Coinjection of CCK and leptin reduces food intake via increased CART/TRH and reduced AMPK phosphorylation in the hypothalamus

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CCK is a gastrointestinal hormone produced by I cells of the proximal small intestine and is released in response to the presence of certain products of digestion, namely fat (14, 31), protein (28), and glucose (29). The CCK receptors CCK1 and CCK2 are a group of G protein-coupled receptors. Satiety signals mediated by CCK are transmitted to the brain via the stimulatory effect of CCK1 on the vagal afferent nerve (37). Although CCK is an anorectic hormone, administration of CCK alone does not decrease body weight, because it reduces meal size but increases food intake frequency (55). Information conveyed by feeding-related signals is transported to the brain not only via the circulation but also via the vagal afferent pathway (7, 51). Vagal afferents reach the nucleus of the solitary tract (NTS) in the hindbrain. CCK elicits neuroendocrine responses via vagally mediated recruitment of NTS neurons, including noradrenergic A2 neurons, that project to the hypothalamus (34, 45). Thus, the neural pathway from hindbrain to hypothalamus is also important in regulating food intake. Synergistic interaction between leptin and CCK reduces short-term food intake (3, 54). In vitro, leptin and CCK synergistically increase phosphorylated STAT3 production in the nodose ganglion, which contains the cell bodies of the vagal afferent neurons (20). Other studies have reported that CCK interacts with leptin at the level of the vagal afferent neurons to control the function of early growth response factor 1 (8, 9). However, the molecular mechanisms of CCK and leptin interaction in the hypothalamus have not been fully elucidated. Here, we first confirmed that coadministration of subthreshold CCK and leptin, which individually have no effect on food intake, significantly reduces food intake. Next, we examined feeding-associated molecules in the hypothalamus, including phosphorylated AMPK, phosphorylated STAT3, and mRNA levels of neuropeptide Y (NPY), agouti-related protein (AgRP), anorectic cocaine- and amphetamine-regulated transcript (CART), thyrotropin-releasing hormone (TRH), and proopiomelanocortin (POMC) after coadministration of CCK and leptin. To elucidate the hypothalamic molecular mechanisms involved in CCK and leptin interaction, we investigated whether the interactive effects of coadministration of CCK and leptin were abolished in rats preadministered agonists or anti-body antagonists for hypothalamic substances involved in these interactive effects. Finally, we investigated food intake reduction and alterations in the production of feeding-related molecules induced by coadministration of these two hormones in rats in which the neural pathways from the hindbrain to the forebrain were cut surgically through bilateral midbrain transection (6, 7, 23).

MATERIALS AND METHODS

Experimental animals. Male Wistar rats (8–10 wk old; Charles River Japan, Shiga, Japan) weighing 300 to 350 g were used for all
experiments. Rats were given standard laboratory chow and water ad libitum. They were housed individually in plastic cages at constant room temperature on a 12:12-h light-dark cycle (lights on 0800 to 2000). All procedures were performed in accordance with the Japanese Physiological Society’s guidelines for animal care. Our experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of the Faculty of Medicine, University of Miyazaki, Japan.

Intracerebroventricular (icv) cannulation and bilateral midbrain transection were performed under isoflurane anesthesia (DS Pharma Animal Health, Osaka, Japan), as described (6, 7, 23). For icv cannulation, a stainless-steel guide cannula (guide cannula, 22-gauge; insert, 28-gauge; Plastics One, Roanoke, VA) was stereotactically placed 0.8 mm posterior to the bregma, 1.2 mm right lateral to the midline, and 5 mm below the outer surface of the skull into the lateral ventricle. Correct cannula placement was confirmed histologically at the end of the study period.

For bilateral midbrain transection, the head was fixed in a stereotaxic instrument in a 2.4-mm nose-down position. A steel knife blade 1.5 mm wide was used to penetrate the brain in the coronal plane at two points: 0.5 mm on either side of the midline and 1 mm in front of the lambdoid suture. At each incision, the blade penetrated to a depth 7.7 mm below the dura. In the sham operation, the skull was exposed but the brain was left intact. Only rats that subsequently exhibited increased weight gain and food intake were selected for subsequent feeding experiments. Previously, we had found that intraperitoneal (ip) injection of ghrelin did not induce food intake in midbrain-transected rats (7). Therefore, to confirm that the transection surgery was successful, we verified before the start of the experiment that ghrelin-induced feeding was disrupted by the transection; we also removed the brains after the feeding tests and histologically verified the exact locations of the lesions.

