

Long-term CCK-leptin synergy suggests a role for CCK in the regulation of body weight

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Matson, Claire A., and Robert C. Ritter. Long-term CCK-leptin synergy suggests a role for CCK in the regulation of body weight. *Am. J. Physiol.* 276 (*Regulatory Integrative Comp. Physiol.* 45): R1038–R1045, 1999.—The gut peptide CCK is a nutrient-related signal important to the control of food intake. In the present studies, we observed that a single intraperitoneal injection of CCK (1–2 µg/kg) given 2–3 h after intracerebroventricular leptin (2–5 µg) reduced body weight and chow intake over the ensuing 48 h more than did leptin alone. CCK alone had no effect on either 48-h chow intake or body weight but significantly reduced feeding during a 30-min sucrose test. However, reduction of 30-min sucrose intake by CCK was not enhanced by prior intracerebroventricular leptin. The present data suggest that CCK can contribute to the regulation of body weight when central leptin levels are elevated.

satiety; obesity; adiposity; food intake; gut peptide

THE GUT PEPTIDE CCK limits meal size (11, 12) by acting at CCK-A receptors (3, 14, 19, 36). CCK acts on small, unmyelinated vagal afferents that transmit the behaviorally relevant satiety signal to the brain stem (4, 5, 28). The satiating actions of intraperitoneal CCK are attenuated by abdominal vagotomy (29), by surgical or capsaicin-induced destruction of small, unmyelinated vagal sensory neurons (23), and by lesions of the vagal sensory termination in the area of the dorsomedial hindbrain (9).

Rats lacking CCK-A receptor expression are obese (10), do not reduce food intake in response to exogenous CCK, and display an altered meal pattern (20). Likewise, chronic administration of CCK antagonists has been reported to increase body weight in rats (17). However, whereas repeated administration of exogenous CCK can persistently reduce individual meal size, the number of meals eaten during this period of time is proportionally increased such that total daily caloric intake is not reduced by CCK (33). The inability of CCK to reduce food intake over an extended period has been interpreted to indicate that, although CCK contributes to the control of meal size, it does not have a role in the regulation of body adiposity. However, these data also reflect the subservience of the control of feeding to the regulation of body weight. Thus, although meal-related signals may not override adipose regulation, they may contribute to body weight regulation when they act in concert with adipose-related

signals such as the adipocyte hormone leptin (1, 6, 16, 34).

Previously, we reported that a single intraperitoneal injection of CCK given to ad libitum-fed mice had no effect on 24-h caloric intake or body weight. However, the same dose of CCK given in the presence of increased plasma leptin levels results in a significantly greater reduction in 24-h caloric intake than after leptin alone (15). This synergistic interaction between CCK and leptin suggests a role for CCK in the long-term control of food intake and the regulation of body weight. We hypothesize that this effect may be mediated, at least in part, by leptin acting in the brain. Therefore, in the present studies, we injected rats with microgram quantities of leptin into either the lateral or the third cerebral ventricle and administered CCK or saline intraperitoneally 2 or 3 h later. We observed that CCK and leptin reduced body weight and chow intake significantly more than did leptin alone. CCK alone had no effect on body weight or cumulative food intake. These data support the hypothesis that CCK contributes to the long-term control of food intake and the regulation of body weight through an interaction with the adipose hormone leptin.

GENERAL METHODS

Naive male Sprague-Dawley-derived rats (300–350 g; Simonsen Laboratories, Gilroy, CA) were housed individually in hanging wire cages in a temperature-controlled room set on a 12:12-h light-dark schedule with lights on at 8:00 AM. Rats were maintained ad libitum on pelleted chow (Harlan Teklad Diet no. 8664, 3.3 kcal/g) except as noted below. Recombinant murine leptin (PeproTech, Rocky Hill, NJ) was diluted in sterile water at a concentration of 1 µg/µl for intracerebroventricular injections. CCK-8 (E. R. Squibb and Sons, Princeton, NJ) solutions were diluted in sterile water to administer 1 µg/kg intraperitoneally. All sucrose tests and training were conducted using a 15% sucrose solution (0.6 kcal/ml) presented in 25-ml glass burette drinking tubes attached to the outside of the animal's cage for 30 min.

Data were analyzed by between-subjects or repeated-measures ANOVAs for overall effect and Fisher's least significant difference post hoc test or repeated-measures *t*-test for specific effects. Significance was set at $P < 0.05$.

EXPERIMENT 1

Methods. Sixteen rats were stereotaxically implanted with unilateral 23-gauge stainless steel cannulas aimed at the lateral cerebral ventricle using the following coordinates: –1.0 mm from bregma, ±1.5 mm from midline, and –3.9 mm from dura. Half of the rats were implanted to the left lateral ventricle, and the other half into the right. An obturator (30 gauge) with a beveled tip was fitted to protrude 0.5 mm from the tip of

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the cannula and remained in place when the cannula was not in use. After 5 days of recovery from surgery, the rats were adapted to an experimental regimen in which the 24-h period began and ended when the rats were weighed and chow was removed and weighed at 8:00 AM. Rats were also allowed access to a 15% sucrose solution for 30 min beginning at 11:45 AM each day. They were adapted to this procedure for 10 days until the intake of individual rats was stable across days, and they were trained to receive intraperitoneal injections of saline (1 ml/kg) immediately before the sucrose test on the 4 days before leptin treatment.

Patency of the lateral ventricle cannulas was assessed by intracerebroventricular injection of ANG II (50 ng/3 μ l sterile water). Only animals that drank more than 5 ml of water in the 30 min after ANG II administration were considered to have a patent ventricular placement. Animals included in the final analysis passed an ANG II test 5 days before and another 7 days after experimental treatment. The final *n* of this preliminary experiment was therefore small. A total of 12 rats were divided into four groups as follows: saline-saline, *n* = 2; CCK-saline, *n* = 3; saline-leptin, *n* = 4; CCK-leptin, *n* = 3.

