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# Leptin Deficiency Induced by Fasting Impairs the Satiety Response to Cholecystokinin\*

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## ABSTRACT

Leptin administration potentiates the satiety response to signals such as cholecystokinin (CCK), that are released from the gut during a meal. To investigate the physiological relevance of this observation, we hypothesized that leptin deficiency, induced by fasting, attenuates the satiety response to CCK. To test this hypothesis, 48-h-fasted or fed rats were injected with ip saline or CCK. Fasting blunted the satiety response to 3.0  $\mu\text{g}/\text{kg}$  CCK, such that 30-min food intake was suppressed by 65.1% (relative to saline-treated controls) in fasted rats vs. 85.9% in the fed state ( $P < 0.05$ ). In a subsequent experiment, rats were divided into three groups: 1) vehicle/fed; 2) vehicle/fasted; and 3) leptin-replaced/fasted; and each group received 3.0  $\mu\text{g}/\text{kg}$  ip CCK. As expected, the satiety response to CCK was attenuated by fasting

in vehicle-treated rats (30-min food intake: vehicle/fed,  $0.3 \pm 0.1$  g; vehicle/fasted,  $1.7 \pm 0.4$  g;  $P < 0.01$ ), and this effect was prevented by leptin replacement ( $0.7 \pm 0.2$  g,  $P < 0.05$  vs. vehicle/fasted;  $P =$  not significant vs. vehicle/fed). To investigate whether elevated neuropeptide Y (NPY) signaling plays a role in the effect of leptin deficiency to impair the response to CCK, we measured the response to 3.0  $\mu\text{g}/\text{kg}$  ip CCK after treatment with 7.5  $\mu\text{g}$  intracerebroventricular NPY. We found that both CCK-induced satiety and its ability to increase c-Fos-like-immunoreactivity in key brainstem-feeding centers were attenuated by NPY pretreatment. We conclude that an attenuated response to meal-related satiety signals is triggered by leptin deficiency and may contribute to increased food intake. (*Endocrinology* 141: 4442–4448, 2000)

**I**NCREASED FOOD INTAKE is an important component of the adaptive response, after fasting, that promotes the recovery of depleted fuel stores. Leptin deficiency is implicated in this response because reduced leptin signaling occurs in fasted animals and is the cause of hyperphagia in genetically obese *ob/ob* and *db/db* mice and *fa/fa* rats. Increased food intake that occurs in leptin-deficient conditions is characterized by increased meal size rather than more frequent meals (1–3), whereas leptin-induced anorexia in normal rodents is characterized by decreased meal size (4, 5) with no change of meal frequency (4). These findings support the hypothesis that leptin reduces energy intake primarily by reducing the size of individual meals, whereas leptin deficiency elicits the opposite effect.

During a meal, neural and humoral signals are generated in response to nutrient ingestion that induce satiety and meal termination. Among these stimuli are satiety factors, one of which is cholecystokinin (CCK). CCK was first isolated in 1964 (6) and has since been extensively studied for its role in ingestive behavior (for a review, see Ref. 7). CCK reduces food intake, within minutes after its release from the upper duodenum and jejunum (8), by binding to CCK-A receptors

on afferent fibers of the vagus nerve. The vagus nerve transmits ingestive information to brainstem areas implicated in satiety, including the nucleus of the solitary tract (NTS), which, in turn, projects fibers to the brainstem's lateral parabrachial nucleus (L-PBN) and the hypothalamic paraventricular nucleus (PVN) (9–11). Thus, systemic CCK administration to rats rapidly inhibits food intake and activates neurons (as measured by c-Fos induction) in brain stem and hypothalamic areas.

Coadministration of CCK with leptin potentiates its ability to reduce food intake (12–15) and to activate neurons in the NTS (13, 14, 16). These findings support a model in which leptin's anorectic effects are mediated, at least in part, by enhancing the response of brainstem neurons to CCK. We therefore hypothesized that reduced leptin signaling, induced by fasting, attenuates the response to satiety signals, which, in turn, increases food intake. To test this hypothesis, we first measured the effect of fasting on the satiety response to ip CCK. To explore the specific role of leptin deficiency in the effect of food deprivation, we performed a second experiment in which fasted animals were infused systemically with a dose of leptin intended to maintain circulating leptin at non-fasted levels, and the satiety response to CCK was compared with that of fasted animals receiving no leptin replacement.

One mechanism by which decreased leptin signaling may increase food intake is via increased synthesis of hypothalamic peptides that stimulate feeding, such as neuropeptide Y (NPY). We therefore hypothesized that NPY increases food intake, at least in part, by attenuating the response of neurons in the brainstem, to satiety signals such as CCK. This hypothesis was investigated by determining the effect of intracerebroventricular (icv) NPY pretreatment on both CCK-

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induced satiety and on the ability of CCK to induce c-Fos expression in areas of the brain that regulate food intake.

