

# Cholecystokinin and leptin act synergistically to reduce body weight

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**Matson, Claire A., Dana F. Reid, Todd A. Cannon, and Robert C. Ritter.** Cholecystokinin and leptin act synergistically to reduce body weight. *Am J Physiol Regulatory Integrative Comp Physiol* 278: R882–R890, 2000.—Leptin, the product of the obese gene, reduces food intake and body weight in rats and mice, whereas administration of the gut-peptide CCK reduces meal size but not body weight. In the current experiments, we report that repeated daily combination of intracerebroventricular leptin and intraperitoneal CCK results in significantly greater loss of body weight than does leptin alone. However, leptin plus CCK treatment does not synergistically reduce the size of individual 30-min sucrose meals during this period, and the effect of leptin-CCK combination on daily chow intake, while significant, is small compared with the robust effects on body weight loss. This synergistic effect on body weight loss depends on a peripheral action of CCK and a central action of leptin. These data suggest a previously unsuspected role for CCK in body weight regulation that may not depend entirely on reduction of feeding behavior and suggest a strategy for enhancing the effects of leptin in leptin-resistant obese individuals.

satiety; obesity; meal size; food intake; adiposity

MULTIPLE FACTORS CONTROL THE size of meals, including signals generated from the gut in response to consumed nutrients and signals proportional to body weight or adiposity. The meal-related signal CCK is a peptide secreted from the intestine in response to specific nutrients in the gut (3, 18, 19). There is compelling evidence that CCK can potentially reduce the size of individual meals (reviewed in Ref. 37); however, there has been little evidence to suggest that CCK contributes to the regulation of body weight. Rather, previous studies have suggested that CCK is not able to reduce body weight, even while reducing meal size (27, 48, 50).

The inability of exogenous CCK to reduce body weight potentially in rodents is in contrast to the effects of the hormone leptin, which can produce profound loss of body fat (20, 21, 34). Whereas leptin treatment reduces meal size (10, 16, 24), increased activity and metabolic changes also are necessary for the full expression of body weight loss in leptin-treated animals (20, 27).

We previously have reported that a single treatment of leptin followed by CCK in mice did not reduce 30-min

meal intake compared with CCK alone, but did reduce total daily caloric intake significantly more than leptin alone (29). We also reported that a single injection of leptin into either the lateral or the third cerebral ventricle (intracerebroventricularly), when followed 2–3 h later by intraperitoneal CCK, significantly reduced feeding during the subsequent 48 h and produced significantly greater loss of body weight than leptin alone. In other words, CCK and leptin acted synergistically to reduce body weight (29). This enhanced body weight loss following leptin plus CCK does not appear to depend on reduced meal size (28, 29). The present experiments were undertaken to determine 1) whether the synergistic loss of body weight following leptin plus CCK is sustained over several days (5–9) of repeated treatment and 2) whether leptin and CCK act in the brain or in the periphery to mediate leptin-CCK synergy on body weight.

## GENERAL 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. Unilateral 23-gauge stainless steel cannulas were implanted stereotaxically into the lateral ventricle at the coordinates –1.0 mm from bregma, ±1.5 from midline, –3.9 from dura. Cannula placement and patency were assessed by measuring water intake following intracerebroventricular ANG II (30 µg/2 µl) both prior to the start of treatment and after collection of data was completed. Rats that drank <5 ml of water in the 30 min following intracerebroventricular ANG II were excluded. Animals that showed any sign of illness throughout the experiment were also excluded from further treatment, and previous data from these animals were not included in analysis.

Rats were adapted to the following daily schedule prior to treatment. Pelleted rodent chow (Harlan Teklad Diet 8664, 3.3 kcal/g) was removed each day at 7:45 AM, and the rats were weighed. After recovery from surgery and prior to the beginning of treatment, they were accustomed to 30-min access to 15% sucrose (0.6 kcal/ml) every day beginning at 11:45 AM for 10 days until a stable daily intake was observed. On treatment days, an injection of 0.9% sterile saline or leptin was given at 8:30 AM, and a second injection of either saline or CCK-8 was given 3 h later at 11:30 AM, immediately prior to sucrose presentation. Chow was returned each day after the sucrose test at 1:00 PM.

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Treatment always began on *day 0* after body weight and chow were collected. Therefore, the first sucrose test in the presence of drug occurred on *day 0*, whereas the first body weight and daily chow intake data taken after onset of treatment were assessed at the start of *day 1*.

Murine leptin (PeprTech, Rocky Hill, NJ) was diluted in sterile distilled water for intracerebroventricular injection. The biologically active octapeptide of CCK, CCK-8, was diluted to 2  $\mu\text{g}/\text{ml}$  or 0.5  $\mu\text{g}/\text{ml}$  in sterile 0.9% saline for intraperitoneal injection or to 0.66  $\mu\text{g}/3 \mu\text{l}$  sterile 0.9% saline for intracerebroventricular injection.

Data were analyzed for each day by one-way ANOVA with alpha level set at  $P < 0.05$ , followed by Tukey's post hoc comparisons. The Tukey's procedure made pairwise comparisons between all groups and adjusted for the total number of comparisons made. This test is also fairly stringent and was chosen to minimize the occurrence of Type II errors given the large overall number of pairwise comparisons.

### Experiment 1

Previously, we reported that a single combination of intracerebroventricular leptin and intraperitoneal CCK elicits significantly greater body weight loss than does leptin alone (29). We hypothesized that this synergistic loss of body weight would be maintained over an extended course of treatment. In other words, we expected the loss of body weight elicited by leptin plus CCK to remain significantly greater than the weight loss following leptin alone throughout the duration of the treatment period.

**Methods.** Rats were divided into four weight-matched groups and baseline body weight and food intake data were collected prior to treatment according to the daily schedule described above. During the 5 consecutive days of the treatment period (*days 0–4*), rats were injected with 2  $\mu\text{g}$  leptin or saline into the lateral cerebral ventricle (intracerebroventricular) at 8:00 AM. At 11:00 AM, rats were injected intraperitoneally with either 2  $\mu\text{g}/\text{kg}$  CCK-8 or saline. The final number of rats was 26 (saline/saline  $n = 7$ ; saline/CCK  $n = 7$ ; leptin/saline  $n = 5$ ; leptin/CCK  $n = 7$ ).

**Results.** Rats that were treated with CCK alone each day for 5 days had no change of body weight relative to saline-saline controls. A low dose of leptin alone reduced body weight relative to saline; however, this trend was not significant ( $P = 0.06$  on *days 5* and *6*). Rats that were treated with leptin plus CCK lost significantly more body weight than rats that received saline-saline by *day 2* ( $P < 0.01$ ) and had lost significantly more body weight than leptin-alone rats by *day 3* ( $P < 0.01$ , Fig. 1). By *day 5*, 24 h after the final injections, rats treated with leptin plus CCK had lost four times more body weight than rats that received saline-saline ( $P < 0.01$ ) and two times more than those that received only leptin ( $P < 0.05$ ). Furthermore, the body weight of leptin plus CCK-treated rats remained significantly reduced compared with saline controls until *day 11*, 7 days after the last injection ( $P < 0.05$ ).