On the day of treatment, rats received a single intracerebroventricular injection of either 5 μ l saline or 5 μ g leptin at 8:45 AM. Rats were given either saline or CCK-8 (1 μ g/kg) intraperitoneally at 11:45 AM and immediately presented with 15% sucrose for 30 min. Chow was returned after the sucrose was removed. Rats were sucrose tested again on the following 2 days at 11:45 AM without receiving a prior intraperitoneal injection. Chow intake and body weight data were collected at 8:00 AM 24 and 48 h thereafter.

Results. CCK-leptin combination enhanced the reduction of body weight after leptin, although CCK had no independent effect of body weight loss. Leptin alone caused a reduction of body weight at 24 and 48 h, and the combination of leptin plus CCK caused a significantly greater reduction than after leptin alone at both time points. There was a significant overall effect of treatment on body weight change from baseline at 24 and 48 h after treatment [$F(3,8) = 15.2$, $P < 0.01$, and $F(3,8) = 10.0$, $P < 0.01$, respectively]. CCK-leptin-treated rats lost more weight than did saline-leptin-treated rats at 24 and 48 h after treatment ($P < 0.01$ for both, Fig. 1). The body weight loss was not attenuated 48 h after CCK-leptin treatment compared with 24 h after treatment by paired Student's *t*-test ($P = 0.90$, Fig. 1). Body weight loss of saline-leptin-treated rats was significantly greater than that of saline-saline-treated rats 24 and 48 h after treatment ($P < 0.01$ for both). However, unlike CCK-leptin-treated rats, in saline-leptin rats this effect was attenuated after the first 24 h because the weight loss at 24 h after saline-leptin was significantly greater than the remaining loss at 48 h after treatment by paired Student's *t*-test ($P < 0.05$). There was no effect of CCK-saline on body weight change relative to saline-saline at 24 or 48 h after treatment ($P = 0.8$ and 0.7 , respectively).

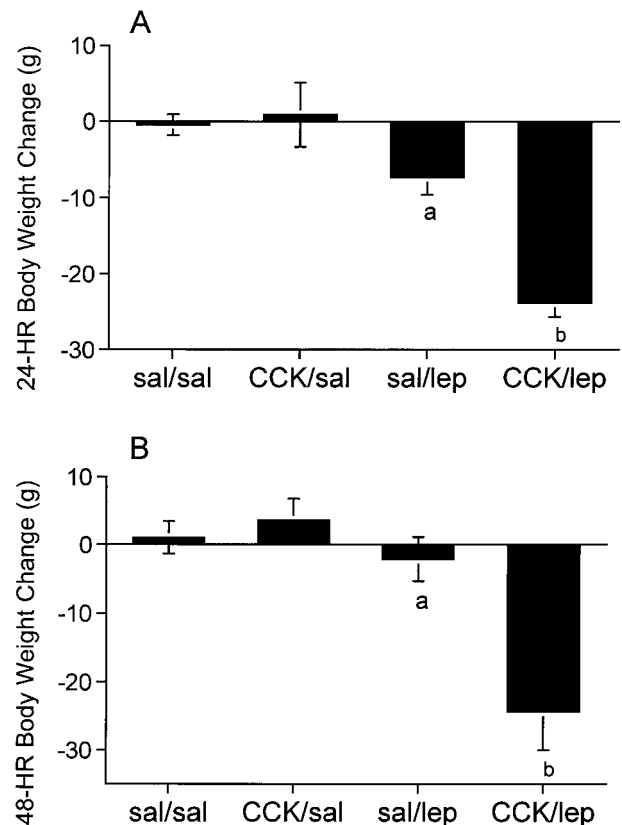


Fig. 1. CCK-leptin (lep) treatment reduces body weight significantly more than does saline (sal)-lep at 24 (A) and 48 h (B) after treatment. In *experiment 1*, CCK (1 μ g/kg) was given intraperitoneally and leptin (5 μ g) was given intracerebroventricularly into the lateral ventricle. ^aSal-lep-treated rats lost significantly more body weight (compared with baseline day before treatment) than sal- or CCK-treated rats ($P < 0.01$ for both); ^bCCK-lep-treated rats lost significantly more weight than sal-lep-treated rats ($P < 0.01$).

CCK also enhanced the reduction of daily chow intake after leptin, although CCK alone had no independent effect on daily chow intake. There was a significant overall effect of treatment on 24-h chow intake [$F(3,8) = 8.5$, $P < 0.01$]. Both saline-leptin and CCK-leptin significantly reduced 24-h chow intake compared with saline-saline ($P < 0.01$ for both). CCK-leptin did not reduce chow intake in the first 24-h period reliably more than saline-leptin ($P = 0.06$). There was no effect of CCK-saline on 24-h chow intake ($P = 0.57$). There was a significant overall effect of treatment on chow intake on the second day after treatment (48-h intake) [$F(3,8) = 13.8$, $P < 0.01$]. CCK-leptin treatment reduced 48-h chow intake significantly more than saline-leptin treatment, and saline-leptin reduced 48-h chow intake significantly more than saline-saline ($P < 0.01$ for both, Fig. 2). Cumulative 48-h chow intake was also significantly affected by treatment [$F(3,8) = 16.3$, $P < 0.01$]. CCK-leptin reduced cumulative 48-h intake to 50% of that consumed after saline-leptin, and saline-leptin reduced intake to 30% of that consumed after saline-saline ($P < 0.01$ for both, Fig. 2). There was no reliable effect of CCK-saline compared with saline-saline on chow intake after 24, 48, or cumulative 48 h ($P = 0.6$, 0.5 , and 0.4 , respectively).

