Postprandial neuronal activation in the nucleus of the solitary tract is partly mediated by CCK-A receptors

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Glatzle, Jörg, Martin E. Kreis, Kazuya Kawano, Helen E. Raybould, and Tilman T. Zittel. Postprandial neuronal activation in the nucleus of the solitary tract is partly mediated by CCK-A receptors. Am J Physiol Regulatory Integrative Comp Physiol 281: R222–R229, 2001.—CCK-A receptors and neurons of the nucleus of the solitary tract (NTS) are involved in the regulation of food intake, and in rats, there is evidence for involvement of an intestinal vagal afferent pathway. Studies investigating the role of CCK-A receptors in activation of NTS neurons using highly selective CCK-A receptor agonists and antagonists have yielded conflicting data. In the present study, we investigated CCK-induced and postprandial activation of NTS neurons, together with food intake studies, in CCK-A receptor-deficient Otsuka Long-Evans Tokushima fatty (OLETF) rats. Activated NTS neurons were detected using immunohistological staining for c-Fos protein. Exogenous CCK increased the number of c-Fos protein-positive cells in the NTS of Sprague-Dawley and CCK-A receptor-intact Long-Evans Tokushima Otsuka (LETO) rats but had no effect in CCK-A receptor-deficient OLETF rats. Food intake-induced c-Fos protein expression in NTS neurons was significantly reduced in CCK-A receptor-deficient OLETF rats compared with Sprague-Dawley or LETO rats. Postprandial c-Fos protein expression in the NTS was also significantly decreased after pretreatment with the CCK-A receptor antagonist MK329 after both short- and long-term fasting periods. Exogenous CCK decreased cumulative food intake in Sprague-Dawley and CCK-A receptor-intact Long-Evans Tokushima Otsuka rats (LETO) but had no effect in CCK-A receptor-deficient Otsuka Long-Evans Tokushima fatty (OLETF) rats. Activated NTS neurons were detected using immunohistological staining for c-Fos protein. Exogenous CCK increased the number of c-Fos protein-positive cells in the NTS of Sprague-Dawley and CCK-A receptor-intact Long-Evans Tokushima Otsuka (LETO) rats but had no effect in CCK-A receptor-deficient OLETF rats. Food intake-induced c-Fos protein expression in NTS neurons was significantly reduced in CCK-A receptor-deficient OLETF rats compared with Sprague-Dawley or LETO rats. Postprandial c-Fos protein expression in the NTS was also significantly decreased after pretreatment with the CCK-A receptor antagonist MK329 after both short- and long-term fasting periods. Exogenous CCK decreased cumulative food intake in Sprague-Dawley and LETO rats but not in OLETF rats. These data demonstrate that CCK-A receptors are involved in the CCK- and food-induced c-Fos protein expression in the NTS. Taken together with the results of the food intake studies, this suggests that activation of CCK-A receptors is involved in the postprandial activation of NTS neurons and in the regulation of food intake.

Regulation of food intake; cholecystokinin; cholecystokinin-A receptor; cholecystokinin-A receptor blockade; MK329; Long-Evans Tokushima Otsuka rats; Otsuka Long-Evans Tokushima fatty rats; c-Fos protein; satiety; brain-gut axis

FOOD INTAKE IS REGULATED BY a variety of mechanisms including gastrointestinal hormones such CCK, which is postprandially released from mucosal I cells (3, 30). Inhibition of food intake by exogenous CCK is well established and was first described more than 20 years ago (12). CCK-induced inhibition of food intake can be observed in a variety of mammalian species including mice, rats, hamster, rabbits, pigs, sheep, monkeys, and humans (3, 17, 25). It is now widely accepted that endogenous CCK contributes to the regulation of short-term food intake and is involved in the mediation of satiety, because pretreatment with a potent CCK-A receptor antagonist increased food intake and almost completely blocked the satiety effects of exogenous CCK injection (11, 21).