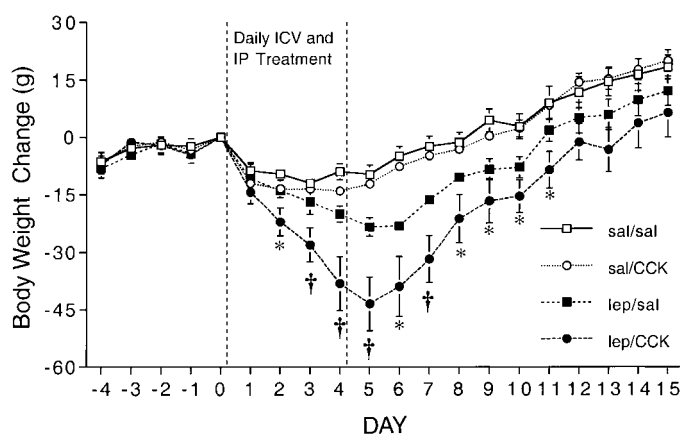


Fig. 1. Repeated leptin (Lep) plus CCK enhanced body weight loss. **Experiment 1:** groups were weight matched on *day 0* [mean body weights (g)  $\pm$  SE: saline (Sal)/Sal 380.5  $\pm$  5.5; Sal/CCK 377.5  $\pm$  13.2; Lep/Sal 376.3  $\pm$  9.5; Lep/CCK 378.3  $\pm$  5.9]. Rats were treated for 5 consecutive days, shown as days within dashed lines, beginning on *day 0* after body weight was measured. Rats treated with Lep plus CCK each day for 5 days had significantly greater weight loss than did rats treated with Lep alone on *days 3–5* and *7* ( $\dagger P < 0.05$ ). Lep-plus-CCK-treated rats also reduced body weight significantly compared with Sal controls by *day 2* and remained significantly underweight until *day 11*, 7 days after treatment was discontinued ( $* P < 0.05$ ). Although Lep alone also reduced body weight compared with Sal controls, this reduction did not reach significance ( $P = 0.06$  on *days 5* and *6*). CCK alone had no effect on body weight change compared with Sal. ICV, intracerebroventricular; IP, intraperitoneal.

Leptin alone reduced daily chow intake to 35% of that of saline controls ( $P < 0.001$  on *day 5*). However, the significant body weight loss of leptin-plus-CCK-treated rats compared with leptin-alone rats, apparent by *day 3*, preceded any significant difference in the daily chow intake of these two groups. Compared with leptin alone, daily chow intake was reduced significantly more by leptin plus CCK only on *day 5* ( $P < 0.05$ ). Daily chow intake of rats treated with CCK alone was not significantly different from saline-saline controls at any time (Fig. 2).

CCK alone significantly reduced 30-min sucrose intake compared with saline on all treatment days ( $P < 0.01$ , Table 1). Leptin alone also reduced 30-min sucrose intake, but this reduction was significant only on *day 3* ( $P < 0.05$ ). The combination of leptin plus CCK did not reduce sucrose intake significantly more than CCK alone. However, the dose of CCK used was sufficiently effective that further suppression may not have been detectable.

**Discussion.** The present data are consistent with our previous reports of synergy between CCK and leptin in mice (29) and rats (29). Interestingly, the enhanced reduction of body weight following leptin plus CCK compared with leptin alone may not depend exclusively on reduced food intake. The relatively small difference between the chow intake of the leptin group and the leptin plus CCK group and the absence of apparent synergy on 30-min intake suggest that the CCK-leptin interaction on body weight might not depend entirely on CCK's well-described ability to reduce meal size. That is, the combination of leptin plus CCK may enhance body weight loss through mechanisms other

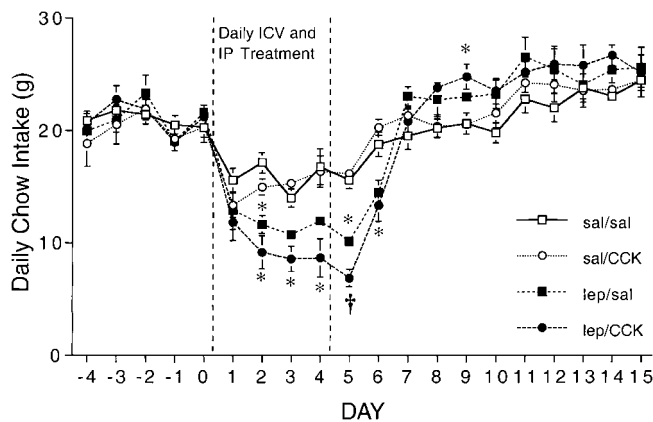


Fig. 2. Repeated Lep plus CCK did not consistently reduce chow intake compared with Lep alone. *Experiment 1*: 5 consecutive days of treatment are shown as area within dashed lines. Lep-plus-CCK-treated rats reduced chow intake more than Sal-treated rats on days 2–6 and were significantly hyperphagic compared with Sal on day 9 ( $*P < 0.05$ ). Lep alone also reduced chow intake compared with Sal on days 2 and 5 ( $P < 0.05$ ). Lep plus CCK reduced chow intake significantly more than Lep alone only on day 5 ( $\dagger P < 0.05$ ). CCK alone had no effect on daily chow intake.

than or in addition to feeding behavior, such as increased metabolic rate, thermogenesis, or decreased efficiency in the absorption and storage of ingested nutrients.

#### Experiment 2

The effects of intraperitoneal CCK to reduce meal size are likely mediated via a peripheral, endocrine, or

Table 1. Effect of intracerebroventricular leptin and intraperitoneal CCK on 15% sucrose intake during a daily 30-min test

	Sal/Sal (n=7)	Sal/CCK (n=7)	Lep/Sal (n=5)	Lep/CCK (n=7)
Baseline, ml	14.3 ± 1.2	15.1 ± 1.2	14.2 ± 2.3	13.8 ± 1.5
Day 0—T	94.6 ± 13.7	50.4 ± 16.7	71.9 ± 6.8	4.5 ± 1.8*
Day 1—T	78.9 ± 7.6	26.3 ± 9.3*	88.2 ± 11.6	2.7 ± 0.5*
Day 2—T	77.6 ± 6.5	19.1 ± 4.8*	56.6 ± 7.5	5.5 ± 2.0*
Day 3—T	90.2 ± 13.2	25.3 ± 4.3*	44.6 ± 9.3*	6.0 ± 2.2*
Day 5—R	84.8 ± 8.0	68.6 ± 12.1	75.2 ± 13.1	51.6 ± 11.6
Day 6—R	100.5 ± 6.7	75.2 ± 13.1	90.5 ± 9.6	74.7 ± 13.6
Day 7—R	122.5 ± 11.2	103.8 ± 6.1	95.2 ± 12.3	94.7 ± 13.3
Day 8—R	108.8 ± 14.9	93.3 ± 14.2	99.1 ± 13.7	86.6 ± 14.0
Day 9—R	115.1 ± 8.6	103.7 ± 10.3	100.0 ± 7.6	91.7 ± 11.1