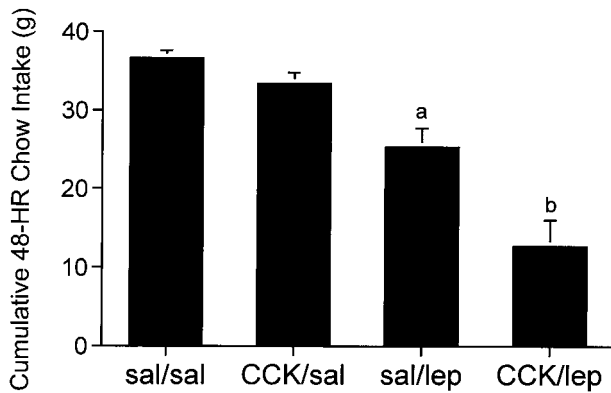


Fig. 2. CCK-lep treatment reduces 48-h cumulative chow intake significantly more than sal-lep treatment. In *experiment 1*, CCK (1 $\mu\text{g}/\text{kg}$) was given intraperitoneally and leptin (5 μg) was given intracerebroventricularly into lateral ventricle. ^aSal-lep significantly reduced intake compared with sal-sal, $P < 0.01$; ^bCCK-lep reduced intake compared with sal-lep, $P < 0.01$.

CCK and leptin did not synergistically suppress the size of an individual sucrose meal compared with the suppression after 1 $\mu\text{g}/\text{kg}$ CCK alone. There was a significant overall effect of treatment on 30-min sucrose intake as a percent of baseline sucrose intake [$F(3,14) = 6.0$, $P < 0.05$, Table 1]. Both CCK alone and leptin alone reduced sucrose intake as a percent of baseline significantly more than saline-saline ($P < 0.01$ and $P < 0.05$, respectively). There was no reliable difference between the percent baseline sucrose intake of CCK-leptin-treated and CCK-saline-treated rats ($P = 0.28$), with CCK-leptin-treated rats drinking slightly more sucrose than CCK-saline-treated rats. There was no significant overall effect of treatment on sucrose intake 24 or 48 h after treatment [$F(3,8) = 1.6$, $P = 0.27$, and $F(3,8) = 2.2$, $P = 0.17$, respectively, Table 1].

Table 1. Reduced meal intake of 15% sucrose after CCK is not significantly enhanced by leptin-CCK combination

	Baseline	Day of Treatment	24 h After Treatment	48 h After Treatment
<i>Experiment 1</i>				
Saline-saline	8.4 \pm 3.2	11.9 \pm 4.5	10.5 \pm 3.1	11.0 \pm 1.6
CCK-saline	11.1 \pm 1.7	3.3 \pm 1.0	5.7 \pm 0.8	11.5 \pm 1.0
Saline-leptin	8.9 \pm 1.0	6.6 \pm 0.8	9.2 \pm 0.6	10.4 \pm 1.4
CCK-leptin	10.8 \pm 0.5	5.8 \pm 2.6	9.9 \pm 2.8	8.0 \pm 0.6
<i>Experiment 2</i>				
Saline-saline	12.3 \pm 1.1	7.8 \pm 1.3	9.6 \pm 1.1	10.2 \pm 0.9
CCK-saline	12.0 \pm 2.5	3.9 \pm 1.5	10.9 \pm 0.7	10.4 \pm 1.1
Saline-leptin	13.1 \pm 0.6	8.0 \pm 1.0	9.5 \pm 0.2	9.5 \pm 0.7
CCK-leptin	13.8 \pm 2.6	1.1 \pm 0.5	10.4 \pm 2.1	11.6 \pm 1.7
<i>Experiment 3</i>				
Saline-saline	12.4 \pm 1.3	8.8 \pm 1.0	10.2 \pm 1.1	10.9 \pm 1.4
CCK-saline	12.5 \pm 1.4	2.9 \pm 1.3	10.5 \pm 1.0	11.1 \pm 1.5
Saline-leptin	13.1 \pm 1.1	6.8 \pm 0.7	8.9 \pm 0.7	9.7 \pm 0.7
CCK-leptin	12.3 \pm 1.2	1.2 \pm 0.3	8.9 \pm 0.9	8.7 \pm 1.0

Values are mean sucrose intakes (in ml) \pm SE. In *experiment 1*, CCK (1 $\mu\text{g}/\text{kg}$) was given intraperitoneally and leptin (5 μg) was given intracerebroventricularly into lateral ventricle. In *experiments 2* and *3*, CCK (2 $\mu\text{g}/\text{kg}$) was given intraperitoneally and leptin (2 μg) was given intracerebroventricularly into third ventricle. There was no significant synergistic effect of CCK-leptin on a single meal compared with CCK-saline in any of the 3 experiments.

Discussion. CCK-leptin treatment produced a greater reduction of body weight and a greater reduction of daily chow intake than did saline-leptin (Figs. 1 and 2). During the first 24 h after treatment, CCK-leptin rats did not reduce chow intake significantly more than saline-leptin-treated rats (although there was a trend in that direction), but they reduced their body weight by threefold more than did saline-leptin-treated rats during this interval and more than 10-fold more than saline-leptin rats after 48 h. These results suggest that reduced caloric intake may not entirely account for the difference in body weight loss between the saline-leptin and CCK-leptin-treated rats.

The relatively long-term nature of these effects on daily food intake and body weight is consistent with the results of our previous report (15). Although CCK-leptin did not produce a significant reduction in chow intake compared with saline-leptin during the first 24 h after treatment, the combination of these two peptides did reduce intake significantly more than after saline-leptin during the second day after treatment (48 h) and during the cumulative 48 h after treatment. Furthermore, whereas the effect of leptin alone on the reduction of body weight was attenuated 48 h after treatment, the reduction in body weight after CCK-leptin was maintained through the second day.

Finally, reduction of 30-min sucrose intake by CCK was not enhanced by prior intracerebroventricular leptin (Table 1). This result is also consistent with our previous report (15), in which leptin given into the periphery 2 and 15 h before intraperitoneal CCK in mice did not significantly enhance the reduction of intake of an individual 30-min Ensure meal.