Feeding experiments. Rats were sufficiently habituated to handling before the experiments. CCK8-(26-33) amide (Peptide Institute, Osaka, Japan) was dissolved in 0.9% saline, and this solution (0.1 to 10 nmol/kg in a volume of 100 μl) was administered ip between 0930 and 1000 to naive rats that had been fasted overnight. We then measured food intake at 0.5, 1, and 2 h after injection. Previously, we had tested various doses of leptin (from 18.8 to 62.5 nmol/kg) in a feeding experiment in naive rats. We found that ip injection of 62.5 nmol/kg leptin significantly decreased food intake 2 h after injection (P < 0.05 vs. saline); neither 18.8 nmol/kg nor 35.7 nmol/kg leptin reduced feeding (41). Another study had also found that 18.8 nmol/kg leptin does not induce phosphorylation of STAT3 in the hypothalamus (46). Therefore, to investigate the combined effect of CCK and leptin on food intake, we used doses of 0.1 nmol/kg CCK and 18.8 nmol/kg rat recombinant leptin (Sigma Aldrinch, St. Louis, MO), neither of which decreased food intake when administered alone. We allocated each of four groups of rats (12 rats per group) to one treatment: single injection of saline, single injection of CCK (0.1 nmol/kg), single injection of leptin (18.8 nmol/kg), and coinjection of CCK (0.1 nmol/kg) and leptin (18.8 nmol/kg).

Feeding experiments were conducted in icv-cannulated, midbrain-transected, or sham-operated rats 1 wk after surgery. Rats (n = 6 per group) were given an icv injection of saline (5 μl); the AMPK activator AICAR (5′-aminomimidazole-4-carboxyamide ribonucleotide); 10 nmol/5 μl), anti-TRH antibody (0.5 μg/2.5 μl; Progen Biotechnik, Heidelberg, Germany) + anti-CART (55-102) antibody (0.5 μg/2.5 μl; Phoenix Pharmaceuticals, Belmont, CA); or normal rabbit serum IgG (Sigma-Aldrich, 1 μg/5 μl) between 0900 and 1000. All compounds were dissolved in 0.9% saline. A previous study had shown that the dose of AICAR did not affect food intake per se (22). One hour after icv administration, the rats were injected ip with saline or CCK (0.1 nmol/kg) + leptin (18.8 nmol/kg), and then food intake was measured at 0.5, 1, and 2 h after injection.

To investigate the effect of CCK and leptin on food intake in midbrain-transected rats, we allocated each of four groups of rats to one treatment, namely single injection of saline in sham-operated rats, coinjection of CCK (0.1 nmol/kg) + leptin (18.8 nmol/kg) in sham-operated rats, single injection of saline in midbrain-transected rats, and coinjection of CCK (0.1 nmol/kg) + leptin (18.8 nmol/kg) in midbrain-transected rats. Rats were injected between 0930 and 1000 with saline or CCK plus leptin ip, and then food intake was measured at 0.5, 1, and 2 h after injection.

Western blotting. The hypothalami were rapidly removed 0.5 h after ip injection of saline, CCK, leptin, or CCK + leptin into naive rats or sham-operated or midbrain-transected rats (n = 4 to 5 per group). Thirty micrograms of total protein per sample was analyzed using SDS-PAGE (8% acrylamide gel) and electroblotted onto polyvinylidene difluoride membranes (Immobilon-P, Millipore, Tokyo, Japan). The membranes were blocked for 1 h at room temperature with Blocking One-P (Nacalai Tesque, Kyoto, Japan), probed overnight at 4°C with primary antibody against STAT3, phosphorylated STAT3, AMPK, or phosphorylated AMPK (Cell Signaling Technology, Danvers, MA), and incubated for 1 h at room temperature with horseradish peroxidase (HRP)-conjugated secondary antibodies (Epitomics, Burlingame, CA). Specific proteins were detected by chemiluminescence with Western BLOT Quant HRP substrate (Takara Bio, Otsu, Japan) in accordance with the manufacturer’s instructions and then exposed to X-ray film (Fuji Film, Tokyo, Japan). Western blotting was quantified by densitometry relative to STAT3 or AMPK by using NIH Image J software (National Institutes of Health, Bethesda, MD).