Values are means ± SE. Results from *experiment 1*, expressed as percent of baseline. Baseline intake for each rat was its own mean intake on the 2 days prior to beginning treatment. Rats were treated (T) for 5 consecutive days beginning prior to the sucrose test on day 0. Intraperitoneal injection of CCK-8 (2 µg/kg) or saline was given immediately prior to presentation of sucrose for each rat. Although rats were given access to sucrose on day 4, intake data were not recorded. CCK alone significantly reduced sucrose intake on days 1–3 ( $*P < 0.05$ ,  $P = 0.06$  on day 0). Leptin alone also reduced sucrose intake, although this was significant only on day 3 ( $*P < 0.05$ ). Leptin plus CCK reduced intake compared with saline on all treatment days; however, leptin plus CCK did not significantly reduce intake as compared with CCK alone (Tukey's post hoc tests for saline (Sal)/CCK vs. leptin (Lep)/CCK: day 0,  $P = 0.06$ ; day 1,  $P = 0.15$ ; day 2,  $P = 0.27$ ; day 3,  $P = 0.35$ ). Although both body weight and chow intake were still significantly altered by Lep alone and Lep plus CCK treatment for several days after treatment was discontinued, there was no effect of prior treatment on sucrose intake during the recovery (R) period.

paracrine action of CCK on CCK-A receptors (reviewed in Ref. 37). The satiety signal ascends to the brain stem via small, unmyelinated fibers of the vagus nerve that synapse in the nucleus of the solitary tract (NTS) (30, 36, 38, 44). Reduction of meal size by peripheral CCK injection is abolished by destruction of the abdominal vagal sensory innervations by either vagotomy (42, 43) or intraperitoneal capsaicin treatment (30, 36). Hence, a peripheral site of action for control of meal size by CCK seems well established.

Nonetheless, centrally acting and centrally produced CCK also have been implicated in the control of feeding (9 and reviewed in Ref. 37). For example, CCK has been reported to reduce meal size when administered directly into the cerebral ventricles of rats (39) and sheep (7). Furthermore, the synergy between leptin and CCK we observed previously (29) and in *experiment 1* might not rely on the same substrates that mediate CCK's reduction of meal size. In other words, it is possible that CCK injected into the periphery acts directly in the brain to mediate leptin-CCK synergy on body weight. Therefore, in *experiment 2*, we gave the same dose of CCK, which we had previously given intraperitoneally, directly into the lateral cerebral ventricle to determine whether centrally administered CCK could mediate significant leptin-CCK synergy.

**Methods.** Rats were divided into four weight-matched groups, and baseline body weight and food intake data were collected prior to treatment according to the daily schedule described above. During the 7 consecutive days of the treatment period (days 0–6), rats were injected with 2 µg leptin or saline into the lateral cerebral ventricle (intracerebroventricularly) at 8:30 AM, and then at 11:30 AM rats were again injected intracerebroventricularly into the lateral ventricle with either 2 µg/kg (0.66 µg) CCK-8 or saline immediately prior to sucrose presentation. The final number of rats was 42 (saline/saline  $n = 11$ ; saline/CCK  $n = 10$ ; leptin/saline  $n = 10$ ; leptin/CCK  $n = 11$ ).

**Results.** In contrast to the results of *experiment 1*, when CCK was given peripherally, CCK injected into the lateral ventricle did not enhance body weight loss when paired with daily leptin treatment. Seven consecutive days of treatment with a low dose of leptin alone or leptin plus CCK reduced body weight relative to saline on days 6–10 ( $P < 0.05$ ) and 6–13 ( $P < 0.05$ , Fig. 3), respectively. However, there was no significant difference between the body weight loss of leptin alone and leptin plus CCK rats at any time. Rats that were treated with CCK alone exhibited no change of body weight relative to saline-saline controls (Fig. 3).

Both leptin alone and leptin plus CCK reduced daily chow intake compared with saline on days 3–7 ( $P < 0.05$ ). However, there was no significant difference between the daily intake of leptin alone and leptin-plus-CCK-treated rats at any time, nor was there a difference between CCK alone and saline-saline controls (Fig. 4).

A dose of CCK (2 µg/kg), which potently reduced sucrose intake when given in the periphery (Table 1), had no effect on 30-min sucrose intake when given

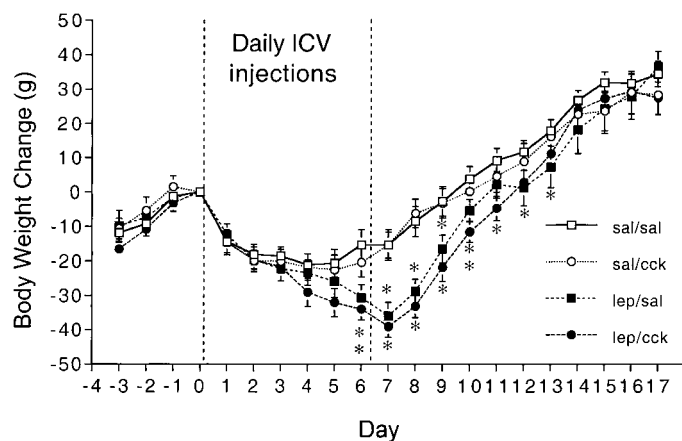


Fig. 3. Intracerebroventricular CCK does not mediate Lep-CCK synergy on body weight. *Experiment 2*: groups were weight matched on day 0 [mean body weights (g)  $\pm$  SE: Sal/Sal 330.2  $\pm$  6.7; Sal/CCK 333.8  $\pm$  5.2; Lep/Sal 331.6  $\pm$  5.0; Lep/CCK 338.3  $\pm$  5.1]. Rats were treated for 7 consecutive days, shown as days within dashed lines, beginning on day 0 after body weight was measured. Rats treated with Lep or Lep plus CCK each day for 7 days had significantly greater weight loss than did rats treated with Sal on days 6–10 and 6–13, respectively ( $*P < 0.05$ ). However, there was no enhancement of body weight loss of Lep-plus-CCK-treated rats compared with Lep alone. CCK alone had no effect on body weight change compared with Sal.

directly into the lateral ventricle (Table 2). There also was no effect of leptin alone or leptin plus CCK on 30-min sucrose intake compared with either saline or CCK-alone, respectively.

**Discussion.** The same dose of CCK that elicited significant synergy when given intraperitoneally (*experiment 1*) was not sufficient to mediate leptin-CCK synergy on body weight when given directly into the brain. This result strongly suggests that CCK given into the periphery acts in the periphery and not in the brain to elicit significant leptin-CCK synergy. It is plausible that the peripheral action of CCK in leptin-CCK synergy may be mediated by the vagus nerve, as is the effect of CCK on meal size; however, further experi-

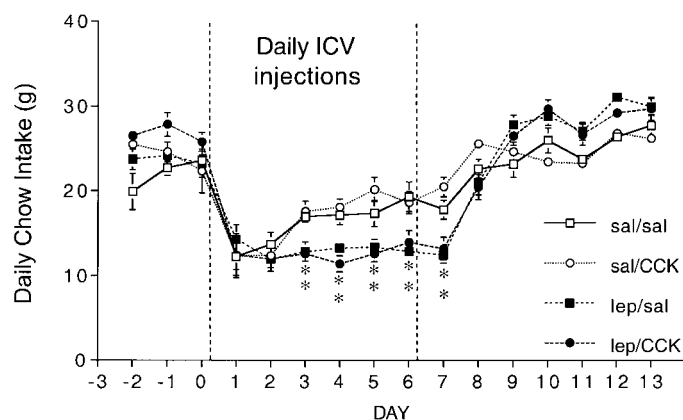


Fig. 4. Intracerebroventricular CCK does not mediate Lep-CCK synergy on food intake. Seven consecutive days of treatment are shown as area within dashed lines. Both Lep- and Lep-plus-CCK-treated rats reduced chow intake more than Sal-treated rats on days 3–7 ( $*P < 0.05$ ). There was no difference between chow intake of Lep- and Lep-plus-CCK-treated rats. CCK alone had no effect on daily chow intake.