## Materials and Methods

### Animals

All studies used male Wistar rats weighing 300–350 g (Simonsen Laboratories, Inc., Gilroy, CA), housed individually in polycarbonate cages (Experiments 1, 2, and 3) or wire-mesh hanging cages (Exp 4) in a temperature-controlled vivarium, on a 12-h light, 2-h dark schedule. Unless otherwise specified, animals were given free access to pelleted rat chow (Ralston Purina Co., St. Louis, MO) and water at all times. All procedures were performed according to institutional guidelines and were approved by the Animal Care and Use Committee at the Puget Sound Veterans Affairs Health Care Center and the University of Washington.

### Exp 1: Effect of fasting on CCK-induced satiety

Forty-five rats were weighed and handled daily and habituated to ip saline vehicle injections 1 week before the beginning of the experiments.

**Ad libitum fed protocol.** Food hoppers were removed from cages at 1500 h and weighed. Animals were weight-matched into treatment groups and injected ip between 1745 h and 1800 h, in randomized order, with sulfated CCK octapeptide (Peninsula Laboratories, Inc., Belmont, CA), at doses of 0.5, 0.8, and 3.0  $\mu\text{g}/\text{kg}$ , diluted up to 2 ml in 0.9% saline solution, or saline for control. Each rat was allowed access to chow immediately after the injection, at a time corresponding to the beginning of the dark cycle (1815 h). Food consumption was measured 30 min, 90 min, and 4 h after CCK administration.

**Fasted protocol.** Food hoppers were removed from cages, at 1500 h, 2 days before the feeding experiment. On the day of the experiment, fasted animals were weight-matched into treatment groups, injected ip in randomized order with either saline or CCK, and monitored for intake of chow as described above.

Half of the animals were divided into four groups and received one of the three doses of CCK or vehicle in the fed condition, followed by the same treatment in the fasted condition 10 days later. The remaining animals received the same injection treatments in the fasted condition first; and 10 days later, they were injected in the fed condition. This cross-over design was repeated after 10 days recovery, and the results were analyzed together.

### Exp 2: Effect of leptin replacement on the response to fasting

**Intrajugular catheter placement.** Rats were habituated with daily handling for 8 weeks before surgery. After anesthesia, induced by ip injection of ketamine/xylazine (60 mg/kg ketamine and 8 mg/kg xylazine), a sterile silicon catheter (ID, 0.020; OD, 0.037 inches) was placed into the right jugular vein using the method of Steffens (17). Two weeks of surgical recovery were allowed before performing the experiment, during which time rats were weighed daily and habituated to the blood sampling procedure.

**Study protocol.** Delivery of infusate via minipump was achieved by attaching a polyethylene Lynch coil (18), filled with a precise volume of either vehicle or peptide. Twenty-three jugular-catheterized animals were weight-matched into three groups (average BW = 406.4  $\pm$  7.0 g): vehicle/fed, vehicle/fasted, and leptin/fasted. After anesthesia, induced by ip injection of ketamine/xylazine, all rats were implanted with sc minipumps with attached Lynch coils that delivered 0.9% saline for the first 48 h after minipump placement, so that infusion of test substances did not commence until after this postsurgical recovery period. After this initial 48-h period, food was removed for 48 h from the vehicle/fasted (n = 8) and leptin/fasted (n = 7) groups, during which time the vehicle/fed group (n = 8) was allowed free access to food. Minipumps delivered either 5 mM sodium citrate vehicle sc, or 100  $\mu\text{g}/\text{kg}\cdot\text{day}$  sc of murine leptin (Peprotech, Inc., Rocky Hill, NJ) dissolved in sodium citrate vehicle at pH 4.0, a rate chosen according to a protocol

used previously (19). A jugular vein blood sample was collected from each rat, 44 h into the fast, to measure plasma leptin levels.

The infusions of leptin or vehicle were designed to finish just before the beginning of the dark cycle (1715 h) on the second day of the fast and were followed by a 0.9% saline infusion delivered by the minipumps for the remainder of the study. At 1400 h, food was removed from the cages of the vehicle/fed group. All animals were injected, in randomized order, with 3  $\mu\text{g}/\text{kg}$  ip CCK, between 1645 h and 1655 h, and food was immediately returned to all cages. Food intake was measured at 30 min, 90 min, 4 h, and 24 h after injection.

To determine the effect of the leptin replacement protocol on food intake in the absence of CCK, the experiment was repeated after 1 month recovery, using ip saline instead of CCK, in a subset of these animals (n = 17, average BW = 425.3  $\pm$  8.1 g). Each animal was maintained in its previous treatment group.

### Exp 3: Effect of icv NPY on CCK-induced satiety and c-Fos induction

**Cannula placement.** Rats were habituated with daily handling for 1 week before surgery. After anesthesia, induced by ip injection of ketamine/xylazine, a 21-gauge cannula (Plastics One, Roanoke, VA) was placed stereotaxically into the third ventricle using a previously described method (20, 21). Cannula placement was verified, 1 week after surgery, by icv injection of 10 ng angiotensin II (American Peptide Co., Sunnyvale, CA) diluted in 1  $\mu\text{l}$  cerebrospinal fluid (CSF). Animals not consuming at least 5 ml water within 30 min post injection were excluded as cannulation failures (4% of all rats).