Quantitative PCR. The hypothalami were rapidly removed 0.5 h after ip injection of saline, CCK, leptin, or CCK + leptin (n = 5 in each group). Total RNAs were extracted with TRizol Reagent (Invitrogen, Carlsbad, CA) and reverse-transcribed using a Superscript III First-Strand Synthesis System Kit (Invitrogen). Real-time PCR for NPY, AgRP, CART, TRH, and POMC was conducted with a LightCycler system (Roche Diagnostics, Mannheim, Germany) and a SYBR Premix Ex Taq II Kit (Takara Bio). Specific primers were designed using a primer design tool (http://www.primer3.org) and used to amplify mouse-specific gene expression. All primers were synthesized by Sigma-Aldrich (St. Louis, MO). The primers were designed to amplify a single PCR product of the expected size. The PCR conditions were as follows: 95°C for 10 s, 50°C for 10 s, and 72°C for 10 s for 40 cycles. The melting curves and relative concentrations of the PCR products derived from the target genes were calculated using LightCycler analysis software.

Table 1. Primers used for quantitative PCR

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<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
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<tr>
<td>NPY</td>
<td>CTCTGTTGGAACCTGGACCTGCTTATT</td>
<td>CATCTTCTGCGCTGGGCTCTCG</td>
</tr>
<tr>
<td>AgRP</td>
<td>TCTGAAGAAAGAGCAAGCACACGAGA</td>
<td>AGCCACGCGGAGAGACAGCT</td>
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<tr>
<td>CART</td>
<td>ATGGAGACGCCTCGGGCTCG</td>
<td>CAGCTTCTTCTATGGGAC</td>
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<tr>
<td>TRH</td>
<td>GTCCTACCCCTGAGACTCC</td>
<td>CGAGGAGCTCAAAGGACGAC</td>
</tr>
<tr>
<td>POMC</td>
<td>GAGCTGACACCAAGAAAGGAGACCTG</td>
<td>ACGGGCTGCGGAGATTTTTCAGTGGAGG</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GCAGAAGGAAGTCTAGGGAAAA</td>
<td>AGTCTCTCAGGGGAGAGCCAG</td>
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food intake. We first tested various doses of CCK ranging from 0.1 to 10 nmol/kg ip in a feeding experiment in naïve rats. A single injection of 1 or 10 nmol/kg CCK significantly reduced food intake 0.5, 1, and 2 h after injection compared with injection of saline (CCK 1 nmol/kg, P < 0.05 at 0.5, 1, and 2 h; CCK 10 nmol/kg, P < 0.001 at 0.5 and 1 h, P < 0.01 at 2 h; n = 7 to 9 per group); however, CCK at 0.1 nmol/kg did not reduce food intake (Fig. 1A). Therefore, in our examination of the food intake-reducing interaction of CCK and leptin we used 0.1 nmol/kg CCK and 18.8 nmol/kg leptin, neither of which decreased food intake when administered alone. Coinjection of 0.1 nmol/kg CCK ip and 18.8 nmol/kg leptin ip significantly reduced food intake 0.5, 1, and 2 h after injection compared with saline (P < 0.05 at 0.5 h and 2 h, P < 0.01 at 1 h, n = 7 per group; Fig. 1B).

Phosphorylation of STAT3 and AMPK. To investigate the molecular mechanism involved in coadministration of CCK and leptin, we examined phosphorylation of STAT3 and AMPK in the hypothalamus. There were no significant differences in phosphorylation of STAT3 among the groups (Fig. 2A), but phosphorylation of AMPK in the hypothalamus was dramatically lower after coinjection of CCK and leptin than after single injection of saline (P < 0.01; n = 5 per group; Fig. 2B).

mRNA expression levels. To examine the roles of anorectic or orexigenic substances produced in the hypothalamus after coinjection of CCK and leptin, we evaluated the mRNA levels of NPY, AgRP, CART, TRH, and POMC after ip injection of rats with saline, CCK, leptin, or CCK + leptin. There were no significant differences in NPY, AgRP, or POMC mRNA levels among the groups (Fig. 3, A, B, and E). In contrast, CART and TRH mRNA levels were significantly higher after coinjection of CCK and leptin than after injection of saline (P < 0.001, n = 5 per group; Fig. 3, C and D).

Role of hypothalamic AMPK and CART/TRH after coinjection of CCK and leptin. To determine whether AMPK inhibition was necessary for the anorectic effect of CCK + leptin, we examined food intake after ip injection of CCK + leptin after icv injection of the AMPK activator AICAR. Following central administration of saline, ip coinjection of CCK and leptin significantly decreased food intake 0.5, 1, and 2 h later compared with icv and then ip injection of saline (P < 0.01 at 0.5 h and 2 h, P < 0.001 at 1 h, n = 6 per group; Fig. 4A). However, when AICAR was given before coinjection of CCK and leptin, the interactive effect on food intake was abolished (Fig. 4A).