Table 2. Effect of intracerebroventricular Lep and intracerebroventricular CCK on 15% sucrose intake during a daily 30-min test

	Sal/Sal (n=11)	Sal/CCK (n=10)	Lep/Sal (n=11)	Lep/CCK (n=11)
Baseline, ml	8.2 $\pm$ 0.7	7.2 $\pm$ 1.1	6.1 $\pm$ 0.8	6.7 $\pm$ 1.3
Day 0—T	104.0 $\pm$ 11.3	101.3 $\pm$ 26.2	157.182 $\pm$ 14.0	112.6 $\pm$ 27.0
Day 1—T	106.3 $\pm$ 19.8	92.0 $\pm$ 23.5	104.8 $\pm$ 18.7	64.1 $\pm$ 13.6
Day 2—T	92.1 $\pm$ 14.6	79.3 $\pm$ 13.5	112.4 $\pm$ 15.3	119.1 $\pm$ 22.1
Day 3—T	107.5 $\pm$ 22.7	117.5 $\pm$ 24.8	163.8 $\pm$ 25.8	141.8 $\pm$ 27.5
Day 4—T	130.3 $\pm$ 20.8	114.5 $\pm$ 13.7	152.5 $\pm$ 35.8	156.3 $\pm$ 48.5
Day 5—T	143.1 $\pm$ 22.8	160.7 $\pm$ 25.1	177.6 $\pm$ 24.7	156.6 $\pm$ 33.4
Day 6—T	148.1 $\pm$ 18.3	208.4 $\pm$ 39.6	186.5 $\pm$ 21.0	157.6 $\pm$ 29.8
Day 7—R	138.8 $\pm$ 26.9	130.0 $\pm$ 17.7	162.9 $\pm$ 20.7	162.1 $\pm$ 22.2
Day 8—R	139.9 $\pm$ 31.4	174.2 $\pm$ 27.7	186.5 $\pm$ 30.8	212.9 $\pm$ 47.1

Values are means  $\pm$  SE. Results from *experiment 2*, expressed as percent of baseline. Baseline intake for each rat was its own mean intake on the 2 days prior to beginning treatment. Rats were treated (T) for 7 consecutive days beginning prior to the sucrose test on day 0. Intracerebroventricular injection of CCK-8 (0.66 mg/3  $\mu$ l, equivalent to 2  $\mu$ g/kg) or saline was given immediately prior to presentation of sucrose for each rat. There was no significant effect of intracerebroventricular CCK, Lep, or the combination of the 2 peptides on 30-min sucrose intake.

ments are needed to test this hypothesis directly. Furthermore, whereas these results suggest that centrally produced and released CCK does not elicit significant enhancement of body weight loss during leptin treatment, they cannot exclude this possibility.

### Experiment 3

Leptin receptors are present both in the periphery and the central nervous system (CNS) (6, 8, 11, 15, 31, 40, 45). Central sites of leptin action apparently elicit effects complementary to, but distinct from, peripheral leptin actions (5, 23, 32). We hypothesized that the leptin-CCK synergy we observed previously and in *experiment 1* is elicited by leptin acting directly in the brain. However, it remained possible that the leptin given into the lateral ventricle diffused out of the brain and acted at some peripheral site. Therefore, in *experiment 3*, we administered leptin by intraperitoneal injection, at the same dose as is effective in eliciting leptin-CCK synergy when it is given intracerebroventricularly.

**Methods.** Male rats were divided into four weight-matched groups, and baseline body weight and food intake data were collected prior to treatment according to the daily schedule described above. During the 5 consecutive days of the treatment period (days 0–4), rats were injected intraperitoneally with 2  $\mu$ g leptin or saline in 0.3 ml at 8:30 AM, and then at 11:30 AM rats were again injected intraperitoneally with either 2  $\mu$ g/kg CCK-8 or saline immediately prior to sucrose presentation. The final number of rats was 23 (saline/saline  $n = 6$ ; saline/CCK  $n = 6$ ; leptin/saline  $n = 5$ ; leptine/CCK  $n = 6$ ).

**Results.** There was no significant effect of either 2  $\mu$ g leptin or 2  $\mu$ g/kg CCK injected intraperitoneally on body weight, nor was there a significant effect of leptin plus CCK (Fig. 5). There was also no significant effect of any treatment on daily chow intake (data not shown).

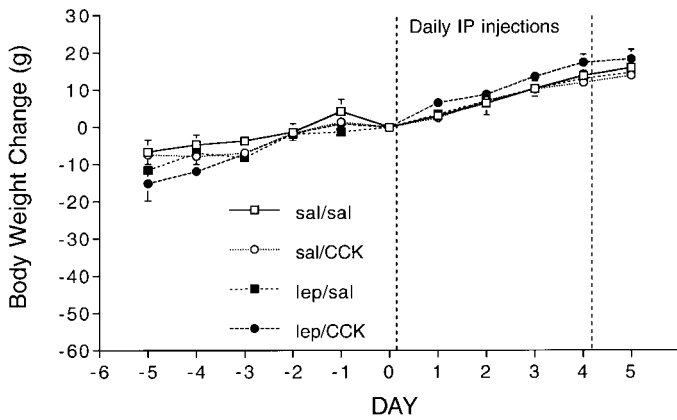


Fig. 5. Low-dose peripheral Lep does not mediate Lep-CCK synergy. *Experiment 3*: groups were weight-matched on *day 0* [mean body wt (g)  $\pm$  SE: Sal/Sal 388.3  $\pm$  7.6; Sal/CCK 393.3  $\pm$  8.1; Lep/Sal 398.7  $\pm$  7.4; Lep/CCK 396.3  $\pm$  7.4]. Rats were treated for 5 consecutive days, shown as days within dashed lines, beginning on *day 0* after body weight was measured. There was no effect of either 2  $\mu$ g Lep, or 2  $\mu$ g/kg CCK injected intraperitoneally on body weight, nor was there a significant effect of Lep plus CCK.

Both CCK alone and CCK plus leptin reduced sucrose intake compared with saline on all treatment days ( $P < 0.01$ ; Table 3). At no time was the reduction of 30-min sucrose intake following leptin and CCK different from that following CCK alone.