**Exp 3.1.** To determine the effect of icv NPY pretreatment on CCK-induced inhibition of food intake, 19 rats were weight-matched to 4 groups, 3 weeks after cannula placement in the third ventricle. The 4 groups studied were: icv CSF/ip saline treatment (n = 4), icv CSF/ip CCK (n = 5), icv NPY/ip saline (n = 5), and icv NPY/ip CCK (n = 5). At 1400–1500 h, each animal received an icv injection of 3.5- $\mu\text{l}$  vol of either synthetic CSF or 7.5  $\mu\text{g}$  NPY in the same volume of CSF, and food was removed from the cages. At approximately 1745–1755 h, either ip saline or 3.0  $\mu\text{g}/\text{kg}$  ip CCK was administered in randomized order, and food was immediately returned to the cages. Food intakes were collected 30 min, 90 min, 4 h, and 24 h after returning food to the cages. After a 10-day recovery period, the same animals were reassigned to groups such that those that had previously received an ip saline treatment now received ip CCK and *vice versa*, while the icv treatment groups remained as before. In this way, each animal served as its own control for the ip treatments. The results of the 2 trials were similar, and the data were therefore analyzed together.

**Exp 3.2.** To determine the effect of icv NPY pretreatment on CCK-induced c-Fos staining, a different group of 29 rats was habituated to ip injections and weight-matched into the following 4 groups after cannulation of the third ventricle: icv CSF/ip saline (n = 8), icv CSF/ip CCK (n = 7), icv NPY/ip saline (n = 7), and icv NPY/ip CCK (n = 7). Third-ventricle injections of either 3.5  $\mu\text{l}$  CSF or 7.5  $\mu\text{g}$  NPY, in an equal volume of CSF, were administered to all rats, followed 1 h later by an ip injection of either ip saline or 3  $\mu\text{g}/\text{kg}$  ip CCK. Immediately afterwards, each animal was returned to its home cage with food hoppers and water bottles removed. After 110 min, rats were anesthetized with ketamine/xylazine and transcardially perfused with PBS of neutral pH, followed by a 4% paraformaldehyde solution. Brains were removed immediately and postfixed in paraformaldehyde, for 1 week, before assay for c-Fos-like-immunoreactivity (cFLI).

### Assays and data analyses

**Leptin assay.** Plasma leptin levels were determined by RIA using an antibody with 100% cross-reactivity for both rat and murine leptin (Linco Research, Inc., St. Louis, MO) (22).

**cFLI.** Each postfixed brain was rinsed 2–3 times in PBS and sectioned, at 50  $\mu\text{m}$ , on an oscillating tissue slicer in a PBS bath. Coronal sections were taken from the forebrain (PVN) and from the hindbrain (L-PBN, NTS, and area postrema, AP) and were processed for cFLI as described in detail elsewhere (23). Sections were mounted on slides, and the

number of cFLLI-positive cell nuclei was quantified in selected brain areas by an investigator blinded to the treatment groups. Quantification was performed using the MCID computer grain counting system (Imaging Research, Inc., St. Catherine's, Ontario, Canada).

**Statistical analyses.** All statistical analyses were carried out using Prism 2.01 (GraphPad Software, Inc., San Diego, CA) statistical software. Data are presented as group mean values ( $\pm$ SEM). For experiments with greater than two study groups, comparisons were performed with one-way ANOVA and Neuman-Keuls *post hoc* test. A paired, two-tailed Student's *t* test was used for two-group comparisons in Exp 1. A *P* value = 0.05 between group mean values was considered statistically significant.

## Results

### Exp 1: Effect of fasting on CCK-induced satiety

To determine the effect of fasting on CCK-induced satiety, 30-min food intake was measured after ip injection of saline or one of three doses of CCK in rats that were either fasted 48 h or fed *ad libitum*. The average food intake for saline-treated rats fed *ad libitum* was  $3.2 \pm 0.3$  g; and for 48-h-fasted rats,  $4.3 \pm 0.2$  g in the first 30 min after lights out ( $P < 0.05$ ). CCK reduced food intake over this time period in a dose-dependent manner in both the fed and fasted groups, but the effect of CCK (relative to saline-treated controls) was significantly greater in fed, compared with fasted, rats at the 3.0- $\mu$ g/kg dose ( $0.5 \pm 0.1$  g fed,  $1.5 \pm 0.3$  g fasted,  $P < 0.001$  for each, compared with ip saline). The percent suppression of 30-min food intake in fed and fasted animals at this dose was 85.9% and 65.1%, respectively, compared with saline controls (Fig. 1,  $P < 0.05$  for percent saline control-fed *vs.* percent saline control-fasted).