EXPERIMENT 2

Methods. A naive group of comparable rats was accustomed to the following schedule. The beginning of each 24-h period was at 8:00 AM, when body weights were taken and chow was removed and weighed; a 30-min sucrose test was given at 11:30 AM, chow was returned after the sucrose test, and rats were allowed ad libitum access to chow until 8:00 AM the following day. Rats were adapted to this schedule until a stable daily intake of the sucrose was observed (10 days).

Rats were then implanted with a 23-gauge stainless steel cannula aimed at the third ventricle using the following coordinates: from bregma, AP -2.2 mm; ML 0 mm; and -8.0 mm ventral from dura. An obturator (30 gauge) remained in place when the cannula was not in use. The rats were allowed to recover for 3–5 days on ad libitum chow and water before reintroduction to the daily testing schedule described above. Cannula placement and patency were confirmed by a drinking response to ANG II (50 ng/3 μl) 6–8 days after surgery, as described in *experiment 1*. The rats were maintained on the sucrose testing schedule for 8 days after reinstatement and before experimental treatment.

Rats were divided into four weight-matched groups: saline-saline, $n = 5$; CCK-saline, $n = 4$; saline-leptin, $n = 4$; CCK-leptin, $n = 5$. All rats received two identical intracerebroventricular injections of either 2 μg leptin

or 2 μ l saline at 5:30 PM on the day before and at 8:00 AM on the day of treatment. Baseline body weight was collected as usual at 8:00 AM in the morning, and the first intracerebroventricular injection was given that evening (at 5:30 PM). The second intracerebroventricular injection was followed by either CCK (2 μ g/kg) or sterile saline intraperitoneally at 11:45 AM. The intraperitoneal injection was immediately followed by the presentation of the sucrose for 30 min. Chow was returned after the sucrose test, and body weight and chow intake were measured 24 and 48 h afterward.

Results. As in the previous experiment, CCK-leptin combination enhanced the reduction of body weight after leptin. The combination of leptin and CCK caused a significantly greater reduction of body weight than after leptin alone at both 24 and 48 h after intraperitoneal injection of 2 μ g/kg CCK. There was a significant overall effect of treatment on body weight change from baseline during the first 24 h after treatment [$F(3,14) = 5.1$, $P < 0.05$]. There was no reliable effect of either CCK or leptin only compared with saline-saline ($P = 0.30$ and $P = 0.17$, respectively). CCK plus leptin, however, caused a significantly greater loss of body weight than either saline-saline ($P < 0.01$), CCK-saline ($P < 0.01$), or saline-leptin ($P < 0.05$). There was also a significant overall effect of treatment on body weight change from baseline 48 h after treatment [$F(3,14) = 9.8$, $P < 0.01$, Fig. 3]. As at 24 h, there was no reliable effect of either CCK-saline ($P = 0.18$) or saline-leptin ($P = 0.15$) on body weight change 48 h after treatment. CCK-leptin caused a significantly greater loss of body weight 48 h after treatment than either saline-leptin ($P < 0.05$), CCK-saline ($P < 0.01$), or saline-saline ($P < 0.01$).

Consistent with the results of *experiment 1*, CCK again enhanced the reduction of daily chow intake after leptin, although CCK alone had no effect on daily chow intake. There was a significant overall effect of treatment on 24-h chow intake [$F(3,14) = 5.1$, $P < 0.01$]. Only CCK-leptin treatment reduced 24-h intake compared with saline-saline ($P < 0.01$). Neither CCK-saline nor saline-leptin significantly reduced chow intake at 24 h ($P = 0.41$ and 0.06 , respectively). CCK-leptin did not significantly reduce 24-h chow intake compared with saline-leptin ($P = 0.17$). There was a significant overall effect of treatment on chow intake the second day after treatment (48-h intake) [$F(3,14) = 12.4$, $P < 0.01$]. CCK-leptin treatment reduced 48-h chow intake significantly more than did saline-leptin ($P < 0.05$), whereas saline-leptin reduced 48-h chow intake significantly more than did saline-saline ($P < 0.05$). There was no effect of CCK-saline compared with saline-saline ($P = 0.24$). There was also a significant overall effect of treatment on cumulative chow intake during the 48 h after treatment [$F(3,14) = 15.4$, $P < 0.01$, Fig. 4]. CCK-leptin reduced cumulative 48-h chow intake significantly more than did saline-leptin ($P < 0.05$), and saline-leptin significantly reduced feeding compared with saline-saline ($P < 0.05$) to 20% of that after saline-saline. There was no reliable effect of CCK-saline on cumulative 48-h intake ($P = 0.80$).