**Discussion.** The results of *experiment 3* support the hypothesis that leptin acts in the brain to mediate the synergy on body weight and food intake observed in *experiment 1* and in previous reports (28, 29). The importance of centrally acting leptin is consistent with the hypothesized role of central leptin receptors in the integration of the regulation of body weight (12, 13). However, although the low dose of leptin used in these experiments did not act in the periphery, this does not exclude the possibility of important peripheral interactions between leptin and CCK. Endogenous leptin-CCK synergy may involve both central and peripheral sites of leptin action. These results do exclude the possibility that the low-dose leptin given in *experiment 1* and in our previous report (29) acts directly on peripheral

Table 3. Effect of intraperitoneal Lep and CCK on 15% sucrose intake during a daily 30-min test

	Sal/Sal (n=6)	Sal/CCK (n=6)	Lep/Sal (n=5)	Lep/CCK (n=6)
Baseline, ml	10.6 $\pm$ 1.1	13.5 $\pm$ 1.9	13.0 $\pm$ 1.3	14.4 $\pm$ 1.0
Day 0–T	119.4 $\pm$ 15.4	60.7 $\pm$ 17.4*	88.1 $\pm$ 16.7	42.4 $\pm$ 13.2*
Day 1–T	108.8 $\pm$ 7.7	21.0 $\pm$ 14.0*	74.5 $\pm$ 13.9	40.8 $\pm$ 16.9*
Day 2–T	95.4 $\pm$ 11.8	14.5 $\pm$ 6.0*	82.8 $\pm$ 6.5	14.1 $\pm$ 8.8*
Day 3–T	100.8 $\pm$ 17.8	23.1 $\pm$ 13.4*	74.2 $\pm$ 6.5	5.1 $\pm$ 2.0*
Day 4–T	116.4 $\pm$ 18.0	36.6 $\pm$ 14.9*	93.1 $\pm$ 11.5	29.7 $\pm$ 12.9*

Values are means  $\pm$  SE. Results from *experiment 3*, expressed as percent of baseline. Baseline intake for each rat was its own mean intake on the 2 days prior to beginning treatment. Rats were treated (T) for 5 consecutive days beginning on *day 0*. Intraperitoneal injection of CCK-8 (2  $\mu$ g/kg) or saline was given immediately prior to presentation of sucrose for each rat. Both CCK alone and Lep plus CCK significantly reduced sucrose intake on all treatment days as compared with Sal (\* $P < 0.01$ ). However, Lep plus CCK did not significantly reduce intake as compared with CCK alone.

tissues such as leptin-sensitive vagal afferents suggested to play a role in short-term leptin-CCK synergy in mice (2, 46, 47).

#### Experiment 4

Exogenous CCK reduces food intake over a wide range of doses (reviewed in Ref. 37). Therefore, in *experiment 4*, we gave two different doses of CCK (2 and 0.5  $\mu$ g/kg) to determine whether a lower CCK dose is capable of mediating leptin-CCK synergy.

**Methods.** Male rats were divided into five weight-matched groups, and baseline body weight and food intake data were collected prior to treatment according to the daily schedule described above. During the 9 consecutive days of the treatment period (*days 0–8*), rats were injected intracerebroventricularly with 2  $\mu$ g leptin in 3  $\mu$ l or saline at 8:30 AM, and then at 11:30 AM rats were injected intraperitoneally with either 2  $\mu$ g/kg CCK-8, 0.5  $\mu$ g/kg CCK-8, or saline immediately prior to sucrose presentation. The final number of rats was 32 (saline/saline  $n = 8$ ; saline/CCK high  $n = 6$ ; leptin/saline  $n = 6$ ; leptin/CCK high  $n = 6$ ; leptin/CCK low  $n = 6$ ).

**Results.** Both low- and high-dose CCK synergistically enhanced the reduction of body weight following leptin treatment. The synergistic enhancement of weight loss by leptin-CCK combination was comparable for both doses of CCK used. A low dose of CCK (0.5  $\mu$ g/kg) plus leptin was as effective for enhancing leptin's body weight reducing effects as leptin plus a 2- $\mu$ g/kg CCK dose (Fig. 6). Furthermore, rats that received leptin

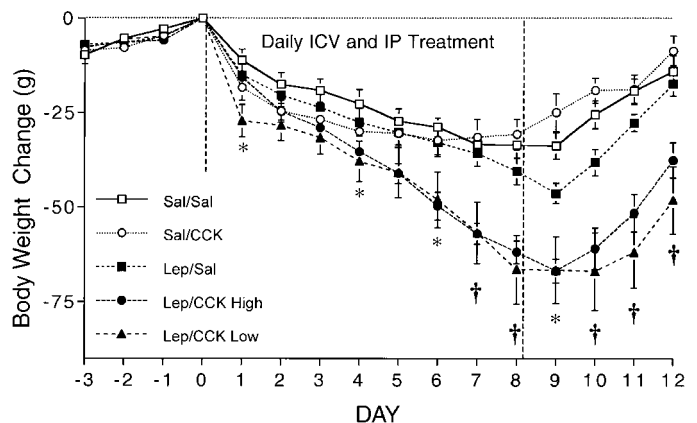


Fig. 6. Low-dose CCK (Low; 0.5  $\mu$ g/kg) mediates Lep-CCK synergy on body weight. *Experiment 4*: groups were weight matched on *day 0* [mean body wt (g)  $\pm$  SE: Sal/Sal 362.5  $\pm$  6.4; Sal/CCK 361.5  $\pm$  5.4; Lep/Sal 358.4  $\pm$  4.9; Lep/CCK Low 360.4  $\pm$  3.3; Lep/high-dose CCK (High; 2  $\mu$ g/kg) 363.5  $\pm$  6.1]. Rats were treated for 9 consecutive days, shown as days within dashed lines, beginning on *day 0* after body weight was measured. Lep-plus-High-CCK-treated rats reduced body weight significantly compared with Sal controls on *days 6–12*, whereas Lep-plus-Low-CCK-treated rats had significantly lower body weight than Sal controls on *days 1, 4, and 6–14* (\* $P < 0.05$ ). Rats treated with Lep plus either High or Low CCK each day for 9 days had significantly greater weight loss than did rats treated with Lep alone on *days 7, 8, 11, and 15* and *days 7, 8, and 10–15*, respectively ( $\dagger P < 0.05$ ). Although Lep alone also reduced body weight compared with Sal controls, this reduction was not significant ( $P = 0.36$  on *day 9*). CCK alone had no effect on body weight change compared with Sal.

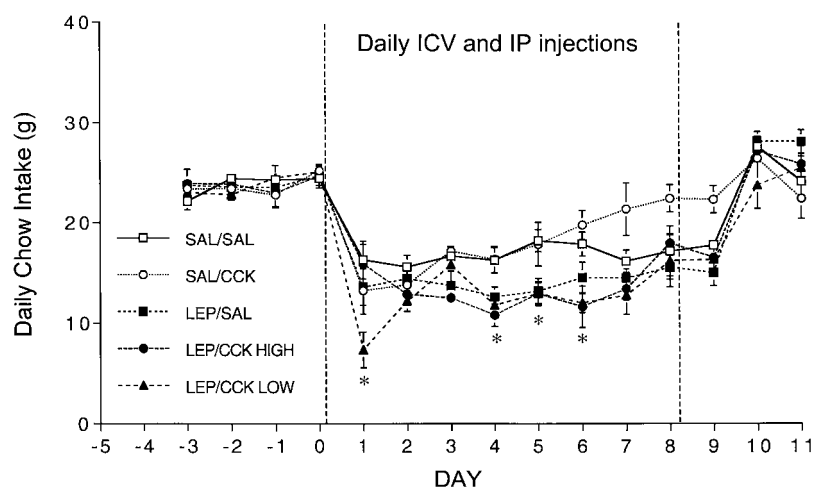


Fig. 7. Lep-CCK synergy affects food intake less than body weight. *Experiment 4*: 9 consecutive days of treatment are shown as area within dashed lines. Lep plus High or Low CCK reduced daily chow intake compared with Sal on days 4 and 6 and 1 and 5, respectively ( $P < 0.05$ ). Lep alone did not significantly reduce daily chow intake. Lep plus either dose of CCK did not reduce intake significantly more than Lep alone on any day. CCK alone had no effect on daily chow intake.

plus either a low ( $0.5 \mu\text{g}/\text{kg}$ ) or high ( $2 \mu\text{g}/\text{kg}$ ) dose of CCK lost significantly more body weight than rats that received saline or leptin alone ( $P < 0.05$ ). By contrast, rats that were treated with intracerebroventricular saline and the high dose of CCK each day for 9 days had no change of body weight relative to saline-saline controls (Fig. 6). Nine days of treatment with a low dose of leptin alone slightly reduced body weight relative to saline; however, this reduction did not reach significance ( $P = 0.36$  on day 9; Fig. 6).