### Exp 2: Effect of leptin replacement on the response to fasting

To investigate whether leptin deficiency, induced by fasting, contributes to the attenuated satiety response to CCK, the effect of leptin infusion at a dose intended to replace basal levels in fasted rats was determined. Leptin levels were reduced by 82% in vehicle/fasted *vs.* vehicle/fed rats ( $0.3 \pm 0.1$  *vs.*  $1.6 \pm 0.2$  ng/mL;  $P < 0.001$ ) and this effect was prevented by sc leptin infusion ( $1.7 \pm 0.2$  ng/mL;  $P < 0.001$  *vs.* vehicle/

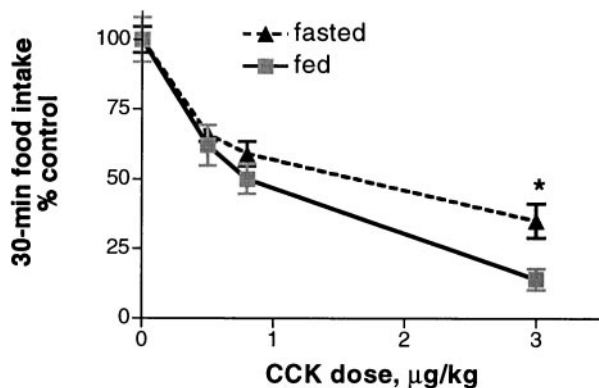


FIG. 1. Food intake, measured for 30 min after ip administration of either saline vehicle or 0.5, 0.8, or 3.0  $\mu$ g/kg CCK, in rats fed *ad libitum* or fasted for 48 h. Data are expressed as percent of the intake in vehicle-treated controls. \*,  $P < 0.05$  for fasted *vs.* fed animals at the 3.0- $\mu$ g/kg dose.

fasted, Fig. 2). Food consumed after 3.0  $\mu$ g/kg ip CCK was greater in vehicle/fasted, compared with vehicle/fed, rats (30-min food intake: vehicle/fasted,  $1.7 \pm 0.4$  g,  $P < 0.01$ ; vehicle/fed,  $0.3 \pm 0.1$  g), in agreement with Exp 1, and this increase was markedly attenuated in leptin/fasted rats ( $0.7 \pm 0.2$  g,  $P < 0.05$  *vs.* vehicle/fasted;  $P$  = not significant *vs.* vehicle/fed, Fig. 2). This pattern persisted over the 24-h refeeding period (data not shown).

To determine whether leptin replacement affects 30-min food independently of CCK, the experiment was repeated in a subset of the same animals, substituting ip injections of saline for that of CCK. As in the previous trial, leptin levels were markedly reduced in vehicle/fasted rats (by 71% in vehicle/fasted *vs.* vehicle/fed rats;  $0.7 \pm 0.1$  *vs.*  $2.6 \pm 0.3$  ng/mL;  $P < 0.01$ ), and this effect was again prevented by sc leptin infusion ( $2.2 \pm 0.5$  ng/mL,  $P < 0.01$  *vs.* vehicle/fasted) (Fig. 3). In the absence of CCK, 30-min food intake in the two groups of fasted animals was greater than that of fed rats, and leptin replacement did not significantly affect this response (30-min food intake:  $4.7 \pm 0.3$  and  $4.2 \pm 0.6$  g in vehicle/fasted and leptin/fasted groups *vs.*  $1.9 \pm 0.4$  g in vehicle/fed animals,  $P < 0.01$  for both fasted *vs.* fed groups) (Fig. 3). This pattern persisted over the 24-h refeeding period (data not shown).

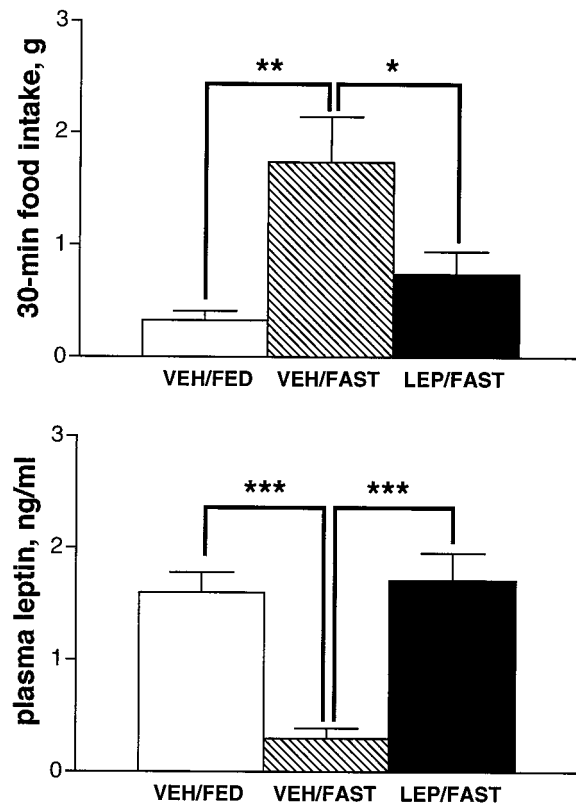


FIG. 2. Top, Thirty-minute food intake, measured after ip administration of CCK (3.0  $\mu$ g/kg ip) in rats fed *ad libitum* that received sc vehicle (VEH) (open bar;  $n = 11$ ) or in rats that were fasted for 48 h and received either sc vehicle (striped bar;  $n = 11$ ) or leptin (LEP) at a dose intended to replace basal levels (filled bar;  $n = 10$ ). Bottom, Plasma leptin levels, measured 4 h before CCK administration, in the same groups. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .