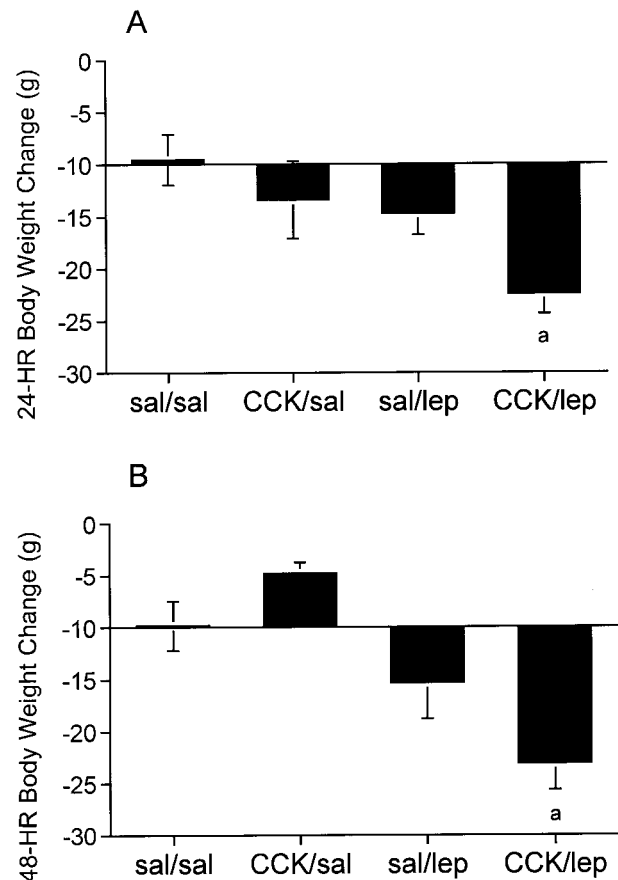


Fig. 3. CCK-lep-treated rats lost significantly more body weight 24 (A) and 48 h (B) after treatment than did sal-lep-treated rats. In *experiment 2*, CCK (2 μ g/kg) was given intraperitoneally and leptin was given intracerebroventricularly into third ventricle in 2 widely spaced bolus injections of 2 μ g each. ^aCCK-lep-treated rats lost significantly more body weight than sal-lep, $P < 0.05$.

Finally, there was again no significant synergy between leptin and CCK to reduce the size of an individual sucrose meal, because reduction of 30-min sucrose intake by CCK was not enhanced by prior intracerebroventricular leptin. There was a significant

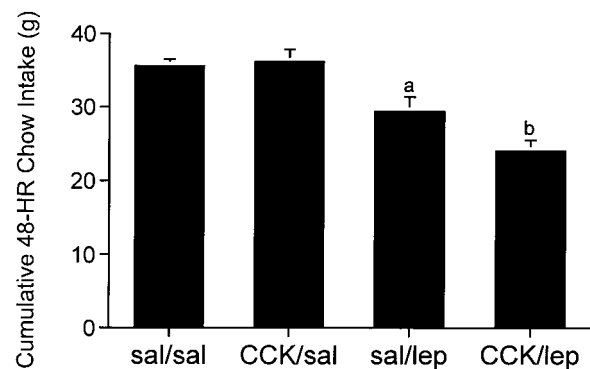


Fig. 4. CCK-lep reduced cumulative 48-h chow intake significantly more than did sal-lep. In *experiment 2*, CCK (2 μ g/kg) was given intraperitoneally and leptin was given intracerebroventricularly into third ventricle in 2 widely spaced bolus injections of 2 μ g each. ^aSal-lep significantly reduced chow intake compared with sal-sal, $P < 0.05$; ^bCCK-lep reduced chow intake significantly more than did sal-lep, $P < 0.05$.

overall effect of treatment on sucrose intake as a percent of baseline intake on the day of treatment [$F(3,14) = 6.6$, $P < 0.01$, Table 1]. CCK-saline did not reliably reduce sucrose intake compared with saline-saline, although there was a trend in that direction ($P = 0.06$). There was no reliable difference between saline-saline and saline-leptin ($P = 0.68$). There was no reliable difference between the reduction of intake after CCK-saline and CCK-leptin; however, there was a trend in that direction ($P = 0.09$), and CCK-leptin did significantly reduce intake compared with saline-saline ($P < 0.01$). There was no effect of treatment on sucrose intake on the day after treatment [$F(3,14) = 1.1$, $P = 0.38$].

Discussion. The results of this experiment are comparable to those obtained in *experiment 1* in that CCK-leptin treatment produced a robust, prolonged reduction of body weight and chow intake compared with saline-leptin treatment. Suppression of sucrose intake after CCK-leptin treatment was not significantly enhanced compared with the suppression after CCK-saline on either the day of treatment or on the following days. These results suggest that the CCK-leptin interaction observed in these experiments may be independent of the well-described effects of CCK on meal size. Furthermore, they are consistent with the results of our previous report (15), in which we administered the leptin in two widely spaced injections. There did not appear to be any difference in the effectiveness of leptin to mediate this interaction when it was administered via the third as opposed to the lateral ventricular route.

EXPERIMENT 3

Methods. The rats used in *experiment 2* were maintained on the same daily schedule: chow was removed and weighed, and body weights were collected at 8:00 AM. Rats were allowed 30-min sucrose access beginning at 11:00 AM. Beginning 1 wk after the completion of *experiment 2*, rats received the four basic treatments (saline-saline, CCK-saline, saline-leptin, CCK-leptin) in counterbalanced order. For treatment days, rats were given an intracerebroventricular injection of either saline or 2 μ g leptin at 5:00 PM on the day before and the same solution (saline or leptin) at 7:00 AM on the day of testing. CCK (2 μ g/kg ip) or saline was given immediately before presentation of the sucrose at 11:00 AM. Chow was returned immediately after the 30-min access to sucrose, and intake was recorded at 24 and 48 h after treatment.

There was a minimum interval of 5 days between each treatment condition, allowing recovery of basal levels of chow intake. However, even after 8 days, rats given saline-leptin or CCK-leptin initially did not regain sufficient body weight to match that of rats given the saline-saline or CCK-saline initially, although the rate of body weight growth appeared comparable. This lasting effect of leptin treatment on body weight has been reported previously (8). Therefore, body weight data were not analyzed in this repeated-measures design. Only animals that completed all four testing

conditions and passed ANG II tests before and after completion were included in the analysis ($n = 12$).

Data were analyzed using repeated-measures ANOVAs and repeated-measures t -tests between conditions.

Results. As in the two previous experiments, there was significant synergy between leptin and CCK to reduce daily chow intake compared with leptin alone. The overall effect of treatments on 24-h chow intake was significant [$F(3,33) = 13.80$, $P < 0.01$, Fig. 5]. CCK-leptin treatment reduced chow intake significantly compared with saline-leptin [$t(11) = 2.49$, $P < 0.05$] and saline-saline [$t(11) = 6.02$, $P < 0.01$]. Saline-leptin treatment significantly reduced 24-h chow intake compared with saline-saline [$t(11) = 3.06$, $P = 0.01$]. CCK alone had no reliable effect on 24-h chow intake [$t(11) = 1.00$, $P = 0.34$].