Furthermore, to investigate the relationship between CART/TRH and the interaction of CCK and leptin, we evaluated the effects of anti-CART and anti-TRH antibodies on the reduction in food intake induced by coinjection of CCK and leptin. Following central administration of control IgG, ip coinjection of CCK and leptin significantly decreased food intake 0.5, 1, and 2 h after injection compared with icv IgG and then ip saline (P < 0.01 at 0.5 h, P < 0.001 at 1 and 2 h, n = 6 per group; Fig. 4B). However, when anti-CART and anti-TRH antibodies were given before coinjection of CCK and leptin, the interactive effect on feeding was abolished (Fig. 4B).

Food intake in midbrain-transected rats. To investigate the importance of the neural pathway from the hindbrain to the hypothalamus in the interaction of CCK and leptin, we examined food intake after coinjection of CCK and leptin in bilaterally midbrain-transected rats. Coinjection of CCK and leptin significantly decreased food intake in sham-operated rats compared with that in saline-injected sham-operated rats 0.5, 1, and 2 h after coinjection (P < 0.01 at 0.5 h, P < 0.001 at 1 and 2 h, n = 7 per group).
Phosphorylation of AMPK in midbrain-transected rats. Phosphorylation of AMPK in the hypothalamus of sham-operated rats was dramatically and significantly reduced after coinjection of CCK and leptin ($P < 0.01$ vs. sham saline, $n = 4$ per group; Fig. 4B). No reduction in AMPK phosphorylation occurred in the hypothalamus of midbrain-transected rats after coinjection (Fig. 5B).

mRNA levels in midbrain-transected rats. The mRNA levels of CART and TRH after coinjection of CCK and leptin and into sham-operated rats were significantly higher than those after injection of saline ($P < 0.001$, $n = 4$ per group), whereas the mRNA levels of CART and TRH after coinjection did not increase in the midbrain-transected rats (Fig. 5C).

DISCUSSION

CCK is released postprandially and elicits satiety signals in the short term. Here, we first examined the dose-response effects of CCK on food intake. We showed that 1 and 10 nmol/kg CCK significantly reduced food intake in naïve rats 0.5, 1, and 2 h after ip injection, whereas 0.1 nmol/kg CCK administered ip did not decrease food intake. Consistent with our findings, some studies have shown that the threshold dose of CCK for reduction of food intake in rats is 1 nmol/kg (17, 18). Interaction between leptin and CCK reduces short-term food intake (3, 39, 56). In agreement with these previous findings, we demonstrated here that coinjection of subthreshold doses of CCK (0.1 nmol/kg) and leptin (18.8 nmol/kg) significantly decreased food intake. However, the molecular mechanisms responsible for the interaction between CCK and leptin in the hypothalamus were not clear.

To clarify whether coinjection of CCK and leptin enhances leptin signaling, we examined the phosphorylation of STAT3 in the hypothalamus after coinjection. Coinjection of subthreshold CCK and leptin did not increase phosphorylation of STAT3 (Fig. 2); this result was similar to the effect of CCK or leptin alone. These data indicate that the reduction of food intake induced by CCK and leptin together does not occur through the direct intensification of JAK-STAT3 signaling in the hypothalamus. AMPK acts as a general energy sensor and integrator of nutrient or hormonal signals in the hypothalamus (21, 43). Hypothalamic AMPK activity is decreased by the action of anorectic hormones, including leptin (1, 33). Here, we demonstrated that coinjection of subthreshold CCK and leptin dramatically decreased the phosphorylation of AMPK. We also used pretreatment with the AMPK activator AICAR to evaluate whether AMPK inhibition was necessary for the anorectic effect of CCK plus leptin. When AICAR was given before coinjection of CCK and leptin, the interactive effect on food intake was abolished. These data imply that coinjection of CCK and leptin reduces food intake, at least in part, by decreasing the phosphorylation of AMPK in the hypothalamus.