Two receptors for CCK have been identified: the CCK-A and the CCK-B receptor; in addition, it is likely that at least two affinity states exist for the CCK-A receptor (4, 6, 21). There is evidence that exogenous and endogenous CCK inhibit food intake via the CCK-A receptor in the high-affinity state (21), although central CCK-B receptors might also be involved (7). Peripheral CCK-induced inhibition of food intake is thought to be mediated, in part, by vagal afferent nerve fibers, which have been shown to express CCK-A receptors, because CCK-induced inhibition of food intake is reduced after truncal vagotomy (15, 28, 40) or functional ablation of capsaicin-sensitive vagal afferents (36). CCK could activate neurons of the nucleus of solitary tract (NTS) via stimulation of CCK-A receptors on vagal afferent nerve fibers (23), which derive from the nodose ganglion, supply the gastrointestinal tract, and have monosynaptic contact with NTS neurons (28). Beside activating vagal afferent nerve fibers, CCK may also act on CCK-A receptors in the area postrema (AP), a region of the brain stem that has a leaky blood-brain barrier and monosynaptic connection to the NTS (19).

Short-term stimulation of nerve cells is followed by transcriptional and translational activity of selected genes including the c-fos oncogene, resulting in the production of intracellular regulatory factors like Fos protein (14, 24). c-Fos protein expression in neurons...
indicates neuronal activation and can be used to trace pathways of neuronal activation (8, 31). We have shown that food intake activates c-Fos protein expression in the NTS, with maximal expression 90 min after the start of food intake (44). Others have shown maximal c-Fos protein expression after intraperitoneal (ip) injection of CCK at this time point (13, 39). Studies to elucidate the role of CCK-A receptors in the regulation of food intake using postprandial c-Fos protein expression in the NTS have yielded contradictory results. CCK-A receptor blockade had no effect on the postprandial activation of NTS neurons in one study (9), whereas others have shown a significant reduction (2, 4, 6, 44).

In the present study, we tested two hypotheses: 1) CCK- and food-induced activation of NTS neurons is dependent on CCK-A receptors and 2) a reduced postprandial activation of NTS neurons is associated with increased food intake. Rather than using pharmacological means to establish a role for CCK-A receptors, we used a rat strain deficient in CCK-A receptors. Neuronal activation in different topographical regions of the NTS was measured in these CCK-A receptor-deficient Otsuka Long-Evans Tokushima fatty (OLETF) rats to assess the role of CCK-A receptors in NTS neuronal activation. These data were compared with those obtained with selective CCK-A and CCK-B receptor blockade using specific and potent receptor antagonists. In addition, food intake was measured in parallel experiments to correlate reduced activation of NTS neurons with alterations of food intake.

METHODS

Animals. Male Sprague-Dawley rats (Charles River, Kieslegg, Germany), Long-Evans Tokushima Otsuka (LETO) rats (Tokushima, Japan), and OLETF rats (Tokushima, Japan), weighing 270–340 g and ~3 mo old, were used for experiments. The OLETF rats constitute a strain deficient in the CCK-A receptor by spontaneous mutation. OLETF rats arose from the LETO rat strain, which shows normal expression of the CCK-A receptor (10). Rats were housed under controlled conditions of illumination (12:12-h light-dark cycles starting at 10:00 AM), humidity (60–70%), and temperature (21°C). The institutional guidelines for the care and use of laboratory animals were followed throughout the study.

CCK-induced c-Fos protein expression in the NTS. c-Fos protein expression in the NTS was investigated in Sprague-Dawley rats (n = 4), LETO rats (n = 5), or OLETF rats (n = 5) 90 min after ip injection of CCK, dissolved in 0.1% carboxymethylcellulose (Sigma, Deisenhofen, Germany). After a 4-h fasting period with free access to water, rats were injected with CCK (4 μg/kg ip) immediately before the dark phase. After 90 min, rats were deeply anesthetized with ketamine hydrochloride (100 mg/kg, Park Davis, Berlin, Germany) and xylazine (15 mg/kg, Bayer, Leverkusen, Germany). The brain stem was removed and processed for immunohistochemistry of c-Fos protein expression in the NTS as described below.

Postprandial c-Fos protein expression in the NTS. Postprandial c-Fos protein expression in the NTS was investigated in Sprague-Dawley rats (n = 7), LETO rats (n = 5), or OLETF rats (n = 5) 90 min after the start of food intake. After a 4-h fasting period with free access to water, rats were given access to food at the beginning of the dark period. After 90 min, rats were deeply anesthetized and the brain stem was removed. c-Fos protein expression was measured 90 min after the start of food intake, because we have previously shown maximal postprandial c-Fos protein expression in the NTS at that time point (44). The amount of food intake during the 90-min feeding period was recorded using the methods described in Measurement of food intake.