Also consistent with the previous two experiments, there was no synergy between leptin and CCK to reduce the size of a sucrose meal. The overall effect of treatment on 30-min sucrose intake was significant [$F(3,33) = 14.041$, $P < 0.01$, Table 1]. CCK-saline treatment significantly reduced sucrose intake compared with saline-saline [$t(11) = 3.74$, $P < 0.01$]. There was no independent or synergistic effect of leptin treatment on 30-min sucrose intake, and there was no reliable difference between the saline-leptin and saline-saline conditions [$t(11) = 1.421$, $P = 0.18$] or between the CCK-saline and CCK-leptin conditions [$t(11) = 1.161$, $P = 0.27$].

Discussion. The results of these experiments indicate that when intracerebroventricular leptin injection is followed by peripheral injection of CCK, rats lose significantly more body weight than when leptin alone is given. In two separate experiments, the CCK enhancement of leptin-induced weight loss was significant both 24 and 48 h after a single injection of CCK. CCK-leptin-treated rats reduced their body weight by threefold more than did saline-leptin-treated rats at 24 h and more than 10-fold more than saline-leptin rats after 48 h in *experiment 1*. Furthermore, although rats treated with 5 μ g leptin administered into the lateral ventricle were beginning to regain lost weight by 48 h, rats treated with CCK and leptin did not begin to regain

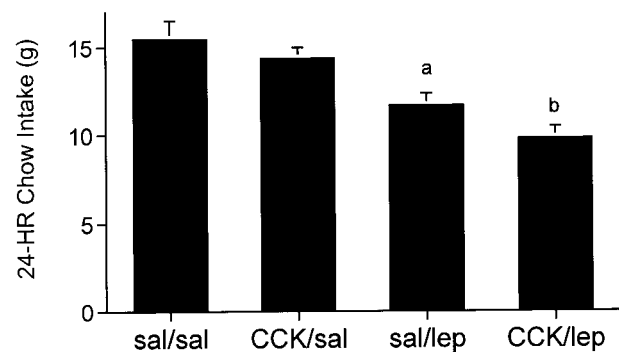


Fig. 5. CCK-lep reduces 24-h chow intake significantly more than does sal-lep. In *experiment 3*, CCK (2 μ g/kg) was given intraperitoneally and leptin was given intracerebroventricularly into third ventricle in 2 widely spaced bolus injections of 2 μ g each. ^aSal-lep reduced intake compared with sal-sal, $P < 0.05$; ^bCCK-lep reduced intake compared with sal-lep, $P < 0.05$.

weight during this same interval. These results suggest that in addition to producing significantly greater weight loss, CCK and leptin given in combination may also extend the length of time over which leptin reduces body weight.

In the three separate experiments presented above, CCK-leptin treatment produced a greater reduction of chow intake than did saline-leptin. In both *experiments 1* and *2*, the effect of CCK-leptin treatment was significantly greater than that of saline-leptin during the second day after treatment (48 h) as well as over the cumulative 48 h after treatment. Furthermore, in a repeated-measures design (*experiment 3*), CCK-leptin treatment reduced 24-h chow intake significantly more than did saline-leptin during the same interval. These data are consistent with our previous report using systemically administered leptin in mice (15) and support the hypothesis that the effects of peripheral CCK-leptin treatment can be mediated, at least in part, by leptin acting in the brain. Although these data do not rule out a contribution of peripherally acting leptin to the long-term CCK-leptin interaction, they do indicate that leptin administered into the brain is sufficient to mediate this effect.

Although the combination of CCK and leptin produced a profound reduction of 48-h food intake, reduced caloric intake may not entirely account for the difference in body weight loss between saline-leptin and the CCK-leptin-treated rats. During the first 24 h after treatment in *experiments 1* and *2*, rats that received leptin and CCK did not reduce chow intake significantly more than rats treated with leptin alone. However, as discussed above, CCK-leptin-treated rats lost significantly more body weight than rats treated with leptin alone during this same interval. These results suggest that the CCK-leptin interaction on body weight observed in these experiments might not depend entirely on the well-described effects of CCK to reduce the size of an individual meal. If so, it is possible that the combination of leptin and CCK might enhance metabolic rate or thermogenesis as well as reduce feeding behavior.

Finally, although there may have been an independent effect of leptin on the sucrose meal, leptin did not enhance the suppression of sucrose intake by CCK. This result is consistent with our previous report of long- but not short-term synergy in mice when leptin was administered 2 and 15 h before CCK. However, short-term synergy between leptin and CCK has been reported in mice (2, 30, 31) after simultaneous administration of the two peptides. Taché and colleagues (2) observed that when leptin and CCK were administered simultaneously into the periphery of fasted mice, the mice that received both CCK and leptin significantly reduced their intake of pelleted chow at 1 and 2 h after presentation compared with saline-treated mice. Mice that received either CCK or leptin alone did not reduce their intake. The intake of the CCK- and leptin-treated mice was no longer reduced by 6 h after treatment, suggesting that this effect is not a long-term interaction. This short-term synergy may be mediated

by an enhancement of CCK signaling via the vagus in the presence of increased extracellular leptin (31). Taché and colleagues have not reported whether the short-term synergy they observed is also apparent at the level of an individual meal, as their first time point is 1 h after chow presentation. The absence of synergy between CCK and leptin at the level of the single, scheduled meal in our previous report (15) and in the experiments reported above, suggests that the long-term CCK-leptin interaction is probably not dependent on the same mechanisms as the short-term synergistic effect on chow intake after simultaneous peripheral leptin and CCK administration (2, 30, 31). Furthermore, as we have observed that direct intracerebroventricular administration of leptin is sufficient to mediate this effect, the long-term CCK-leptin interaction we report here should not depend on direct leptin action on the peripheral vagus.