To investigate which molecules in the hypothalamus were involved in the food intake reduction induced by CCK plus leptin, we examined the mRNA expressions of NPY, AgRP, CART, TRH, and POMC, which are hypothalamic neuropeptides associated with food intake. Coinjection of CCK and leptin, or single injection of saline, CCK, or leptin, did not affect the mRNA expressions of NPY, AgRP, or POMC. In contrast, although neither CCK nor leptin alone affected CART or TRH at subthreshold doses, coinjection of subthreshold doses of CCK and leptin significantly increased CART and TRH expression. To further evaluate the relationship between the interaction of CCK plus leptin and the induction of CART/TRH, we examined whether preadministration of CART and TRH antibodies blocked the reduction in food intake caused by coinjection of CCK and leptin. When anti-CART and anti-TRH antibodies were given before coinjection of CCK and leptin, the interactive effect on feeding was abolished. These data imply that coinjection of CCK and leptin reduces food intake, at least in part, by increasing CART and TRH and decreasing the phosphorylation of AMPK in the hypothalamus.

Although CART was originally identified in the striatum through the increased expression of its mRNA in response to cocaine or amphetamine administration, CART is widely distributed in the central nervous system (24, 25). It is well known that CART is produced by a lateral population of neurons in the hypothalamic arcuate nucleus; these neurons project widely throughout the brain (10–12). CART is also expressed in several other hypothalamic nuclei, including the paraventricular nucleus and lateral hypothalamic area (5). In addition, double-label in situ hybridization has shown that CART is coexpressed with TRH in neurons of the hypothalamic paraventricular nucleus (5). CART stimulates the hypothalamic-pituitary-thyroid axis via axosomatic and axodendritic interac-
Injections in the TRH neurons of the paraventricular nucleus (15, 53). Barrachina et al. showed that coinjection of CCK and leptin enhances the number of Fos-positive cells in the paraventricular nucleus, whereas injection of leptin or CCK alone does not modify Fos expression (3). These findings suggest that signals produced by coinjection of CCK and leptin affect CART/TRH neurons located in the paraventricular nucleus.

Previous studies have shown that CCK1 receptors are abundant on peripheral vagal afferent fibers and that peripheral injection of CCK decreases food intake through activation of the vagal afferent pathway (44, 49). Indeed, subdiaphragmatic vagotomy or perivagal application of capsaicin, a specific afferent neurotoxin, blocks CCK-induced food intake reduction (48, 50). Thus, the vagus nerve plays an important role in transmitting the anorectic signals of CCK. Furthermore, we used midbrain-transected rats to investigate the role of the neural pathway from hindbrain to hypothalamus in the interaction of CCK and leptin to reduce food intake. Coinjection of subthreshold doses of CCK and leptin in midbrain-transected rats did not reduce food intake. This finding suggests that the neural pathway from the hindbrain to the hypothalamus plays an important role in the food intake reduction induced by the interaction of CCK and leptin. We also demonstrated that coinjection of subthreshold doses of CCK and leptin dramatically decreased the phosphorylation of AMPK in naive and sham-operated rats, whereas this effect was blocked in midbrain-transected rats. These findings imply that the neural pathway from hindbrain to hypothalamus contributes to the feeding reduction of CCK and leptin.

Leptin, an adipose tissue- or stomach-derived circulating hormone, plays a key role in regulating food intake by conveying anorectic information to the brain (2, 16). ObRb receptors are expressed in several hypothalamic nuclei, including the...
Arcuate nucleus, ventromedial hypothalamic nucleus, and dorsomedial hypothalamic nucleus (36). ObRbs are also expressed in vagal afferent neurons (39, 40) and the hindbrain (27, 36), which contains the nucleus of the NTS, and the area postrema, which plays a role in controlling the entry of blood-borne substances into the neurons of the brain stem (30, 42). These observations indicate that various sites are potential targets of leptin. We can therefore surmise that there are several kinds of interactive mechanisms that induce food intake reduction upon coinjection of CCK and leptin. Our finding that the interactive effect of CCK and leptin on food intake was abolished in midbrain-transected rats suggests that there are at least three mechanisms. The first is that CCK and leptin signals are integrated in the hindbrain; the second is that the CCK signal reaching the NTS enhances the activity of leptin-sensitive neurons in the hypothalamus through the neural pathway from the hindbrain; and the third is that the descending fibers originating from leptin-sensitive neurons in the hypothalamus enhance the CCK signal reaching the NTS. Indeed, previous studies have shown that the potentiating effect of leptin on CCK-induced satiety involves local actions of leptin on CCK-sensitive neurons within the nodose ganglion and the hindbrain (19, 32). In contrast, Morton et al. (35) showed that injection of adenovirus expressing the ObRb gene into the hypothalamic arcuate nucleus of leptin receptor-deficient rats reduces meal size and enhances peripheral CCK-induced anorectic signals in the hindbrain. They hypothesized that projection of leptin-sensitive hypothalamic neurons to the hindbrain is critical in the food intake reduction induced by coinjection of CCK and leptin. However, considering that subthreshold leptin did not affect the phosphorylation of STAT3, it is unlikely that leptin signaling in the hypothalamus enhances the CCK signal or is enhanced by the CCK signal. Additional studies are required to clarify how and where CCK and leptin signals are integrated. It is also important to focus on the interaction of CCK with stomach-derived leptin. Elucidation of the mechanisms of the interactions among the various feeding-related hormones, as

