect of leptin alone may have been close to the maximal rate of weight loss that is possible in a healthy rat, creating a basement effect that obscured enhancement of weight loss by CCK (Fig. 1D). We observed that at 10 days, there was a complete absence of abdominal adiposity in both leptin-treated groups at this dose of leptin (data not shown). At lower doses, some adiposity remained in retroperitoneal, mesenteric, and epididymal depots, although it appeared diminished and perirenal fat was sometimes absent.

Leptin treatment was accompanied by a significant reduction in voluntary feeding for the highest dose of leptin used (1.0 $\mu\text{g}/\text{day}$). At this dose, there was a significant main effect of leptin treatment on *days 2–6*, *8*, and *10*. Despite enhanced body weight loss, there was no significant interaction between CCK and leptin on food intake at any dose of leptin (Fig. 1). As observed previously (13), daily intake began to return to baseline levels during leptin treatment at a time when body weight had plateaued at a lower level or was still descending. The addition of CCK did not enhance the reduction of food intake or affect the tendency of intake

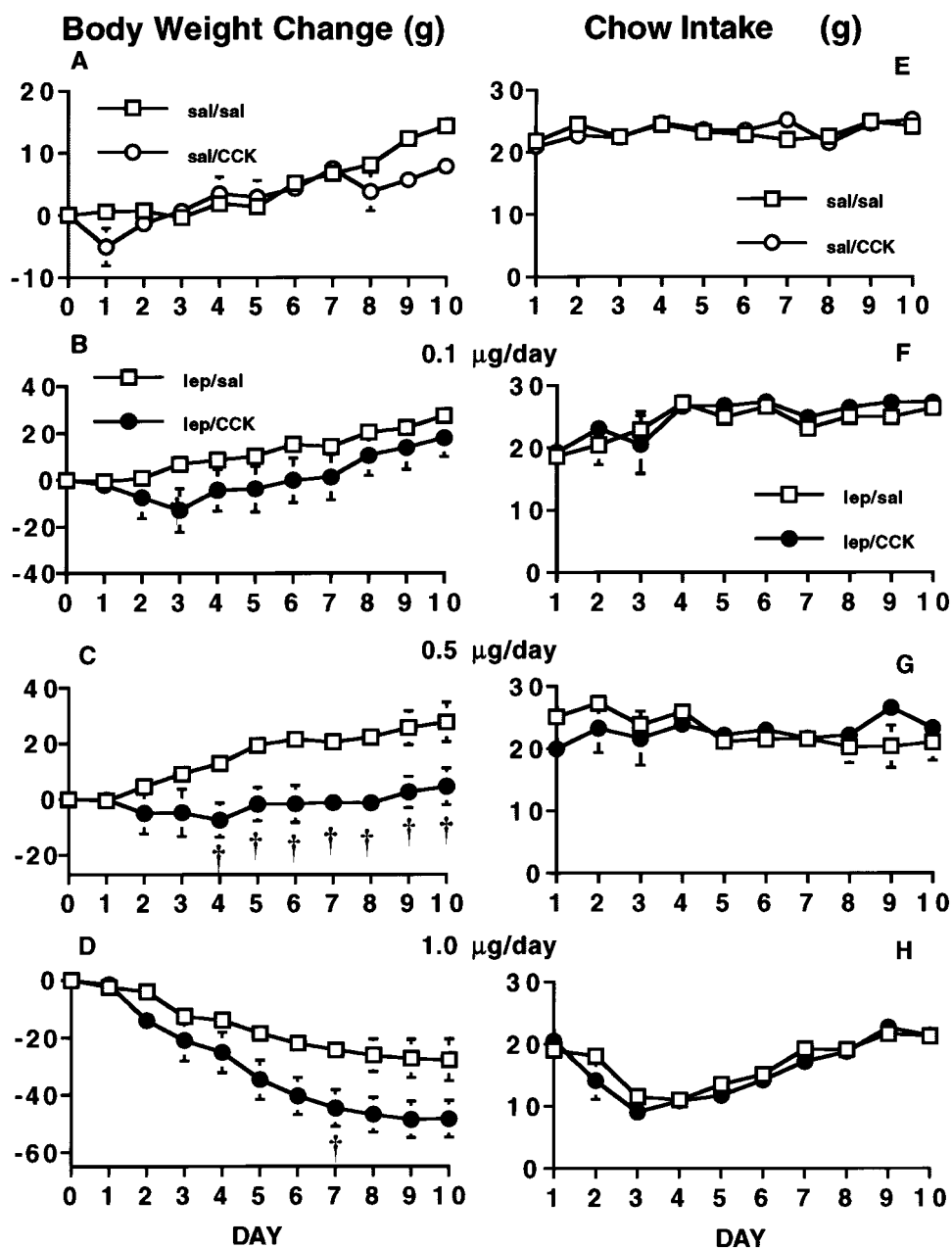


Fig. 1. CCK enhanced leptin-induced body weight loss but did not reduce daily food intake. The 10 consecutive days of treatment are shown (B–D). Weight loss during treatment with leptin was significantly enhanced by a single daily injection of a low dose of CCK (0.5 µg/kg) (†interaction of leptin and CCK, $P < 0.05$). There was a significant main effect of 1.0 µg/day leptin on body weight loss on days 2–9 and of 0.1 mg/day leptin on days 8 and 10, $P < 0.05$. The highest dose of leptin had a significant effect on daily intake, $P < 0.05$ (H); although there was no CCK-by-leptin interaction on daily intake at any time or dose (F–H). There was no effect of CCK on daily intake. Sal, saline; lep, leptin.