There was no significant difference between the daily chow intake by leptin-alone rats and the intake by rats treated with leptin plus either dose of CCK, despite the large difference in body weight loss among these groups. A low dose of leptin alone did not significantly reduce daily chow intake compared with saline controls (Fig. 7). However, daily chow intake was reduced by leptin

plus a low dose of CCK compared with saline controls on days 1 and 5 ( $P < 0.05$ ) and by leptin plus a high dose of CCK on days 4 and 6 ( $P < 0.05$ ). The daily chow intake of rats treated with CCK alone was never significantly different from saline-saline controls (Fig. 7).

A high dose ( $2 \mu\text{g}/\text{kg}$ ) of CCK alone significantly reduced sucrose intake compared with saline ( $P < 0.05$ ; Table 4). Leptin plus a high or low dose of CCK also reduced intake compared with saline ( $P < 0.05$ ), but at no time did leptin plus either dose of CCK significantly reduce intake compared with CCK ( $2 \mu\text{g}/\text{kg}$ ) alone.

#### GENERAL DISCUSSION

We report here that a single daily intraperitoneal injection of CCK octapeptide synergistically enhances

Table 4. Effect of intracerebroventricular leptin and Low or High intraperitoneal CCK on 15% sucrose intake during a daily 30-min test

	Sal/Sal (n=8)	Sal/CCK (n=6)	Lep/Sal (n=6)	Lep/CCK Low (n=6)	Lep/CCK High (n=6)
Baseline, ml	16.4 ± 1.4	16.8 ± 2.1	14.8 ± 1.0	14.8 ± 1.0	16.4 ± 1.5
Day 0—T	72.3 ± 10.3	12.8 ± 5.2*	57.8 ± 15.0	18.6 ± 9.5*	25.4 ± 11.4*
Day 1—T	49.6 ± 9.1	18.0 ± 7.9	53.8 ± 5.3	38.8 ± 10.9	21.0 ± 6.2
Day 2—T	60.8 ± 12.4	29.7 ± 7.9*	61.6 ± 11.3	48.9 ± 8.1	20.5 ± 9.6
Day 3—T	70.5 ± 6.4	37.9 ± 10.1*	74.0 ± 9.6	51.0 ± 7.7	21.7 ± 3.3*
Day 4—T	51.2 ± 6.4	29.8 ± 7.9	36.8 ± 10.0	34.6 ± 3.4	34.2 ± 10.7
Day 5—T	60.1 ± 3.4	35.2 ± 6.1*	52.6 ± 4.9	30.5 ± 3.4*	29.5 ± 10.4*
Day 6—T	63.2 ± 5.3	26.4 ± 8.6*	53.2 ± 3.5	34.4 ± 8.9*	19.7 ± 5.9*
Day 7—T	74.8 ± 6.8	33.0 ± 11.1*	55.7 ± 7.8	38.4 ± 7.1*	30.7 ± 12.9*
Day 8—T	61.2 ± 13.1	32.7 ± 8.2	57.5 ± 11.5	30.0 ± 6.3	43.6 ± 14.4
Day 9—R	66.8 ± 8.6	64.9 ± 10.0	58.7 ± 15.5	62.2 ± 8.4	69.3 ± 9.0
Day 10—R	68.9 ± 8.9	67.0 ± 6.0	65.8 ± 12.0	65.6 ± 5.8	89.1 ± 5.2
Day 11—R	88.7 ± 11.6	92.4 ± 15.8	74.9 ± 10.6	71.5 ± 8.2	95.5 ± 3.8
Day 12—R	112.6 ± 11.3	100.1 ± 15.6	82.4 ± 17.6	103.4 ± 10.4	111.8 ± 8.7

Values are means ± SE. Results from *experiment 4*, expressed as percent of baseline. Baseline intake for each rat was its own mean intake on the 2 days prior to beginning treatment. Rats were treated (T) for 9 consecutive days beginning prior to the sucrose test on day 0. Intraperitoneal injection of CCK-8 ( $2 \mu\text{g}/\text{kg}$  or  $0.5 \mu\text{g}/\text{kg}$ ) or saline was given immediately prior to presentation of sucrose for each rat. A high dose (High;  $2 \mu\text{g}/\text{kg}$ ) of CCK alone significantly reduced sucrose intake as compared to Sal on days 0, 2, 3, and 5–7 ( $P < 0.05$ ). Lep plus a high dose of CCK reduced intake compared with Sal on days 0, 3, and 5–7, whereas leptin plus a low dose of CCK reduced intake on days 0, and 5–7 ( $P < 0.05$ ). However, at no time did Lep plus either dose of CCK significantly reduce intake as compared with CCK alone (Tukey's post hoc tests for Sal/CCK high vs. Lep/CCK High: day 0,  $P = 0.93$ ; day 1,  $P = 1.00$ ; day 2,  $P = 0.97$ ; day 3,  $P = 0.61$ ; days 5–6,  $P = 0.96$ ; day 7,  $P = 1.00$ ). There was no significant overall effect of group on days 4 or 8–12. Although both body weight and chow intake remained significantly affected by Lep alone and Lep plus CCK treatment for several days after treatment was discontinued, there was no effect of prior treatment on sucrose intake during the recovery (R) period (days 9–12).

the efficacy of a low intracerebroventricular dose of leptin to reduce body weight. This effect depends on a peripheral action of CCK and a central action of leptin. The robust effect of repeated CCK plus leptin to enhance weight loss observed in *experiment 1* was replicated in *experiment 4* and is consistent with our previous report of CCK-leptin synergy on body weight following a single treatment of these two peptides (29). In addition, the results of *experiment 4* indicate that an even lower dose of CCK (0.5 µg/kg) given into the periphery is sufficient to mediate synergistic body weight loss.

The experiments in this report do not support the existence of an especially robust leptin-CCK synergy on food intake (Fig. 7). In our previous work using a similar paradigm in mice, we observed an effect of leptin-CCK combination on total daily caloric intake (29). Previously we also have observed a significant leptin-CCK synergy in rats on reduction of daily chow intake as well as on body weight, although the effect on food intake was not as robust as the effect on body weight (29). However, in the present experiments, there was not a reliable difference between the food intake of the leptin alone and the leptin plus CCK-treated rats, even when the data were analyzed as cumulative intake over several days. Rather, the results of these studies suggest that while there is some effect of leptin and CCK on long-term food intake, this effect is far less robust than the greater effects of leptin-CCK combination on body weight. Thus leptin-CCK synergy might promote body weight loss through mechanisms largely independent of feeding behavior, such as increased resting metabolic rate, thermogenesis, or decreased efficiency of absorption and storage of ingested nutrients.