Postprandial c-Fos protein expression in the NTS after CCK-A and CCK-B receptor blockade. Postprandial c-Fos protein expression in the NTS was analyzed in Sprague-Dawley rats after ip injection of the CCK-A receptor antagonist MK329 (1 mg/kg dissolved in 0.1% carboxymethylcellulose; Merck, Sharp & Dohme, Munich, Germany), the CCK-B receptor antagonist L-365,260 (10 or 100 μg/kg dissolved in 0.1% carboxymethylcellulose; Merck, Sharp & Dohme), or vehicle (1 ml/kg 0.1% carboxymethylcellulose, Sigma) 15 min before the start of food intake. Rats were deprived of food for either 4 h (MK329, n = 5; L-365,260, 10 μg/kg, n = 4; L-365,260, 100 μg/kg, n = 4; vehicle, n = 5) or 16 h (MK329, n = 4; vehicle, n = 4) with free access to water. After 90 min, rats were deeply anesthetized and the brain stem was removed. The amount of food intake during the 90-min feeding period was recorded using the methods described in Measurement of food intake.

Measurement of food intake. An automatic recording system (Diet-Scan, Omnitech, Columbus, OH) with four separate cages was used to measure food intake. Food consumption was measured with a precision of 0.1 g and with selectable time intervals. Food intake was recorded every 15 min for 4 h, starting at the beginning of the dark phase.

Effects of CCK and CCK-receptor blockade on food intake. Food intake in Sprague-Dawley rats (n = 8), LETO rats (n = 8), and OLETF rats (n = 8) was measured after a 4-h fasting period with free access to water. Either 0.1% BSA (Sigma) or sulfated CCK-8 (dissolved in 0.1% BSA; Sigma) at a dose of 4 μg/kg (~3.5 nmol/kg) was injected ip 5 min before the dark phase. Food intake was also measured in Sprague-Dawley rats after ip injection of either the CCK-A receptor antagonist MK329 (1 mg/kg ip), the CCK-B receptor antagonist L-365,260 (10 or 100 μg/kg ip), or vehicle (ip) 15 min before the start of food intake. Two different fasting periods were tested to elucidate the effects of different hunger intensities on CCK-A receptor blockade of food intake. Rats were fasted for either 4 h (MK329, 1 mg/kg, n = 15; L-365,260, 10 μg/kg, n = 10; L-365,260, 100 μg/kg, n = 10; vehicle, n = 30) or 16 h (MK329, 1 mg/kg, n = 15; vehicle n = 15).

Tissue harvesting and immunohistochemistry. Tissue harvesting and immunohistochemistry were performed as described previously (42, 43). In brief, rats were perfused with 0.9% saline in 0.1 M phosphate buffer (PB; pH 7.4) via the ascending aorta followed by 4% paraformaldehyde in 0.1 M PB. The brain stem was removed, postfixed in 4% paraformaldehyde in 0.1 M PB for 2 h, and cryoprotected in 25% sucrose in 0.1 M PB overnight. Sections were cut with a cryotome (CM 3000, Leica, Wiesbaden, Germany) and then by 0.05% DAB/0.033% 3,3′-diaminobenzidine (DAB) serving as the chromagen. Tissue was incubated overnight at 4°C with Fos Ab-5 antibody (Calbiochem, Bad Soden, Germany) at a dilution of 1:1,000, followed by ABC (Vectastain, Camon, Wiesbaden, Germany) and then by 0.05% DAB/0.033% hydrogen peroxide (Sigma) for 10 and 5 min, respectively. Sections were mounted onto gelatin-coated slides, air-dried, dehydrated, and placed on a coverslip with mounting medium (Eukitt, Kindler, Freiburg, Germany).
Analysis of c-Fos protein expression in the NTS. Immuno-

histochemistry was performed on every third section of the

brain stem (i.e., approximately every 100 μm of the tissue,

50–60 sections per animal). Fos-like immunopositive cells in

the NTS were counted by a video camera and computer-

assisted bright field microscopy system (Quantimed 500,

Leica) at the levels of the obex (interaural about

25.30 mm), the AP (interaural about 24.80 mm), and the level of the

caudal fourth ventricle (interaural about 24.30 mm) accord-

ing to stereotaxic rat brain atlases (27, 38). In each rat, three

sections of each of the three anatomic levels were analyzed.

For each brain stem section analyzed, the area wherein c-Fos protein-positive cells were detected was calculated by a com-

puter program, as described previously (44).