to return to pretreatment levels after the initial reduction.

We observed no significant synergy between CCK and leptin to reduce the size of a sucrose meal (Fig. 2). Both CCK and leptin had a significant main effect on 30-min intake: at the highest dose of leptin, both CCK and leptin significantly reduced intake on all days. At 0.5 µg/day, leptin reduced intake only on day 7, whereas 0.1 µg/day reduced intake on days 1–8. No significant interaction between leptin and CCK was observed on 30-min intake at any dose of leptin on any day.

DISCUSSION

Previously, we demonstrated that CCK enhances body weight loss following single (26, 27) or repeated

(25) bolus injection of leptin. Endogenous plasma leptin levels display some variability that is not correlated to actual changes in fat mass (reviewed in Ref. 28). However, it has been suggested that an integration occurs such that the important “overall” level of leptin is predicted by the amount of body fat. Because we observed enhanced body weight loss with leptin and CCK when the leptin was chronically infused, the present data suggest that it may be this integrated leptin level, rather than large fluctuations, that ultimately leads to enhanced weight loss in the presence of CCK. In other words, phasic elevation of CCK enhances the body weight lost in response to the integrated (background) leptin concentration.

Although CCK enhanced leptin-induced body weight loss, CCK did not enhance the reduction of daily food

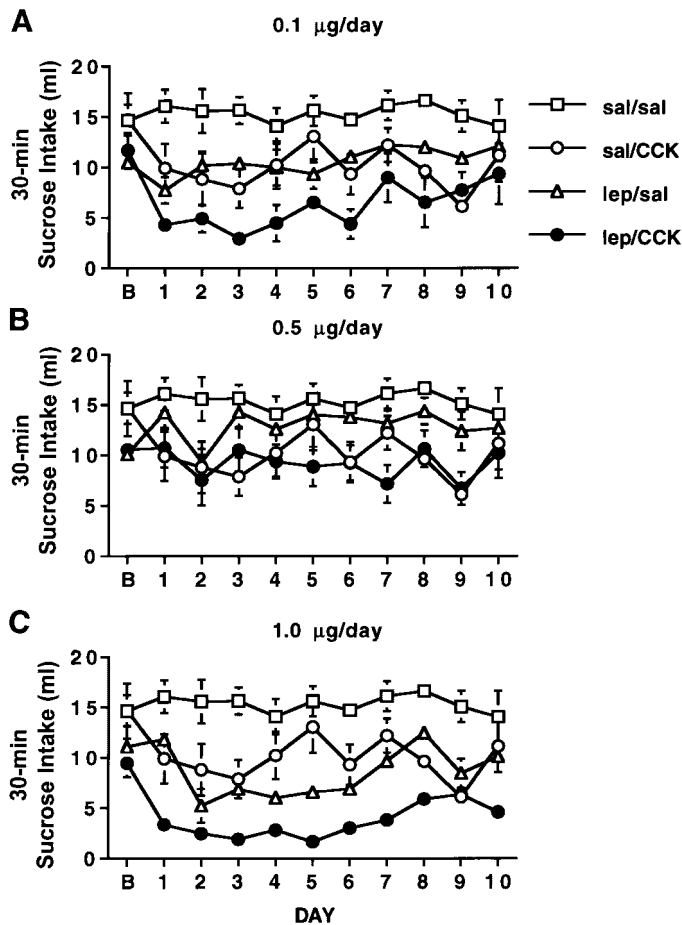


Fig. 2. No leptin-CCK synergy on 30-min meal size. We observed that both leptin and CCK had significant main effects on 30-min sucrose meal size, $P < 0.05$. However, no significant interaction was observed by 2×2 factorial ANOVA.