**Fig. 4. Effects of 5′-aminoimidazole-4-carboxamide ribonucleoside (AICAR; A) and anti-CART + anti-TRH antibodies (B) on the reduction in food intake caused by CCK + leptin. Preadministration of AICAR or anti-CART + anti-TRH antibodies abolished the food intake reduction induced by coinadministration of CCK (0.1 nmol/kg) + leptin (18.8 nmol/kg). Data represent means ± SE (n = 6 each). **P < 0.01 vs. intracerebroventricular (icv) saline and then ip saline or icv IgG and then ip saline; ***P < 0.001 vs. icv saline and then ip saline or icv IgG and then ip saline.**

arcuate nucleus, ventromedial hypothalamic nucleus, and dorsomedial hypothalamic nucleus (36). ObRbs are also expressed in vagal afferent neurons (39, 40) and the hindbrain (27, 36), which contains the nucleus of the NTS, and the area postrema, which plays a role in controlling the entry of blood-borne substances into the neurons of the brain stem (30, 42). These observations indicate that various sites are potential targets of leptin. We can therefore surmise that there are several kinds of interactive mechanisms that induce food intake reduction upon coinjection of CCK and leptin. Our finding that the interactive effect of CCK and leptin on food intake was abolished in midbrain-transected rats suggests that there are at least three mechanisms. The first is that CCK and leptin signals are integrated in the hindbrain; the second is that the CCK signal reaching the NTS enhances the activity of leptin-sensitive neurons in the hypothalamus through the neural pathway from the hindbrain; and the third is that the descending fibers originating from leptin-sensitive neurons in the hypothalamus enhance the CCK signal reaching the NTS. Indeed, previous studies have shown that the potentiating effect of leptin on CCK-induced satiety involves local actions of leptin on CCK-sensitive neurons within the nodose ganglion and the hindbrain (19, 32). In contrast, Morton et al. (35) showed that injection of adenovirus expressing the ObRb gene into the hypothalamic arcuate nucleus of leptin receptor-deficient rats reduces meal size and enhances peripheral CCK-induced anorectic signals in the hindbrain. They hypothesized that projection of leptin-sensitive hypothalamic neurons to the hindbrain is critical in the food intake reduction induced by coinjection of CCK and leptin. However, considering that subthreshold leptin did not affect the phosphorylation of STAT3, it is unlikely that leptin signaling in the hypothalamus enhances the CCK signal or is enhanced by the CCK signal. Additional studies are required to clarify how and where CCK and leptin signals are integrated. It is also important to focus on the interaction of CCK with stomach-derived leptin. Elucidation of the mechanisms of the interactions among the various feeding-related hormones, as

**Fig. 5. Effects of CCK + leptin on food intake reduction in midbrain-transected rats. A: effects of ip administration of saline or of CCK (0.1 nmol/kg) + leptin (18.8 nmol/kg) on food intake in sham-operated and midbrain-transected rats. B: Western blot analysis of pAMPK in hypothalami of sham-operated and midbrain-transected rats after ip administration of saline or of CCK (0.1 nmol/kg) + leptin (18.8 nmol/kg). Band intensity ratio of pAMPK to AMPK in sham-operated rats after saline administration was arbitrarily set to 1.0. C: quantitative PCR analysis of CART (left) and TRH (right) in hypothalami of sham-operated and midbrain-transected rats after ip administration of saline or of CCK (0.1 nmol/kg) + leptin (18.8 nmol/kg). Data were normalized against the amount of GAPDH. Data represent means ± SE (n = 4–7 each). *P < 0.05 vs. sham-operated saline, **P < 0.01 vs. sham-operated saline, ***P < 0.001 vs. sham-operated saline.
shown here, will advance our understanding of the physiological mechanisms of food intake and energy metabolism.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