Blood glucose levels of OLETF rats. Adult OLETF rats

spontaneously develop diabetes mellitus at the age of about 6

mo (16). In separate experiments, blood glucose levels were

measured by puncturing the rat tail vein 90 min after the

start of the food intake to ensure normoglycemic metabolism

at the time of experiments. Blood glucose was measured by a

glucose-oxidase test (Haemo-Glucotest, Boehringer, Mann-

heim, Germany) and is reported in milligrams per 100 ml.

Statistical analysis. Data are presented as means ± SE.

Differences between groups were determined by ANOVA

followed by Student’s t-test using the software package of

JMP (version 3.2.2, SAS Institute, Cary, NC). A probability of

P < 0.05 was taken as significant.

RESULTS

CCK-induced c-Fos expression in the NTS. In vehi-
cle-treated rats fasted for 4 h, few c-Fos-positive cells

were seen throughout the NTS [13 ± 2, 14 ± 3, and 3 ± 1

cells/section at the level of 4th ventricle (interaural

−4.3 mm), the AP (interaural −4.8 mm), and the obex

(interaural −5.3 mm), respectively] as previously pub-

lished (44). CCK injection significantly increased the

number of c-Fos protein-positive cells at all levels of

the NTS of Sprague-Dawley and LETO rats but not in

OLETF rats (Figs. 1 and 2).

CCK induced c-Fos protein expression in the AP in

both Sprague-Dawley and LETO rats but not in

OLETF rats. However, no quantitative analysis was

performed.

Postprandial c-Fos protein expression in the NTS.

Feeding significantly increased the number of c-Fos

protein-positive cells in the NTS of Sprague-Dawley

and LETO rats, with no significant differences between

these two rat strains. In LETO rats, 23 ± 4, 30 ± 3, and

6 ± 1 c-Fos protein-positive cells/section could be de-
tected at the level of the 4th ventricle, the AP, and the

obex, respectively, 90 min after the start of the feeding

process.
period. In OLETF rats, the number of c-Fos protein-positive cells in the NTS 90 min after the start of feeding was significantly lower (14 ± 2, 9 ± 3, and 3 ± 0 cells/section at the level of the 4th ventricle, AP, and obex, respectively), representing a 39, 70, and 50% decrease compared with LETO rats (Fig. 3). Cumulative food intake 90 min after the start of feeding was increased in OLETF rats compared with LETO rats, but the difference was not statistically significant (cumulative food intake g/100 g body wt at 90 min: OLETF rats 1.32 ± 0.10 vs. LETO rats 1.19 ± 0.32; not significant).

Effects of CCK-A and CCK-B receptor blockade on the postprandial c-Fos protein expression in the NTS in Sprague-Dawley rats. Basal c-Fos protein expression in the NTS of rats fasted for 16 h was 7 ± 1, 13 ± 1, and 7 ± 2 cells/section at the levels of the caudal 4th ventricle, the AP, and the obex, respectively. Administration of MK329 significantly decreased the postprandial number of c-Fos protein-positive cells in the NTS in Sprague-Dawley rats. In rats fasted for 4 h, a 54% decrease in c-Fos protein-positive cells was observed in the NTS only at the level of the AP. After a 16-h fasting period, a 31 and 56% decrease in the number of c-Fos protein-positive cells was observed in the NTS at the level of the AP and the obex, respectively. No significant changes were seen in the number of c-Fos protein-positive cells in the NTS at the level of the caudal 4th ventricle after either fasting period (Fig. 4).

In contrast, postprandial c-Fos protein expression in the NTS was not significantly changed after CCK-B receptor blockade by L-365,260 (10 or 100 μg/kg ip) in rats fasted for 4 h, compared with vehicle treatment at any level of the NTS (Fig. 4).

Effects of CCK injection on food intake in Sprague-Dawley, LETO, and OLETF rats. Administration of CCK (4 μg/kg ip) after a 4-h fasting period decreased food intake both in Sprague-Dawley and in LETO rats in the 30 min after the start of the feeding period. No significant difference was observed between the two rat strains. In contrast, administration of CCK had no effect on food intake in OLETF rats (Fig. 5).