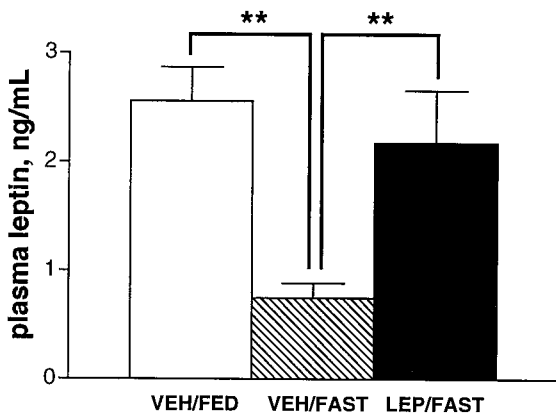
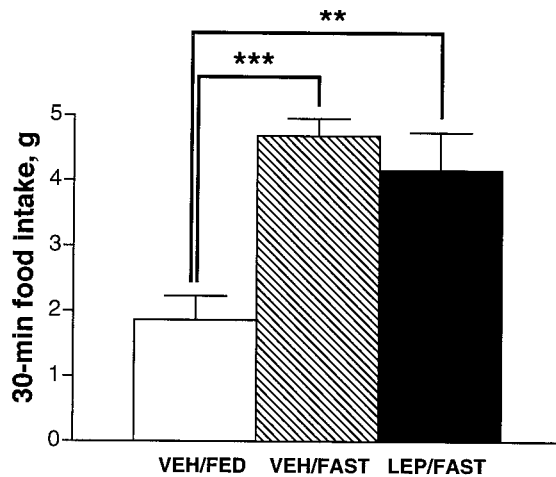


FIG. 3. *Top*, Thirty-minute food intake, measured after ip administration of saline, in rats fed *ad libitum* that were treated with sc vehicle (open bar;  $n = 5$ ), or in rats that were fasted for 48 h and received either sc vehicle (striped bar;  $n = 7$ ) or leptin at a dose intended to replace basal values (filled bar;  $n = 5$ ). *Bottom*, Plasma leptin levels, measured 4 h before CCK administration, in the same groups. \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

#### Exp 3: Effect of icv NPY on CCK-induced satiety and *c-Fos* induction

**Exp 3.1.** To determine whether NPY pretreatment attenuates the feeding response to CCK, rats received either 7.5  $\mu\text{g}$  icv NPY or icv CSF, followed 2 h later by 3.0  $\mu\text{g}/\text{kg}$  ip CCK or ip saline. Average food intake of icv CSF/ip saline-treated rats was  $2.6 \pm 0.5$  g in the first 30 min after lights out (Fig. 4). Similar to previous experiments, CCK reduced 30-min food intake to  $0.2 \pm 0.04$  g ( $P < 0.01$  vs. vehicle) in rats receiving icv CSF vehicle. By comparison, food intake of rats receiving 7.5  $\mu\text{g}$  icv NPY and ip saline was increased to  $5.2 \pm 0.5$  g ( $P < 0.001$  vs. vehicle), whereas food intake of icv NPY/ip CCK-treated rats was similar to that of icv CSF/ip saline-treated rats ( $1.9 \pm 0.4$  g). The relative reduction of 30-min food intake induced by CCK in the icv CSF group was

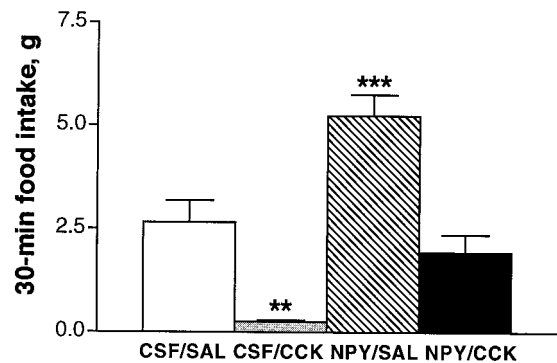


FIG. 4. Thirty-minute food intake, measured after administration of either icv vehicle/ip vehicle (open bar;  $n = 9$ ), icv vehicle/ip CCK (gray bar;  $n = 9$ ), icv NPY/ip vehicle (striped bar;  $n = 10$ ), or icv NPY/ip CCK (filled bar;  $n = 10$ ) in rats. ip CCK and icv NPY doses were 3.0  $\mu\text{g}/\text{kg}$  and 7.5  $\mu\text{g}$ , respectively. SAL, Saline; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

therefore greater than in the group receiving icv NPY (92 vs. 63%;  $P < 0.001$ ).

