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Phenotype of neurons in the nucleus of the solitary tract that express CCK-induced activation of the ERK signaling pathway

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Babic T, Townsend RL, Patterson LM, Sutton GM, Zheng H, Berthoud HR. Phenotype of neurons in the nucleus of the solitary tract that express CCK-induced activation of the ERK signaling pathway. Am J Physiol Regul Integr Comp Physiol 296: R845–R854, 2009. First published January 28, 2009; doi:10.1152/ajpregu.90531.2008.—The satiating potency of CCK has been well characterized, including its mediation by capsaicin-sensitive vagal primary afferents. We have previously shown that peripherally administered CCK activates the MAPK-signaling cascade in a population of neurons of the solitary tract (NTS) neurons and that preventing ERK1/2 phosphorylation partly attenuates CCK’s satiating potency. The aim of this study was to identify the neurochemical phenotypes of the NTS neurons that exhibit CCK-induced activation of ERK1/2. Using confocal microscopy, we demonstrate that intraperitoneal CCK administration increases the number of neurons that express phosphorylated ERK1/2 (pERK1/2) in the medial and commissural subnuclei of the NTS and that CCK-induced expression of ERK1/2 is increased in tyrosine hydroxylase-immunoreactive neurons. Using Western blot analysis, we show that the robust increase in tyrosine hydroxylase phosphorylation obtained with intraperitoneal CCK is significantly attenuated in rats pretreated with the ERK-pathway blocker U0126 injected into the 4th ventricle. In addition, CCK injections increased pERK1/2 expression in POMC neurons in the NTS. In contrast, only the rare GAD67, neuronal nitric oxide synthase, and leptin-responsive neuron exhibited CCK-induced pERK immunoreactivity. We conclude that activation of POMC-immunoreactive neurons and tyrosine hydroxylase activity via the ERK-signaling pathway in the NTS likely contributes to CCK’s satiating effects.

tyrosine hydroxylase; proopiomelanocortin; leptin receptor; mitogen-activated protein kinase; satiety; food intake; obesity

INCREASED FOOD INTAKE IS AN important component of energy balance and a major cause in the development of obesity. Because humans eat relatively few discrete meals, meal size control is a reasonable strategy to limit total food intake (33). Meal size is controlled by the process of satiation and, although considerable progress has been made in identifying the various signals contributing to satiation, the neural mechanisms integrating these signals are poorly understood.

CCK released from enteroendocrine cells lining the upper small intestine has been generally recognized as the archetypal satiation hormone (for a recent review, see Ref. 11). Its signaling pathway via CCK-1 receptor-bearing vagal afferent fibers to the nucleus tractus solitarius (NTS) in the caudal brain stem has been well documented (19, 24, 27, 31, 34, 40). In contrast, it is not clear how activity in the NTS ultimately leads to termination of a meal and how it is related to a certain period of satiety after the meal. On the basis of the well-established expression of the immediate-early gene c-Fos in NTS neurons that goes along with the process of satiation, we had hypothesized that these neurons may change their excitability and synthetic machinery to a “satiated” state through changes in intracellular signaling patterns and ultimately changes at the transcription level (7). In support of this hypothesis, we demonstrated that exogenously administered CCK increases activity of the ERK 1/2 → CREB signaling cascade in NTS neurons and that inhibiting this pathway significantly attenuates CCK-induced food intake suppression in rats (35, 36).

The aim of the present study was to identify the neurochemical phenotypes of the NTS neurons exhibiting CCK-induced ERK activation. Our attention was focused on the A2 catecholaminergic neurons located in the NTS (16) because they have been most strongly implicated in CCK-induced satiation. First, a significant percentage of NTS catecholamine neurons express c-Fos upon stimulation with various satiation signals from the gut (29–31, 37). Second, CCK is less able to suppress food intake in rats with selective immunotoxic ablation of NTS catecholaminergic neurons, and CCK’s capacity to suppress food intake is proportional to the number of surviving catecholaminergic neurons (28). In addition, tyrosine hydroxylase (TH), a key enzyme in the catecholamine synthetic pathway, has a specific ERK-phosphorylation site at Ser 31(12). Therefore, one aim was to assess the number of catecholaminergic neurons exhibiting CCK-induced activation of the ERK pathway and whether CCK is able to activate the ERK-specific phosphorylation site on TH.