Effects of CCK-A and CCK-B receptor blockade on food intake in Sprague-Dawley rats. Administration of MK329 (1 mg/kg ip) significantly increased food intake
Table 1. CCK-A receptor blockade by MK329 significantly increased food intake in Sprague-Dawley rats being deprived of food for 4 or 16 h

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Vehicle (n = 30)</th>
<th>MK329 (n = 15)</th>
<th>Vehicle (n = 15)</th>
<th>MK329 (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cumulative food intake</td>
<td>Δ Food intake</td>
<td>Cumulative food intake</td>
<td>Δ Food intake</td>
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<tr>
<td>15</td>
<td>0.54 ± 0.08</td>
<td>0.78 ± 0.11†</td>
<td>0.84 ± 0.07</td>
<td>1.02 ± 0.06*</td>
</tr>
<tr>
<td>30</td>
<td>0.77 ± 0.13</td>
<td>1.10 ± 0.15*</td>
<td>1.31 ± 0.13</td>
<td>1.65 ± 0.09*</td>
</tr>
<tr>
<td>60</td>
<td>1.02 ± 0.14</td>
<td>1.61 ± 0.22†</td>
<td>1.90 ± 0.21</td>
<td>1.90 ± 0.21</td>
</tr>
<tr>
<td>90</td>
<td>1.36 ± 0.12</td>
<td>2.24 ± 0.29†</td>
<td>2.37 ± 0.26</td>
<td>2.37 ± 0.18</td>
</tr>
<tr>
<td>120</td>
<td>1.66 ± 0.18</td>
<td>2.68 ± 0.32†</td>
<td>2.74 ± 0.28</td>
<td>3.57 ± 0.37†</td>
</tr>
<tr>
<td>150</td>
<td>1.89 ± 0.15</td>
<td>2.96 ± 0.35†</td>
<td>3.08 ± 0.28</td>
<td>4.07 ± 0.41*</td>
</tr>
<tr>
<td>180</td>
<td>2.05 ± 0.15</td>
<td>3.61 ± 0.33†</td>
<td>3.25 ± 0.28</td>
<td>4.48 ± 0.43†</td>
</tr>
<tr>
<td>240</td>
<td>2.77 ± 0.16</td>
<td>4.34 ± 0.38†</td>
<td>3.78 ± 0.37</td>
<td>5.24 ± 0.46*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Cumulative food intake and change in (Δ) food intake per 60-min time interval are given as g/100 g body wt. *P < 0.05, †P < 0.001, MK329 vs. corresponding vehicle.

DISCUSSION

The present study demonstrates, using a strain of rats deficient in CCK-A receptors, that the c-Fos protein expression in the NTS induced by exogenous peripheral administration of CCK is mediated by CCK-A receptors. In addition, the significant reduction in the number of activated neurons in the NTS in OLETF rats after feeding suggests that CCK-A receptors are involved in the activation of NTS neurons in response to a meal. Consistent with this finding in CCK-A-deficient rats, activation of NTS neurons in response to feeding was also markedly reduced by pretreatment with MK329, a potent CCK-A receptor antagonist. In addition, exogenous CCK decreased cumulative food intake in Sprague-Dawley and in LETO rats but had no effect on food intake in CCK-A-receptor-deficient OLETF rats. Thus, in the absence of CCK-A receptors and neuronal activation of NTS neurons, CCK had no effect on food intake, suggesting that activation of NTS neurons is required to initiate the satiety response to CCK. We have previously shown that there is a correlation between the amount of food eaten and the number of activated neurons in the NTS (44). However, this correlation no longer holds in the absence of CCK-A receptors. In OLETF rats, despite eating equivalent amounts of food to either LETO or Sprague-Dawley rats, there was significantly less activation of NTS neurons. Taken together, the results of the present study demonstrate that CCK- and food-induced activation of NTS neurons is dependent on CCK-A receptors and that a reduced postprandial activation of NTS neurons is associated with increased food intake. CCK is released from enteroendocrine cells of the mucosal wall by nutrients in the small intestine, and increased plasma levels of CCK have been measured postprandially in humans and experimental animals including rats (18). There is considerable evidence that CCK is involved in the short-term regulation of food intake. CCK is released postprandially, food intake is significantly reduced after exogenous CCK injection, and blockade of the CCK-A receptor increases food intake and abolishes the inhibitory effect of exogenous CCK (11, 12, 22). Food intake and CCK injection increase the neuronal activity in the NTS, an area in the brain stem known to be involved in the regulation of food intake (20, 44). In our study, neuronal activation in the NTS corresponding to exogenous CCK was observed both in Sprague-Dawley and in LETO rats. Previously, we have shown a dose-dependent increase of c-Fos protein-positive cells in the NTS in response to exogenous CCK, in doses from 0.1 to 4 μg/kg (88 pmol/kg to 3.5 nmol/kg). C-Fos protein expression in the AP was only observed with CCK doses higher than 2 μg/kg (1.75 nmol/kg) (44). The number of activated NTS neurons in Sprague-Dawley rats in response to CCK injection was higher in our experiments than in those of Chen et al. (2), possibly due to the longer interval between the CCK injection and the fixation. However, in CCK-A receptor-deficient OLETF rats, activation in Sprague-Dawley rats after either a 4- or 16-h fasting period (Table 1). After a 4-h fasting period, MK329 increased cumulative food intake by ~58% during the first 60 min, compared with a 25% increase in rats being deprived of food for 16 h.