**Exp 3.2.** In a separate group of rats, the effect of 7.5  $\mu\text{g}$  icv NPY pretreatment on neuronal *c-Fos* staining, induced by 3.0  $\mu\text{g}/\text{kg}$  ip CCK, was determined using a protocol similar to that of Exp 3.1, except that food and water were not available to the animals at any time after the injections. Representative sections showing cFLI in the NTS and AP are shown in Fig. 5A. The mean number of cFLI-positive nuclei in the NTS increased by 960% after treatment with ip CCK, compared with ip saline, among icv CSF-treated animals ( $101.8 \pm 10.2$  vs.  $9.6 \pm 2.5$  cFLI-positive nuclei,  $P < 0.001$ ), and this response was lowered by 35% in rats pretreated with icv NPY ( $65.9 \pm 11.9$  cFLI-positive nuclei,  $P < 0.01$  vs. icv CSF/ip CCK-treated animals) (Fig. 5B). cFLI-positive nuclei were also markedly increased in the AP after ip CCK, compared with ip saline, in the icv CSF group ( $153.0 \pm 6.3$  vs.  $9.6 \pm 2.7$  cFLI-positive nuclei,  $P < 0.001$ ), and this effect was attenuated by 28% in animals pretreated with icv NPY ( $110.2 \pm 21.3$  cFLI-positive nuclei,  $P < 0.05$ ). In contrast, NPY pretreatment did not attenuate CCK-induced *c-Fos* expression in the PVN or the L-PBN, brain areas in which icv NPY and ip CCK elicited similar cFLI patterns (Fig. 5, A and B). NPY given in the absence of CCK also increased for staining in the NTS, L-PBN, and PVN, in relation to icv vehicle.

## Discussion

The recent finding that leptin administration potentiates the satiety response to CCK (12–15) supports a model in which signals involved in long-term energy homeostasis (such as leptin) influence food intake via an interaction with short-term signals (such as CCK) that control the size of individual meals. The observation that *ob/ob* mice with genetic obesity caused by mutation of the leptin gene are relatively insensitive to CCK-induced satiety provides additional support for this concept (24, 25). In the current experiments, we wished to determine whether leptin signaling is required for an intact response to satiety signals in genetically normal animals. We hypothesized that previous observations suggesting that fasting blunts responsiveness to