Proopiomelanocortin (POMC)-containing neurons in the NTS have also been implicated in mediating satiating effects of CCK (1, 10). Peripheral administration of CCK has been shown to increase the expression of c-Fos in POMC neurons in the NTS, and administration of a melanocortin receptor antagon-

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onist into the fourth ventricle attenuated CCK-induced reduction in food intake (10). However, it is not clear whether activation of POMC neurons in the NTS occurs via activation of the ERK1/2 signaling pathway.

In addition, specific populations of NTS neurons expressing glutamic acid decarboxylase (GAD67), neuronal nitric oxide synthase (nNOS), and leptin receptors may also be involved in the satiation process. Therefore, the second aim was to assess the proportion of these specific neuron populations exhibiting CCK-induced activation of the ERK pathway. Because immunohistochemical identification of POMC, GABA, and leptin receptors is difficult, we used transgenic mouse models expressing green fluorescent protein (GFP) under the respective promoters.

MATERIALS AND METHODS

Animals. All animal procedures were approved by the Institutional Animal Care and Use Committee and conformed to the guidelines of the National Institutes of Health. All animals were housed under standard laboratory conditions (12:12-h light-dark cycle, lights on at 0700; 22 ± 2°C). Food and water were available ad libitum except where noted. Male Sprague-Dawley rats weighing 300–350 g were housed individually in hanging wire-mesh cages. All mice were group housed. Both male and female mice were used for experiments. GAD-GFP mice were obtained from The Jackson Laboratory [Bar Harbor, ME; FVB-Tg(GadGFP)45704Swn/J]. ObRb-GFP transgenic founder mice were obtained from Dr. Martin Myers, and offspring were produced by mating mice homozygous for Rosa26-GFP and Lrb-Cre transgenes. POMC-GFP mice were obtained by mating POMC-Cre mice (gift from Dr. Bradford Lowell) (2) with Z/EG-GFP reporter mice from the Jackson Laboratory (22).

Peptides and antibodies. CCK (sulfated octapeptide, CCK 26–33) was purchased from Bachem-Peninsula (San Carlos, CA; cat. # 7183). MEK inhibitor U0126 was purchased from Promega (Madison, WI; cat. # V1121). Recombinant mouse leptin (vial # AFP565) was obtained through NHPP, NIDDK; Dr. A.F. Parlow, Torrance, CA. A complete list of primary antibodies is provided in Table 1.

Immunohistochemical detection of phospho-ERK 1/2 and double-labeling protocols. Rats or mice, four animals per group, were adapted to handling and fasted overnight. On the test day, animals were injected with saline or CCK (5 or 10 μg/kg in 0.9% saline). Twelve minutes after the injection, animals were anesthetized with pentobarbital (3 mg/kg) followed by injection of saline. Leptin injections were made 90 min before perfusion. Sections through the NTS were stained for pERK1/2 immunoreactivity using nickel-enhanced diaminobenzidine (Pierce, Rockford, IL). A second series of sections was processed for pERK1/2 and pSTAT3 double labeling. For pERK1/2 and pSTAT3 double immunohistochemistry, ObRb-GFP mice received either two injections of saline, saline followed by CCK (5 μg/kg), or injection of leptin (3 mg/kg) followed by injection of saline. Leptin injections were made 90 min before perfusion. Twelve minutes after the last injection, mice were anesthetized with pentobarbital sodium (85 mg/kg ip) and 3 min later transcardially perfused. Sections through the NTS were stained for pSTAT3 immunohistochemistry, according to the protocol of Munzberg et al. (21). Sections were incubated in 1% NaOH and 1% H2O2 in potassium PBS (KPBS) for 20 min, followed by 0.3% glycine for 10 min and in 0.03% SDS in KPBS for 10 min. Sections were incubated in 4% normal donkey serum in 0.4% TritonX-100 in KPBS for 2 h and placed in rabbit anti-pSTAT3 antibody diluted 1:1,000. Tissue was washed in 1% normal donkey serum in 0.02% KPBS and placed in biotinylated donkey anti-rabbit antibody (1:250; Jackson ImmunoResearch) for 1 h. Sections were rinsed and placed in avidin-biotin complexes (ABC; Vector AB Elite kit; Vector Laboratories, Burlingame, CA). Reaction product was visualized by placing sections in nickel-enhanced diaminobenzidine (Pierce, Rockford, IL). Following pSTAT3 immunolabeling, sections were pro-