intake produced by leptin alone. In other words, rats treated with leptin plus CCK lost more weight without eating less food compared with those receiving leptin alone. Previously, we observed that CCK enhanced reduction of 48-h food intake following bolus administration of leptin plus CCK (25–27). However, these effects on 48-h intake appeared less robust than the effects of leptin and CCK on loss of body weight (25, 26). The present results are consistent with the interpretation that decreased feeding is not necessary for CCK to enhance leptin-induced weight loss.

We reported no synergy between CCK and bolus leptin to reduce meal size (25–27) via the same route of administration as the present study (lateral ventricle) and using the same dose of CCK (25, 26). It is possible that the timing of the leptin injection in our previous studies prevented us from observing an interaction between CCK and leptin to reduce meal size. Recently, Emond and colleagues (11) demonstrated significantly greater reduction of feeding after CCK and leptin when they shortened the interval between the two injections from 3 to 1 h using doses of leptin and CCK similar to those we employed. In the present study, leptin was chronically infused, but we observed no significant

interaction. It is also unlikely that the dose of leptin or CCK obscured an enhancement. These data do not support the conclusion that synergistic reduction of meal size occurs when both chronic leptin and bolus CCK are present.

To achieve body weight regulation, animals have been observed to employ many distinct strategies. Although feeding behavior is a common strategy that is relatively easy for the animal to control and for the experimenter to measure, it is not the only one. Thus, when one system is not or cannot be invoked to facilitate weight regulation, it can nevertheless occur. For example, when rats are deprived of food or food restricted for several days, they will reattain the body weight of nondeprived controls, despite never ingesting all of the lost calories during subsequent ad libitum feeding (21) or continued food restriction (31). This result clearly indicates the existence of alternative strategies able to compensate for the lost calories. In addition, it underscores that the operation of any individual mechanism, such as feeding, cannot necessarily predict the effect on body weight. Leptin has been recognized as a key participant in body weight regulation for this reason: it has effects on behaviors and physiology in addition to feeding, particularly on the many systems that comprise the category “energy expenditure.”

The observations described in the preceding paragraph, taken together with our data, lead to two hypotheses concerning the putative mechanisms by which leptin and CCK act synergistically on body weight. The first hypothesis is that if CCK participates directly in the regulation of body weight, rather than only the control of feeding, then CCK should enhance noningestive actions of leptin as well as the suppression of voluntary intake. This hypothesis predicts that enhanced body weight loss induced by CCK in the presence of leptin should be possible when no synergistic reduction of food intake is apparent. The appearance of body weight loss that is separable from reduced feeding (Fig. 1) is one of the most significant clues to how CCK and leptin may be interacting in these studies. If CCK enhances leptin-induced increases in energy expenditure, this strongly supports a novel role for CCK in the regulation of body weight in addition to its participation in the control of feeding behavior.

The second hypothesis concerns the probable neural site(s) where the integration of these signals can occur. It remains probable that CCK and leptin can interact in multiple distinct anatomic locations, for example, in the brain or in the periphery (3). We previously demonstrated that CCK acts in the periphery, whereas leptin acts in the brain to mediate synergistic body weight loss (25). We hypothesize that the neural signal generated by CCK in the periphery is communicated to the hindbrain via vagal afferents, and it is then relayed within the CNS to a site of integration with leptin-related afferent information. For the purposes of the regulation of body weight, we propose that the signal is integrated at a central site upstream from the divergence of efferent pathways controlling feeding, thermo-

genesis, metabolic rate, and voluntary energy expenditure.

Perspectives

The interaction between leptin and CCK described here and previously (25–27) was initially unexpected and remains somewhat counterintuitive to the current paradigm for the physiology of CCK. Identified in 1928 (16), our understanding of CCK's role in physiology has evolved conceptually many times. Subsequent to the pioneering report by Gibbs and colleagues (12) of potent reduction of meal size by CCK, an extensive literature has been devoted to CCK. Our lab admittedly shares in the affection for this multitalented peptide. It is in this context that we suggest these data support a novel action of CCK: as a meal-related signal that can facilitate the regulation of body weight via both feeding and feeding-independent mechanisms when it acts in concert with leptin.

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