CCK-B receptor blockade by L-365,260 (10 or 100 μg/kg ip) had no effect on cumulative food intake for 90 min (cumulative food intake g/100 g body wt at 90 min: vehicle, 1.36 ± 0.2; L-365,260, 10 μg/kg, 1.13 ± 0.11; L-365,260, 100 μg/kg, 1.52 ± 0.13; not significant vs. vehicle).

Postprandial blood glucose levels in LETO and OLETF rats. Postprandial blood glucose levels were not significantly different between LETO and OLETF rats [LETO, n = 10, 110 ± 5 mg/dl (198 mmol/ml); OLETF, n = 10, 107 ± 5 mg/dl (193 mmol/ml)]. The blood glucose levels measured in each individual animal were in the normal range, indicating that both rat strains were normoglycemic at the time of experiments.

Area analysis in NTS sections. No area differences in the NTS sections at the anatomic level of the obex (interaural about −5.30 mm), the AP (interaural about −4.80 mm), and the level of the caudal 4th ventricle (interaural about −4.30 mm) were found among all groups investigated (data not shown).
the NTS neurons and AP neurons (data not shown) was not increased by exogenous CCK. In Sprague-Dawley rats, c-Fos mRNA and protein expression in the NTS in response to CCK were suppressed to basal levels by MK329, a potent and specific CCK-A receptor antagonist (2, 4). These data support the evidence that the CCK-A receptor is essential for information transfer from the periphery to the brain stem, because both CCK-A receptor-deficient OLETF rats and Sprague-Dawley rats pretreated with MK329 had no increased neuronal activation in the NTS after exogenous CCK. Because CCK-A receptors are located on vagal afferent nerve fibers and are activated by exogenous CCK (22), it is likely that activation of NTS neurons occurs in response to the monosynaptic vagal afferent input to NTS neurons. However, because we did observe c-Fos expression in the AP, CCK could also act on these neurons resulting in activation of the NTS, because monosynaptic connections between the AP and the NTS exist (19). However, it should be noted that c-Fos protein expression in the AP was seen at higher doses than those required to activate NTS neurons, suggesting that vagal afferent input is important and sufficient for peripheral CCK to activate NTS neurons.

Food intake–induced neuronal activation in the NTS in both Sprague-Dawley and LEKO rats was observed at 90 min after the beginning of the feeding period, the time point at which we have previously shown maximal stimulation (44). In LEKO rats, postprandial neuronal activation in the NTS was significantly reduced compared with LEKO rats, indicating that CCK-A receptors are involved in the postprandial activation of NTS neurons. This was despite consumption of equivalent amounts of food. Similarly, we also found a significant reduction of postprandially activated neurons in the NTS after pretreatment with MK329, although this was less pronounced compared with the findings in OLETF rats. Thus the activation of NTS neurons predicted by the amount of food eaten is reduced in the absence of CCK-A receptor activation. This provides support for the hypothesis that CCK receptor activation of the NTS is important in the regulation of food intake. Taken together, these findings demonstrate that CCK-A receptors are involved in the regulation of food intake, because when CCK-A receptors are absent or blocked, food intake is increased and correspondingly the number of activated NTS neurons is reduced. It has been suggested that the feeding-induced c-Fos expression is largely independent of CCK release (9), because in these observations, the meal-induced c-Fos expression was not completely blocked by pretreatment with CCK-A receptor antagonist. However, no detailed analysis was performed, and the number of c-Fos protein-positive cells in the NTS was not quantified. In the present study, MK329 did not completely block the postprandial c-Fos protein expression in the NTS and, in CCK-A receptor-deficient rats, postprandial activation of NTS neurons was also found, indicating that factors in addition to CCK are involved in the postprandial neuronal activation of the NTS.