There is compelling evidence to suggest that body adiposity is actively maintained at a defended level. For example, both underfeeding (24) and overfeeding (27) produce regulatory responses to regain the previously maintained body weight. Likewise, surgical removal of fat (lipectomy) and surgical introduction of excess fat produce regulatory responses, including hyper- and hypophagia, respectively. If hyperphagia is prevented after weight loss, alterations in resting metabolism and temperature eventually restore the defended level of adiposity (for review, see Ref. 13). The defended level of a mature animal is not a fixed set point per se, in that certain conditions may alter the defended level of adiposity (21). Adipose-related signals such as the adipocyte hormone leptin (6, 16, 33) and the pancreatic hormone insulin, when it acts within the central nervous system (25, 26, 33), inform the brain of the current level of adiposity. Administration of leptin or central insulin results in decreased body adiposity due to hypophagia, increased metabolic rate, and increased thermogenesis (16, 25, 33). Thus animals appear to respond to changes in leptin and central insulin as they would to actual changes in adipose stores.

We hypothesize that non-adipose-related signals such as CCK may also play a role in the regulation of adiposity. However, many previous studies have reported that exogenous CCK has little effect on the control of feeding beyond the individual meal or on body weight. West et al. (32) provided an elegant demonstration that CCK alone does not alter the long-term control of feeding by computer-controlled infusion of CCK at the beginning of each spontaneously initiated meal. They reported that meal size was persistently decreased, but meal frequency was proportionally increased so that cumulative daily intake was unchanged. Furthermore, there was only a very small decrease in body weight (32). However, our present data suggest that CCK can contribute to the long-term control of feeding and the regulation of body weight when central leptin levels are elevated. Such elevation would occur naturally when the level of adiposity is increased. Therefore, we suggest that CCK contributes to body weight regulation when body adiposity is

elevated above its defended level as indicated by excursions in adipose-related signals such as leptin.

The hypothesis that CCK may contribute to the regulation of body adiposity is supported by several recent reports. The Otsuka Long-Evans Tokushima fatty rat is an obese rodent model that was recently reported to have a congenital mutation in the CCK-A receptor gene (10), rendering the obese rats insensitive to CCK (20). Although they may have additional, unknown traits that predispose toward excess body fat, the obesity of these rats suggests that sensitivity to CCK may be necessary for the efficient regulation of body adiposity. Furthermore, although human CCK-receptor mutations appear to be rare (22), there has also been a case report of a congenital defect in CCK-A receptor expression coincident with obesity in a human patient (18), and cholesterol gallstone disease, which has been suggested to involve decreased CCK functioning (22), is highly associated with obesity in the human population. If CCK does play a role in the regulation of body fat, both genetic and nongenetic causes of diminished CCK sensitivity may play an important role in the pathogenesis of human obesity. For example, recently our lab reported that the satiating effects of CCK are attenuated in rats fed a high-fat diet (7). In light of the present results suggesting that CCK may play an important role in the regulation of body weight, this high-fat diet-induced reduction in CCK sensitivity may contribute to the pathogenesis of obesity that often occurs coincident with a high fat content in the diet.

In conclusion, we reported that a single bolus injection of CCK administered 2–3 h after intracerebroventricular injection of microgram quantities of leptin causes significantly greater loss of body weight and a greater reduction of feeding compared with intracerebroventricular leptin alone, whereas CCK alone has no effect on these parameters. We propose that these results are indicative of an important role in the regulation of body adiposity for CCK.