Recently, several reports have indicated that CCK and leptin probably interact in multiple ways. Bado et al. (1) reported that leptin immunoreactivity is present in the stomach and is depleted, concomitant with a rise in plasma leptin levels, following refeeding after a fast or treatment with very large doses of exogenous CCK. Furthermore, two groups have reported short-term synergy between leptin and CCK to reduce feeding behavior (2, 14). A synergistic suppression of chow intake lasting <6 h has been reported in fasted mice when leptin and CCK are given simultaneously into the peritoneal cavity (2). This short-term interaction was apparent following a low dose of peripheral leptin (2) and may be mediated by leptin-sensitive vagal afferents and involve enhanced neuronal activity in the hypothalamus (46, 47). In addition, there have been recent reports of enhanced CCK-mediated satiety during a 30-min Ensure meal 1 h after intracerebroventricular leptin (3.5 or 10 µg) in rats, concomitant with synergistically enhanced *c-fos* expression in dorsal hindbrain nuclei and hypothalamus (14). This experiment used a large group of rats ( $n = 18$ ) in a repeated-measures design (14) and thus had greater statistical power than the present experiments.

However, we previously observed no significant synergy between peripheral leptin and CCK on 30-min

Ensure intake in a similar repeated-measures design with a large group size ( $n = 16$ ) in which the different treatments were given in a randomized, counterbalanced order (29). In eight separate experiments using several different doses of CCK and leptin, we have not observed significant enhancement of CCK's reduction of intake during a single 30-min meal in either mice (29) or rats (present experiments and 29). Wildman et al. (49) also have reported the absence of enhanced CCK action on single-meal intake following leptin treatment using a similar design. The dose of CCK (2 µg/kg) we have used has a robust effect on meal size and may have precluded detection of greater reductions. Furthermore, we have observed both an independent effect of leptin on single meal intake (Table 1) and trends toward decreased sucrose intake following leptin plus CCK compared with CCK (Table 1). However, these trends were not consistently observed (Table 4), suggesting that if leptin plus CCK treatment does reduce individual meal size, this effect is not robust. Thus we have observed long-term enhancement of leptin's suppression of body weight by CCK with no concomitant effect on short-term intake. Therefore, we suggest that the short-term interaction(s) between leptin and CCK on feeding (2, 14) may be related, but are not necessarily essential to, the long-term body-weight effects of CCK-leptin combination.

The present experiments also provide some insight into the possible mechanisms by which CCK and leptin act to mediate this synergy. CCK appears to act in the periphery. It will be of considerable interest to determine whether this interaction is dependent on the same peripheral neural substrates that appear to mediate CCK's effects on meal size. For example, leptin-CCK synergy may be abolished by capsaicin treatment or vagotomy or attenuated by prior treatment with a CCK-A receptor antagonist or lesion of the NTS.

Leptin appears to act within the CNS to mediate leptin-CCK synergy on body weight. Leptin receptors in the hypothalamus have been extensively investigated (6, 8, 11–13, 15, 31, 40, 41, 45), and several neuropeptide systems have been suggested to mediate effects downstream from hypothalamic leptin receptor activation (12, 13). These pathways may also mediate leptin actions important to leptin-CCK synergy. For example, could leptin-CCK synergy be elicited without activation of leptin receptors on the neuropeptide Y (NPY)/agouti-related peptide (AgRP) containing cells in the arcuate nucleus?

The synergistic interaction between leptin and CCK is most likely integrated at one or more sites within the CNS. Recently, Broberger and colleagues (4) have suggested the parabrachial nucleus (PBN) in the pons as a potential site for integration of short- and long-term satiety signals because of a dense projection of NPY/AgRP neurons from the arcuate to the PBN. The NTS, which receives synaptic termination from CCK-activated vagal afferents (35), also provides a dense projection to the PBN (22, 25, 33). Both the NTS and the arcuate nucleus display intense leptin-receptor immunoreactivity (45). The PBN may also supply as-

ending input to the hypothalamus related to feeding behavior or body weight regulation, as CCK immunoreactive cell bodies in the PBN project to the ventromedial hypothalamus (17). However, the PBN is only one of several potential sites of integration that can be proposed based on our current understanding of the neural events initiated by leptin and CCK. The eventual identification of the neural mechanisms underlying leptin-CCK synergy promises to extend our understanding of the roles of CCK and leptin in both feeding and body weight regulation.

### Perspectives

A more complete understanding of the factors that enter into the calculus of the regulation of body weight requires that we identify the key endogenous signals and determine their interaction. The synergistic body weight loss following leptin-CCK combination suggests that interactions between such signals are probably critical to the understanding of the control of food intake and the regulation of body weight. Consideration of these interactions may extend beyond the search for pharmacological "magic bullets" and move us toward a more realistic and informed approach to the complex pathogenesis of obesity.