Table 1. Primary antibodies used

<table>
<thead>
<tr>
<th>Primary Antibody</th>
<th>Host</th>
<th>Description</th>
<th>Source</th>
<th>Product Number</th>
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<td>rabbit</td>
<td>phospho-p44/42 MAP kinase Thr 202/Tyr 204</td>
<td>Cell Signaling Technology, Beverly, MA</td>
<td>9101</td>
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<tr>
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<td>22941</td>
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<tr>
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<td>Tyrosine hydroxylase Phospho-Ser14</td>
<td>Millipore-Chemicon, Temecula, CA</td>
<td>ab5423</td>
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<tr>
<td>nNOS</td>
<td>mouse</td>
<td>Brain, clone NOS-B1 (1–181) monoclonal</td>
<td>Sigma, St. Louis, MO</td>
<td>ab N2280</td>
</tr>
<tr>
<td>GFP</td>
<td>goat</td>
<td>Recombinant, full length, made in &lt;i&gt;Escherichia coli&lt;/i&gt; polyclonal</td>
<td>Abcam, Cambridge, MA</td>
<td>ab5450</td>
</tr>
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NOS, nitric oxide synthase; nNOS, neuronal NOS; GFP, green fluorescent protein.
cessed for pERK1/2 immunohistochemistry as described above using Cy3 as the chromagen.

Neurons immunoreactive for any of the antigens used were counted using conventional light or fluorescence microscopy with a 20× objective. The number of single- or double-labeled neurons was counted bilaterally on 2–3 sections at three rostrocaudal levels of the NTS, immediately rostral to, at the level of the area postrema (AP) and immediately caudal to the AP. The average count of labeled neurons at each rostrocaudal level was calculated for each experimental group. In addition, the average count of pERK1/2-labeled neurons per section for each subnucleus of the NTS was calculated. Since POMC-GFP neurons were observed only in the caudal aspect of the NTS, two series of 20-μm sections through the caudal NTS were processed for pERK 1/2 and POMC-GFP double-labeling. Counts were analyzed by Student’s t-test. In cases in which more than two groups were compared, counts were analyzed by an ANOVA followed by a Student’s t-test. Results are presented as means ± SE.

Differences between groups were considered statistically significant at P < 0.05. In addition, the location of immunoreactive neurons was mapped onto a standard set of drawings of the NTS. Nomenclature of Paxinos (25) and Paxinos and Watson (26) was used for rat and mouse.

**Effects of MEK inhibitor on CCK-induced stimulation of pTH.** Animals were anesthetized with ketamine/xylazine/acepromazine (80/5/1.6 mg/kg sc) and given atropine (1 mg/kg ip). A 24-Ga stainless-steel guide cannula was aimed at the fourth ventricle (2.5 mm anterior to the posterior occipital suture, on the midline, 5.0 mm below the dura). A 30-Ga beveled injector was designed to protrude 1.0 mm from the guide cannula. Rats were given 10 days to recover, after which cannula placement and patency were verified using the 5-thio-glucose test (32), consisting of injecting 5-thio-glucose (210 μg/mL in 3 μL sterile saline) and measuring plasma glucose concentration after 30 min. Only animals responding with an increase of plasma glucose concentration of at least 80 mg/mL were used for experiments.