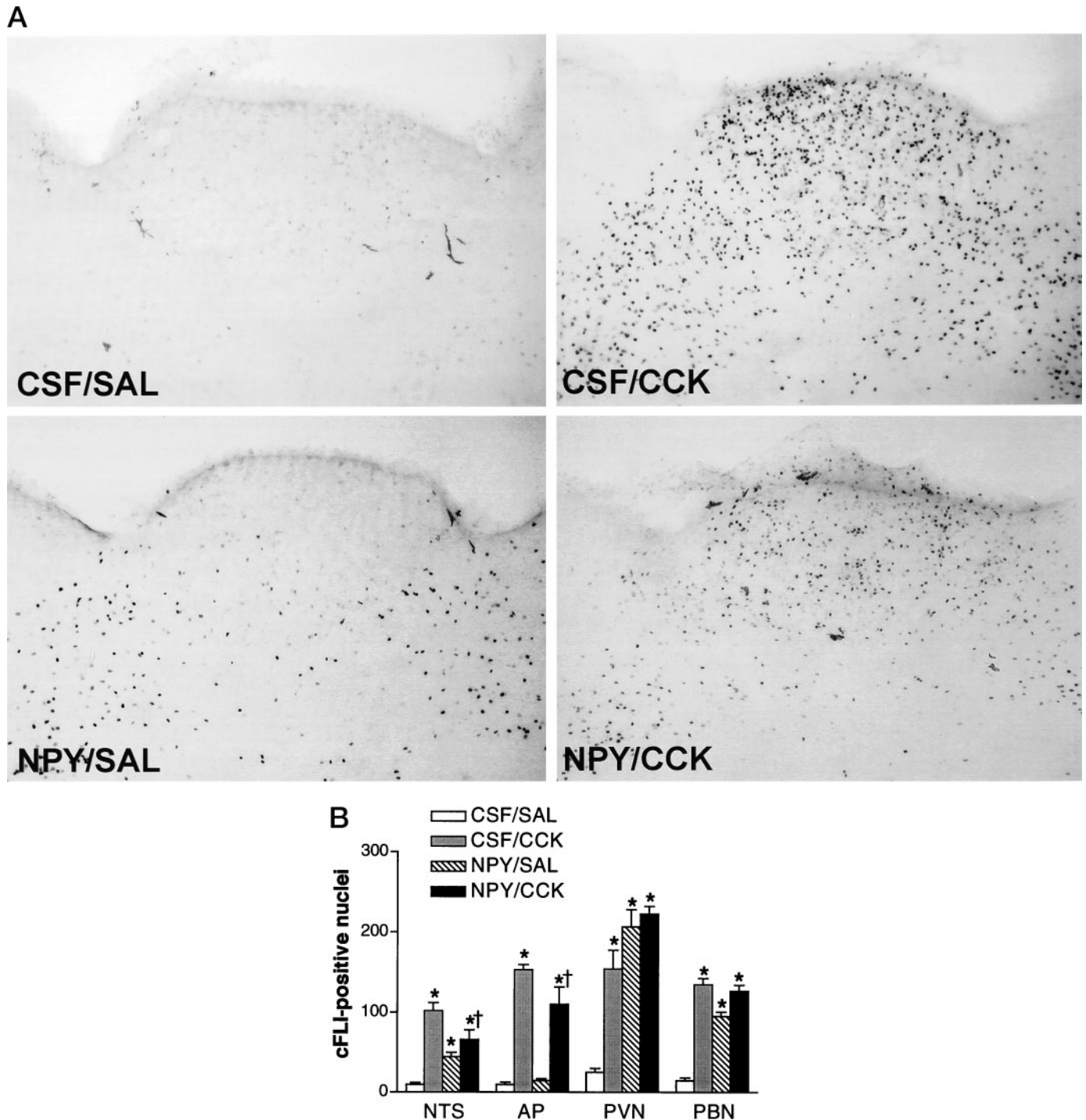


FIG. 5. A, Representative photomicrographs of cFLI in coronal sections of rat brain stem at the level of the NTS and AP in animals from each of the four groups in Exp 3.2. CCK-induced expression of cFLI in both the NTS and AP is attenuated in animals receiving icv NPY pretreatment; B, numbers of cFLI-containing neurons in rat NTS, AP, L-PBN, and PVN measured after administration of either icv vehicle/ip vehicle (open bar), icv vehicle/ip CCK (gray bar), icv NPY/ip vehicle (striped bar), or icv NPY/ip CCK (filled bar) ( $n = 5-8/\text{group}$ ). ip CCK and icv NPY doses were  $3.0 \mu\text{g}/\text{kg}$  and  $7.5 \mu\text{g}$ , respectively. \*,  $P < 0.01$  vs. icv vehicle/ip vehicle; †,  $P < 0.05$  vs. icv vehicle/ip CCK.

exogenous CCK (26–31) may be explained by reduced leptin signaling.

To test this hypothesis, we first confirmed the ability of a 48-h fast to attenuate CCK-induced satiety in rats. We then demonstrated, in a second experiment, that this effect of

fasting was prevented by infusion of leptin at a dose that achieved plasma levels comparable with those of *ad libitum*-fed animals. Thus, leptin deficiency seems to play a key role in the effect of fasting to reduce the response to exogenous CCK. In addition, we showed that the suppression of food

intake elicited by CCK was attenuated by pretreatment with icv NPY, an intervention that also reduced the number of c-Fos-positive nuclei induced by CCK in hindbrain areas strongly implicated in CCK-induced satiety. Taken together, these findings support the hypothesis that the decrease in leptin signaling, induced by fasting, blunts the satiety response to CCK and that fasting-induced increases of NPY may contribute to this response.

Our results are consistent with several previous studies showing that CCK-induced food intake suppression is reduced when animals are fasted (26–31). Leptin replacement reversed the effect of fasting to attenuate the response to CCK, amounting to a recovery of 59% of the response measured in rats fed *ad libitum* ( $P < 0.05$  vs. fasted animals receiving vehicle). The fact that CCK suppression of food intake was incompletely restored by leptin replacement in fasted animals raises the possibility that another circulating factor, such as insulin, may also enhance CCK-induced satiety. This possibility is supported by the observations that, like leptin, insulin levels are markedly decreased by fasting, and icv insulin infusion synergistically reduces food intake when coadministered with systemic CCK (32).

The interaction between leptin infusion and CCK was transient, however. Food intake increased in the leptin-replaced group such that, by 4 h, the fasted groups were no longer significantly different from one another, and the recovery of lost weight after 24 h of refeeding did not differ among leptin- and vehicle-treated groups (data not shown). The latter finding is discordant with the results of two previous studies in which coadministration of leptin with CCK elicited a synergistic suppression of food intake and body weight in lean rats that lasted for 2 days (15, 33), but it is in agreement with a more recent study that did not detect this interaction (34). Our study differs from all three of these previous studies, however, in that we sought to achieve physiological leptin replacement during a fast rather than to administer leptin at supraphysiological doses in *ad libitum*-fed animals.