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### REFERENCES

- Bado, A., S. Levasseur, S. Attoub, S. Kermorgant, J. Laigneau, M. Bortoluzzi, L. Moizo, T. Lehy, M. Guerre-Millo, 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. Martínez, L. X. 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.
- Broberger, C., J. Johansen, C. Johansson, M. Schalling, and T. Hokfelt. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic and monosodium glutamate-treated mice. *Proc. Natl. Acad. Sci. USA* 95: 15043-15048, 1998.
- Cawthorne, M. A., N. M. Morton, A. L. Pallett, Y. L. Liu, and V. Emilsson. Peripheral metabolic actions of leptin. *Proc. Nutr. Soc.* 57: 449-455, 1998.
- Chen, S. C., J. P. Kochan, L. A. Campfield, P. Burn, and R. J. Smeyne. Splice variants of the OB receptor gene are differentially expressed in brain and peripheral tissues of mice. *J. Recept. Signal Transduct. Res.* 19: 245-266, 1999.
- Della-Fera, M. A., and C. A. Baile. CCK-octapeptide injected in CSF decreases meal size and daily food intake in sheep. *Peptides* 1: 51-54, 1980.
- DeMatteis, R., K. Dashtipour, A. Ognibene, and S. Cinti. Localization of leptin receptor splice variants in mouse peripheral tissues by immunohistochemistry. *Proc. Nutr. Soc.* 57: 441-448, 1998.
- Dorre, D., and G. P. Smith. Cholecystokinin (B) antagonist increases food intake in rats. *Physiol. Behav.* 65: 11-14, 1998.
- Eckel, L. A., W. Langhans, A. Kahler, L. A. Campfield, F. J. Smith, and N. Geary. Chronic administration of OB protein decreases food intake by selectively reducing meal size in female rats. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 275: R186-R189, 1998.
- Elmquist, J. K., C. Bjorbaek, R. S. Ahima, J. S. Flier, and C. B. Saper. Distributions of leptin receptor isoforms in the rat brain. *J. Comp. Neurol.* 395: 535-547, 1998.
- Elmquist, J. K., E. Maratos-Flier, C. B. Saper, and J. S. Flier. Unraveling the CNS pathways underlying responses to leptin. *Nat. Neurosci.* 1: 445-450, 1998.
- Elmquist, J. K., C. F. Elias, and C. B. Saper. From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron* 22: 221-232, 1999.
- Emond, M., G. J. Schwartz, E. E. Ladenheim, and T. H. Moran. Central leptin modulates behavioural and neural responsiveness to CCK. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 276: R1545-R1549, 1999.
- Fei H., H. J. Okano, C. Li, G. H. Lee, C. Zhao, R. Darnell, and J. M. Friedman. Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. *Proc. Natl. Acad. Sci. USA* 94: 7001-7005, 1997.
- Flynn, M. C., T. R. Scott, T. C. Pritchard, and C. R. Plata-Salaman. Mode of action of OB protein (leptin) on feeding. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 275: R174-R179, 1998.
- Fulwiler, C. E., and C. B. Saper. Cholecystokinin-immunoreactive innervation of the ventromedial hypothalamus in the rat: possible substrate for autonomic regulation of feeding. *Neurosci. Lett.* 53: 289-296, 1985.
- 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.
- Halaas, J. L., C. Boozer, J. Blair-West, N. Fidahusein, D. A. Denton, and J. M. Friedman. Physiological response to long-term peripheral, and central leptin infusion in lean and obese mice. *Proc. Natl. Acad. Sci. USA* 94: 8878-8883, 1997.
- Halaas, J. L., K. S. Gajiwala, M. Maffei, S. L. Cohen, B. T. Chait, D. Rabinowitz, R. L. Lallone, S. K. Burley, and J. M. Friedman. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269: 543-546, 1995.
- Herbert, H., M. M. Moga, and C. B. Saper. Connections of the parabrachial nucleus with the nucleus of the solitary tract and the medullary reticular formation in the rat. *J. Comp. Neurol.* 293: 540-580, 1990.
- Houseknecht, K. L., and C. P. Portocarrero. Leptin and its receptors: regulators of whole body energy homeostasis. *Domest. Anim. Endo.* 15: 457-475, 1998.
- Kahler, A., N. Geary, L. A. Eckel, L. A. Campfield, F. J. Smith, and W. Langhans. Chronic administration of OB protein decreases food intake by selectively reducing meal size in male rats. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 275: R180-R185, 1998.
- Kapp, B. S., C. G. Markgraf, J. S. Schwaber, and T. Bilyk-Spafford. The organization of dorsal medullary projections to the central amygdaloid nucleus and parabrachial nuclei in the rabbit. *Neuroscience* 30: 717-732, 1989.
- Kopin, A. S., W. F. Mathes, E. W. McBride, M. Nguyen, W. Al-Haider, F. Schwartz, S. Bonner-Weir, R. Kanerak, and M. Beinborn. The cholecystokinin-A receptor mediates inhibition of food intake yet is not essential for maintenance of body weight. *J. Clin. Invest.* 103: 383-391, 1999.
- Levin, N., C. Nelson, A. Gurney, R. Vandlen, and F. de Sauvage. Decreased food intake does not completely account for adiposity reduction after ob protein infusion. *Proc. Natl. Acad. Sci. USA* 93: 1726-1730, 1996.
- Matson, C. A., and R. C. Ritter. Long-term CCK-leptin synergy suggests a role for CCK in the regulation of body weight. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 276: R1038-R1045, 1999.
- 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.

30. **Mercer, J. G., D. A. Farmingham, and C. B. Lawrence.** Effect of neonatal capsaicin treatment on cholecystokinin (CCK-8)-satiety and axonal transport of CCK binding sites in the rat vagus nerve. *Brain Res.* 569: 311–316, 1992.
31. **Mercer, J. G., N. Hoggard, L. M. Williams, C. B. Lawrence, L. T. Hannah, and P. Trayhurn.** Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. *FEBS Lett.* 387: 113–116, 1996.
32. **Niijima, A.** Afferent signals from leptin sensors in the white adipose tissue of the epididymis, and their reflex effect in the rat. *J. Auton. Nerv. Sys.* 73: 19–25, 1998.
33. **Norgren, R.** Projections from the nucleus of the solitary tract in the rat. *Neuroscience* 3: 207–218, 1978.
34. **Pelleymounter, M. A., M. J. Cullen, M. B. Baker, R. Hecht, D. Winters, T. Boone, and F. Collins.** Effects of the *obese* gene product on body weight regulation in ob/ob mice. *Science* 269: 540–543, 1995.
35. **Raybould, H. E., R. J. Gayton, and G. J. Dockray.** Mechanisms of action of peripherally administered cholecystokinin octapeptide on brainstem neurons in the rat. *J. Neurosci.* 8: 3018–3024, 1988.
36. **Ritter, R. C., and E. E. Ladenheim.** Capsaicin pretreatment attenuates suppression of food intake by cholecystokinin. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 248: R501–R504, 1985.
37. **Ritter, R. C., M. Covasa, and C. A. Matson.** Cholecystokinin: proofs and prospects for involvement in control of food intake and body weight. *Neuropeptides* 33: 387–399, 1999.
38. **Ritter, R. C., S. Ritter, W. R. Ewart, and D. L. Wingate.** Capsaicin attenuates hindbrain neuron responses to circulating cholecystokinin. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 257: R1162–R1168, 1989.
39. **Schick, R. R., C. W. Stevens, T. L. Yaksh, and V. L. Go.** Chronic intraventricular administration of cholecystokinin octapeptide (CCK-8) suppresses feeding in rats. *Brain Res.* 448: 294–298, 1988.
40. **Schwartz, M. W., R. J. Seeley, L. A. Campfield, P. Burn, and D. G. Baskin.** Identification of targets of leptin action in rat hypothalamus. *J. Clin. Invest.* 98: 1101–1106, 1996.
41. **Shioda, S., H. Funahashi, S. Nakajo, T. Yada, O. Maruta, and Y. Nakai.** Immunohistochemical localization of leptin receptor in the rat brain. *Neurosci. Lett.* 243: 41–44, 1998.
42. **Smith, G. P., C. Jerome, B. J. Cusin, R. Eterno, and K. J. Simansky.** Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. *Science* 213: 1036–1037, 1981.
43. **Smith, G. P., C. Jerome, and R. Norgren.** Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 249: R638–R641, 1985.
44. **South, E. H., and R. C. Ritter.** Capsaicin application to central or peripheral vagal fibers attenuates CCK satiety. *Peptides* 9: 601–612, 1988.
45. **Tartaglia, L. A., M. Dembski, X. Weng, N. Deng, J. Culpepper, R. Devos, G. J. Richards, L. A. Campfield, F. T. Clark, J. Deeds, C. Muir, S. Sankér, A. Moriarty, K. J. Moore, J. S. Smutko, G. G. Mays, E. A. Woolf, C. A. Monroe, and R. I. Tepper.** Identification and expression cloning of a leptin receptor, OB-R. *Cell* 83: 1263–1271, 1995.
46. **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.
47. **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. Regulatory Integrative Comp. Physiol.* 273: R833–R837, 1997.
48. **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. Regulatory Integrative Comp. Physiol.* 246: R776–R787, 1984.
49. **Wildman, H. F., S. Chua Jr., R. L. Leibel, and G. P. Smith.** Effects of leptin and cholecystokinin in rats with a null mutation of the leptin receptor *Lepr<sup>Fak</sup>*. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* In press.
50. **Wisner, J. R., Jr., S. Ozawa, B. G. Xue, and I. G. Renner.** Chronic administration of a potent cholecystokinin receptor antagonist, L-364,718, fails to inhibit pancreas growth in preweaning rats. *Pancreas* 5: 434–438, 1990.