Intraperitoneal CCK administration induces phosphorylation of ERK 1/2 in the NTS of rats and mice. In both rats and mice, exogenous administration of CCK increased the number of pERK1/2 immunoreactive neurons in the NTS compared with control saline injection (Figs. 1, 2, and 3). In saline-treated control rats and to a lesser extent in mice, a moderate number of neurons spontaneously expressed pERK, particularly in the medial, intermediate, and commissural subnuclei. Administration of either 5 or 10 μg/kg of CCK in rats significantly increased numbers of pERK-positive neurons in the medial and commissural subnuclei (Fig. 3). There were no differences in the number or in the distribution of pERK1/2-immunoreactive neurons between rats that received 5 or 10 μg/kg of CCK (Fig. 3). In the mouse, the number of CCK-induced pERK-positive neurons was generally lower compared with the rat. Administration of 5 μg/kg of CCK significantly increased the number of pERK1/2 immunoreactive neurons in the medial, gelatinous, and ventrolateral subnuclei (Figs. 2 and 3).

Labeled cells were of variable size, and the label typically extended to one or more distal dendrites (for higher magnification examples, see Figs. 4, 7, and 9). Thus, although we did not use cell type-specific markers for positive proof, we assume that most labeled cells are neurons.

CCK induces phosphorylation of ERK1/2 and tyrosine hydroxylase in a population of catecholaminergic NTS neurons in the rat. Some pERK 1/2 immunoreactive neurons in rats also expressed the catecholamine-synthesizing enzyme TH (Figs. 4 and 5). Although some double-labeled neurons were also found after control saline injections, both the number (10.5 ± 0.8, vs. 16.0 ± 1.5 P < 0.05) of double-labeled neurons and the percent of total TH-positive neurons (20.4 ± 2.4 vs. 29.4 ± 1.7 in the NTS, P < 0.05) was significantly higher after 10 μg/kg CCK (Fig. 5). However, double-labeled neurons represented only a fraction of about 20% of the total number of neurons expressing pERK following CCK administration.

Because TH has a selective ERK phosphorylation site (12) and given CCK’s ability to stimulate the ERK-pathway in TH expressing neurons, we speculated that CCK would also stimulate TH activity via the ERK-pathway. In rats, CCK (2 μg/kg ip) produced a significant three-fold increase (P < 0.05) in

**RESULTS**

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phosphorylated Ser(31)TH compared with control saline injection (Fig. 6). To test mediation of Ser(31)TH phosphorylation by the ERK pathway, we pretreated some rats with 4th ventricular injections of the MEK inhibitor U0126 (2 µg/rat). Under these conditions, the CCK stimulatory effect was greatly attenuated and no longer significantly different from intraperitoneal saline injection (Fig. 6). Injection of the MEK blocker alone did not significantly affect baseline p-Ser(31)TH.

CCK induces pERK phosphorylation in a fraction of POMC, but not in GAD67 and nNOS-expressing neurons. In an attempt to characterize the neurochemical phenotype of the many noncatecholaminergic NTS neurons exhibiting CCK-induced pERK, we used double-labeling strategies for the visualization of POMC, leptin receptors (ObRb), and the candidate neurotransmitters GABA and nNOS. Because of the inherent difficulty to immunohistochemically demonstrate POMC, GABA, and ObRb in the medulla, we used GFP-expressing mouse models.