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REFERENCES

- Bado, A., S. Levasseur, S. Attoub, S. Kermorgant, J. Laigneau, M. Bortoluzzi, L. Moizo, T. Lehy, M. Guerremillo, Y. Le Marchand-Brustel, and M. J. M. Lewin. The stomach is a source of leptin. *Nature* 394: 790–793, 1998.
- Barrachina, M. D., V. Martinez, L. Wang, J. Y. Wei, and Y. Taché. Synergistic interaction between leptin and cholecystokinin to reduce short-term food intake in lean mice. *Proc. Natl. Acad. Sci. USA* 94: 10455–10460, 1997.
- Brenner, L., and R. C. Ritter. Peptide cholecystokinin receptor antagonist increases food intake in rats. *Appetite* 24: 1–9, 1995.
- Brenner, L., D. P. Yox, and R. C. Ritter. Suppression of sham feeding by intraintestinal nutrients is not correlated with plasma cholecystokinin elevation. *Am. J. Physiol.* 264 (Regulatory Integrative Comp. Physiol. 33): R972–R976, 1993.
- Calingasan, N., S. Ritter, R. Ritter, and L. Brenner. Low-dose near celiac arterial cholecystokinin suppresses food intake in rats. *Am. J. Physiol.* 263 (Regulatory Integrative Comp. Physiol. 32): R572–R577, 1992.
- Considine, R. V., and J. F. Caro. Leptin and the regulation of body weight. *Int. J. Biochem. Cell Biol.* 29: 1255–1272, 1997.
- Covasa, M., and R. C. Ritter. Rats maintained on high fat diets exhibit reduced satiety in response to CCK and bombesin. *Peptides* 19: 1407–1415, 1998.
- Cusin, I., F. Rohner-Jeanrenaud, A. Stricker-Krongard, and B. Jeanrenaud. The weight-reducing effect of an intracerebroventricular bolus injection of leptin in genetically obese *fal/fa* rats. *Diabetes* 45: 1446–1450, 1996.
- Edwards, G. L., E. E. Ladenheim, and R. C. Ritter. Dorsomedial hindbrain participation in cholecystokinin-induced satiety. *Am. J. Physiol.* 251 (Regulatory Integrative Comp. Physiol. 20): R971–R977, 1986.
- Funakoshi, A., K. Miyasaka, H. Shinozaki, M. Masuda, T. Kawanami, Y. Takata, and A. Komo. An animal model of congenital defect of gene expression of cholecystokinin (CCK)-A receptor. *Biochem. Biophys. Res. Commun.* 210: 787–796, 1995.
- Gibbs, J., R. C. Young, and G. P. Smith. Cholecystokinin decreases food intake in rats. *J. Comp. Physiol. Psychol.* 84: 488–495, 1973.
- Gibbs, J., R. C. Young, and G. P. Smith. Cholecystokinin elicits satiety in rats with open gastric fistulas. *Nature* 245: 323–325, 1973.
- Keese, R. E., and T. L. Powley. The regulation of body weight. *Annu. Rev. Psychol.* 37: 109–133, 1986.
- Leighton, G. E., T. Griesbacher, R. G. Hill, and J. Hughes. Antagonism of central and peripheral anorectic effects of caerulein by L-364,718. *Eur. J. Pharmacol.* 161: 255–258, 1989.
- Matson, C. A., M. F. Wiater, J. L. Kuiper, and D. S. Weigle. Synergy between leptin and cholecystokinin (CCK) to control daily caloric intake. *Peptides* 18: 1275–1278, 1997.
- Matson, C. A., M. F. Wiater, and D. S. Weigle. Leptin and the regulation of body adiposity. *Diab. Rev.* 4: 488–508, 1996.
- Meeris-Schwanke, K., H. Klonowski-Stumpe, L. Herberg, and C. Niederau. Long-term effects of CCK-agonist and -antagonist on food intake and body weight in Zucker lean and obese rats. *Peptides* 19: 291–299, 1998.
- Miller, L. J., E. L. Holicky, C. D. Ulrich, and E. D. Wieben. Abnormal processing of the human cholecystokinin receptor gene in association with gallstones and obesity. *Gastroenterology* 109: 1375–1380, 1995.
- Moran, T. H., P. J. Ameglio, G. J. Schwartz, and P. R. McHugh. Blockade of type A, but not type B, CCK receptors attenuates satiety actions of exogenous and endogenous CCK. *Am. J. Physiol.* 262 (Regulatory Integrative Comp. Physiol. 31): R46–R50, 1992.
- Moran, T. H., L. F. Katz, C. R. Plata-Salaman, and G. J. Schwartz. Disordered food intake and obesity in rats lacking cholecystokinin A receptors. *Am. J. Physiol.* 274 (Regulatory Integrative Comp. Physiol. 43): R618–R625, 1998.
- Mrosovsky, N. *Rheostasis: The Physiology of Change*. New York: Oxford Univ., 1990.
- Nardone, J., I. A. Ferber, and L. J. Miller. The integrity of the cholecystokinin receptor gene in gallbladder disease and obesity. *Hepatology* 22: 1751–1753, 1995.
- Ritter, R. C., and E. Ladenheim. Capsaicin pretreatment attenuates suppression of food intake by cholecystokinin. *Am. J. Physiol.* 248 (Regulatory Integrative Comp. Physiol. 17): R501–R504, 1985.
- Schwartz, M. W., M. F. Dallman, and S. C. Woods. Hypothalamic responses to starvation: implications for the study of wasting disorders. *Am. J. Physiol.* 269 (Regulatory Integrative Comp. Physiol. 38): R949–R957, 1995.
- Schwartz, M. W., D. P. Figlewicz, D. G. Baskin, S. C. Woods, and D. Porte, Jr. Insulin in the brain: a hormonal regulator of energy balance. *Endocr. Rev.* 13: 387–414, 1992.
- Schwartz, M. W., and R. J. Seeley. The new biology of body weight regulation. *J. Am. Diet. Assoc.* 97: 54–60, 1997.
- Seeley, R. J., C. A. Matson, M. Chavez, S. C. Woods, M. F. Dallman, and M. W. Schwartz. Behavioral, endocrine, and hypothalamic responses to involuntary overfeeding. *Am. J.*

- Physiol.* 271 (*Regulatory Integrative Comp. Physiol.* 40): R819–R823, 1996.
28. **Smith, G. P., D. Greenberg, J. D. Falsco, A. A. Avilion, J. Gibbs, R. A. Liddle, and J. A. Williams.** Endogenous cholecystokinin does not decrease food intake or gastric emptying in fasted rats. *Am. J. Physiol.* 257 (*Regulatory Integrative Comp. Physiol.* 26): R1462–R1466, 1989.
29. **Smith, G. P., C. Jerome, B. J. Cushin, R. Eterno, and K. J. Simansky.** Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. *Science* 213: 1036–1037, 1981.
30. **Wang, L., V. Martínez, M. D. Barrachina, and Y. Taché.** Fos expression in the brain induced by peripheral injection of CCK or leptin plus CCK in fasted lean mice. *Brain Res.* 791: 157–166, 1998.
31. **Wang, Y. H., Y. Taché, A. B. Sheibel, V. L. W. Go, and J. Y. Wei.** Two types of leptin-responsive gastric vagal afferent terminals: an in vitro single-unit study in rats. *Am. J. Physiol.* 273 (*Regulatory Integrative Comp. Physiol.* 42): R833–R837, 1997.
32. **West, D. B., D. Fey, and S. C. Woods.** Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats. *Am. J. Physiol.* 246 (*Regulatory Integrative Comp. Physiol.* 15): R776–R787, 1984.
33. **Woods, S. C.** Insulin and the brain: a mutual dependency. *Prog. Psychobiol. Physiol. Psychol.* 16: 53–81, 1995.
34. **Woods, S. C., R. J. Seeley, D. Porte, Jr., and M. W. Schwartz.** Signals that regulate food intake and energy homeostasis. *Science* 280: 1378–1383, 1998.
35. **Yox, D. P., L. Brenner, and R. C. Ritter.** CCK receptor antagonists attenuate suppression of sham feeding by intestinal nutrients. *Am. J. Physiol.* 262 (*Regulatory Integrative Comp. Physiol.* 31): R554–R561, 1992.