Conversely, when saline was given instead of ip CCK, food intake in fasted, saline-treated animals was not significantly attenuated by leptin replacement at any time point. Thus, the restoration of the CCK response seen in fasted, leptin-replaced animals in the first trial was not attributable to an independent effect of leptin on food intake. Moreover, it seems that leptin replacement in fasted animals does not reduce fasting-induced hyperphagia. Preventing the decline in plasma leptin levels may therefore be insufficient, in and of itself, to attenuate hyperphagia in response to fasting, and additional studies are warranted to address this possibility. One potential explanation for this outcome is that fasting lowers basal plasma and duodenal levels of satiety factors such as CCK (35–38), gastrin (35), secretin (36), and glucagon-like-peptide-1 (36). By giving CCK, we may have overridden a fasting-induced reduction of satiety signals, thereby permitting the interaction between leptin and CCK to be detected. In fasted rats that did not receive CCK, however, levels of satiety signals during refeeding may have been too low to interact effectively with leptin.

Several possible mechanisms may be considered to explain an effect of leptin deficiency to inhibit the satiety effect of CCK. The ability of ip CCK to activate NTS neurons is well-documented and is believed to involve stimulation of vagal afferent fibers from the gut that synapse in this brain area (7,

9). Because leptin receptors are present in caudal brainstem areas, such as the NTS (39), the possibility of a local interaction of leptin with CCK at this site may be considered. In this scenario, leptin is proposed to act locally in the NTS to potentiate the activation of hindbrain neurons by CCK or other satiety signals. Alternatively, leptin could act indirectly to influence the response of hindbrain neurons to CCK or other satiety signals. For example, many leptin-sensitive neurons of the ARC project, either directly or indirectly, to the hindbrain, where they may influence the response to CCK and other vagally mediated stimuli (40).

NPY neurons of the ARC provide one example of a pathway downstream of leptin signaling with the potential to mediate some of the effects of leptin deficiency on the response to CCK. Hypothalamic NPY synthesis and release increase during fasting (20, 41, 42), and this response is suppressed by leptin (43). Leptin receptors are present on ARC NPY neurons, especially those that are upregulated with fasting (44), and elevated ARC NPY biosynthesis is implicated in the hyperphagia of leptin-deficient, *ob/ob* mice (45, 46). ARC NPY neurons project to the PVN and other hypothalamic areas with well-defined projections to the NTS (40).

In the current studies, we found that, when rats were pretreated with third ventricular NPY or CSF vehicle 1–2 h before administering ip CCK or saline vehicle, the feeding effects of NPY and CCK offset one another. Thus, NPY/CCK-treated animals ate the same amount of food as vehicle/vehicle-treated animals in the first 30 min after ip injection, in agreement with the hypothesis that NPY blocked CCK-induced satiety. The results of our study, however, could also be explained by an effect of CCK to interfere with the orexigenic actions of NPY, or by independent effects of NPY and CCK on neuronal circuits that control food intake. To investigate whether the response of key hindbrain nuclei to CCK is also attenuated by icv NPY pretreatment, we measured cFLI in key brain areas as an index of regional neuronal activation. The key finding of this experiment was that third-ventricular NPY injection significantly reduced the number of cFLI-positive nuclei induced by CCK throughout the AP and medial NTS. This finding supports the hypothesis that CCK-induced activation of key hindbrain neurons is attenuated by icv NPY administration.

The site of the NPY receptor population where NPY inhibited the response of NTS and AP neurons to CCK cannot be ascertained from this study. It is conceivable that third-ventricular NPY entered CSF in the 4th ventricle, from where it was able to exert inhibitory effects directly on CCK-sensitive hindbrain neurons containing NPY receptors. Alternatively, NPY may have acted upon forebrain sites involved in food intake regulation, such as the PVN. This brain area contains NPY receptors (47), is richly supplied with NPY-containing fibers emanating from the ARC (48, 49), and is highly sensitive to NPY's orexigenic effects (50). Because PVN neurons are located adjacent to the third ventricle and project directly to the NTS (reviewed in Ref. 40), NPY inhibition of CCK-responsive neurons could have occurred indirectly via this highly integrative hypothalamic nucleus. The role of the PVN and other neuronal subsets, as mediators of the inhibitory effect of NPY on brainstem responses to CCK, warrants additional study.

In conclusion, our findings support the hypothesis that fasting attenuates the satiety response to CCK via a mechanism



involving decreased leptin signaling. These findings support a model in which CCK's satiety effects are modulated in proportion to changes in energy balance, and that leptin signaling plays a key role in this process. In addition, our data suggest that the opposing effects of NPY and CCK on food intake involve interactions with specific subsets of neurons in the brain stem and provide a novel mechanism whereby NPY may increase food intake. Thus, the ability of reduced leptin signaling to attenuate the response to exogenous CCK may involve hypothalamic mediators such as NPY. Because sc leptin injections are limited in their ability to reduce body weight in humans (51), our findings suggest a potential application for drugs targeting the control of short-term food intake, given in combination with those that regulate long-term energy balance, as an approach to obesity treatment.

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