POMC-GFP neurons were observed in the caudal portion of the mouse NTS, primarily in the commissural subnucleus (Fig. 7). Quantitative analysis showed that CCK administration significantly increased the number of POMC neurons expressing
pERK (5.8 ± 2.3 vs. 14.6 ± 2.3 neurons/section, \( P < 0.05 \)) and the number of double-labeled neurons expressed as a percentage of POMC neurons (11.5 ± 4.3 vs. 34.5 ± 7.6%, \( P < 0.05 \)) compared with saline (Fig. 8).

The GFP-labeled GAD67 mouse model was used to visualize GABA-containing neurons. Although there were many GFP-labeled GAD67 neurons throughout the NTS, very few (<1%) expressed pERK, and this percentage was not different in saline- and CCK-treated mice. (Fig. 9, A and B).

Neuronal NOS was stained immunohistochemically in CCK-injected rats, but again, pERK 1/2 immunoreactivity was colocalized in few neurons, mainly in the rostral NTS. CCK-induced pERK was almost absent from the central subnucleus of the NTS, which contains a high density of NOS neurons (Fig. 9, C and D).

GFP-labeled ObRb immunoreactive neurons were observed in the NTS of mice, with the majority of these neurons localized at the level of the area postrema (Figs. 10 and 11). However, only a few of these neurons also contained pERK1/2 immunoreactivity after CCK administration (Fig. 10). Because we found relatively few GFP-labeled ObRb neurons in the NTS, a group of mice also received intraperitoneal injections of leptin, and sections of the NTS were labeled for pSTAT3, a marker for leptin-responsive neurons (21). As described previously (9, 14, 15), leptin injections induced expression of pSTAT3 in the NTS. Although only about 25% of all pSTAT3-

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**Fig. 3.** Quantitative analysis of pERK immunoreactive neurons in rat (left) and mouse (right) NTS subnuclei following intraperitoneal injections of CCK or saline. Average number of neurons per section in each subnucleus was computed. * \( P < 0.05 \) compared with respective saline control (based on ANOVA followed by Bonferroni-adjusted multiple-comparisons test). For abbreviations, see Fig. 2.

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**Fig. 4.** Colocalization of CCK-induced pERK in catecholaminergic neurons of the rat NTS. Lower (A–A′′)- and higher (B–B′′)-power confocal image stacks (15 × 1.5 μm) were taken separately for pERK immunoreactivity (red) and tyrosine hydroxylase (TH) immunoreactivity (green), and merged images are shown on the right. Scale bar = 50 μm for A–A′′; scale bar = 25 μm for B–B′′.
immunoreactive neurons also contained GFP, nearly all GFP neurons were positive for pSTAT3 (Fig. 10). However, only a small number of pERK 1/2 immunoreactive neurons also contained either pSTAT3 or GFP (Fig. 11). Injections of CCK alone had no effect on the number of GFP-labeled or pSTAT3-immunoreactive neurons, and leptin treatment prior to CCK injections did not alter the number of pERK 1/2-immunoreactive neurons in the NTS (Fig. 11).

DISCUSSION

Activation of pERK by CCK in the NTS has previously been implicated in the control of satiation (36). The aim of this study was to identify the phenotype of NTS neurons that show CCK-induced ERK activation. The results demonstrate that peripheral CCK administration increases the expression of pERK1/2 in a fraction of TH- and POMC-containing NTS neurons. In contrast, NTS neurons expressing nNOS and GAD67, as well as leptin-responsive and leptin receptor-expressing neurons did not show increased pERK1/2 activation following peripheral CCK administration. Together with our previous finding that 4th ventricular administration of an ERK-pathway blocker attenuated CCK-induced suppression of food intake (36), the results suggest that CCK-induced satiation is partly mediated through activation of the ERK pathway in NTS neurons expressing TH and POMC, but not in neurons expressing nNOS, GAD67, or leptin receptor.