The relative reduction of postprandially activated c-Fos protein-positive cells in the NTS and the relative increase of food intake after pretreatment with MK329 were more evident in rats fasted for 4 h than in rats fasted for 16 h. On the other hand, the absolute reduction of activated neurons in the NTS is bigger after a 16-h fasting period. However, it remains to be observed which is the more relevant measure for the brain regarding the regulation of food intake.

CCK-A receptors in rats are expressed in functionally distinct subpopulations of neurons innervating the gut, including enteric neurons and vagal afferent neurons (3). CCK released from endocrine intestinal cells might activate CCK-A receptors at afferent vagal nerve terminals (23), which innervate the gastrointestinal tract and make monosynaptic contact with NTS neurons (1, 29). Several studies have shown that the effect of CCK on food intake is mediated, at least in part, via the vagus nerve and the NTS. In addition, hormones such as serotonin [5-hydroxytryptamine (5-HT)], which is also released from the gut wall by a meal, might also be involved in the regulation of food intake (32). 5-HT receptors are found on vagal afferent nerve fibers, indicating that activation of NTS neurons in response to food intake could be partly mediated by serotonin (34). NTS neurons in response to food intake are likely activated by a variety of mediators, because food intake is regulated by a complex and redundant system that includes multiple transmitters and receptors in the gut as well as in the brain (35).

In this context, it is important to know that we only examined the response of the NTS in OLETF rats to CCK. It is possible that NTS activation may be altered by a number of different factors unrelated to alteration of the lack of CCK-A receptors. A “positive” control measurement, for example, of the response to another neuroactive peptide would enable us to demonstrate that the defect is specific for CCK. However, because of the interrelationship of many factors, both in the periphery and brain stem, the identity of a candidate for a reliable positive control is unclear.

CCK-A receptor blockade by MK329 has been shown to increase food intake in rats (11). In the present study, we compared the effect of MK329 on food intake in rats deprived of food for either 4 or 16 h. MK329 increased the total amount of food intake in rats fasted for 4 h by ~58% compared with a 25% increase in rats fasted for 16 h, whereas no differences could be observed in the absolute amount of food-intake increase between these two groups. Endogenous CCK release after food intake is probably more efficient in the regulation of food intake after short fasting periods. CCK-B receptors might also be involved in the regulation of food intake, because increased food intake could be measured in satiated rats after CCK-A or CCK-B receptor blockade, and this was more efficient after CCK-B receptor blockade (7). CCK-B receptor blockade by L-365,260 at a dose of 10 μg/kg did not affect food intake or neuronal activation in the NTS in our experiments in rats being fasted for 4 h, whereas in presatiated rats, food intake was significantly increased (7).
CCK-B receptors might be involved in the central regulation of food intake in partially satiated or satiated rats, because both the paraventricular nucleus and the ventromedial nucleus of hypothalamus, areas of the brain known to be involved in regulation of body weight, contain high densities of CCK-B receptors (41).

CCK significantly decreased food intake in Sprague-Dawley rats within 60 min after the beginning of food intake, similar to other studies (5, 11). CCK also reduced food intake in LETO rats, but no effect could be observed in CCK-A receptor-deficient OLETF rats, indicating that the CCK-A receptor is essential in the CCK-induced inhibition of food intake. OLETF rats are known to be hyperphagic and obese, consistent with the interpretation that the lack of CCK-A receptors results in a satiety deficit that promotes hyperphagia and obesity (33). However, it is unlikely that the absence of CCK-A receptors in the brain-gut axis alone is responsible for the hyperphagia and obesity observed in these rats.

In summary, our data indicate that the C-Fos protein expression in the NTS after exogenous CCK injection is mediated by CCK-A receptors and that in the postprandial activation of NTS neurons, CCK-A receptors are, at least in part, involved. This study provides further evidence that CCK and the NTS are interacting parts in the regulation of short-term food intake.

**Perspectives**

Regulation of food intake is a very complex mechanism in which a variety of different factors and regulation pathways is involved. In the present study, we demonstrated that CCK-A receptors and NTS neurons are interacting parts of the food-intake regulation system. It is important to gain further knowledge of the physiological interaction among food intake, the sensory transduction mechanism of the gut corresponding to different nutrients, the feedback regulation to the brain, and the induction of satiety, which terminates food intake, because in a variety of diseases, this interaction is disturbed and obesity is a common problem in our society.

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