However, since only a small fraction of neurons exhibiting CCK-induced pERK also expressed TH or POMC, it is likely that other neurons of an unknown neurochemical phenotype are also involved. Identification of transmitters, peptides, receptors, and other functional markers in NTS neurons has been a challenge. In studies demonstrating CCK’s neuronal activation pattern in the NTS with c-Fos, a similarly large fraction of activated neurons remained neurochemically undefined. However, TH- (28) and POMC- (10) expressing NTS neurons have been strongly implicated in the control of satiation, and the present results suggest that ERK-signaling in these neurons is involved.

Some differences in the location of NTS neurons activated by CCK were observed between rats and mice. In rats, CCK increased the number of pERK1/2 immunoreactive neurons only in the commissural and medial subnuclei of the NTS, whereas in mice, CCK increased the number of pERK1/2 immunoreactive neurons in the gelatinous, medial, and ventrolateral subnuclei of the NTS. While reasons for these differences are not readily apparent, previous reports have also shown species differences in the neurochemistry of NTS neurons (15). Distribution of GLP-1-immunoreactive neurons in the NTS has been shown to differ between rats and mice. Additionally, leptin injections induced expression of pSTAT3 in GLP-1-immunoreactive neurons in mice, but not in rats (15). Taken together, these observations suggest that differences in the distribution of pERK 1/2-immunoreactive neurons in the NTS may reflect either differences in the sensitivity of the two species to CCK or differences in anatomical distribution of neurons that respond to CCK. The possibility also exists that species differences observed in the present study were due to the strain of mouse used. Although direct comparison of the location of different neuronal populations between different strains of mice cannot be made, location and number of neurons in the NTS expressing pERK1/2 after injections of CCK did not differ between strains used in this study.

The major findings of the present study are that intraperitoneal injections of CCK increase the number of TH and
POMC neurons expressing pERK 1/2 and induces an ERK-mediated phosphorylation of TH in the NTS. In contrast, CCK injections did not increase phosphorylation of ERK 1/2 in NTS neurons expressing nNOS, GAD67, or in leptin-responsive neurons. It has previously been shown that CCK increases the expression of c-Fos in TH immunoreactive neurons in the NTS (30, 31) and stimulates catecholamine release at their projection sites (8). It has also been demon-

Fig. 7. Colocalization of pERK in NTS neurons expressing POMC in mice injected with CCK (5 μg/kg). Lower (A–A′)- and higher (B–B″)-power confocal images showing pERK and POMC-GFP separately and merged. Examples of double-labeled neurons are indicated by arrows. Confocal image stacks consisted of 15 × 1.5 μm for A, and 6 × 1 μm for B. The distribution of POMC-GFP neurons in the NTS is shown in C. Scale bar = 60 μm for A–A″; scale bar = 20 μm for B–B″. For abbreviations, see Fig. 2.

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Fig. 8. Quantitative analysis of pERK expression in POMC-GFP neurons of the mouse caudal NTS following either saline (open bars) or CCK (5 μg/kg ip; solid bars) injections. Left: numbers of pERK- and POMC-GFP-immunoreactive neurons per section are shown. Right: numbers of double-labeled neurons and percentage of POMC-GFP and pERK neurons are shown. Neurons were counted in the caudal NTS from two series of sections. *P < 0.05, based on individual t-tests.
strated that TH-immunoreactive neurons partly mediate satiating effects of CCK (28). The present observations extend these previous findings by implicating participation of the ERK signaling pathway in a subpopulation of catecholaminergic NTS neurons. The results suggest that intraperitoneally injected CCK leads to activation of the MEK → ERK signaling cascade and subsequent stimulation of tyrosine hydroxylase production. The fact that the ability of exogenously administered CCK to suppress food intake depends on the integrity of abdominal vagal afferents (20, 34) and NTS catecholamine neurons (28) suggests that catecholamine neuron activation is mediated by vagal afferents, probably via the release of glutamate from central terminals of vagal afferents and NMDA and other glutamate receptors on catecholaminergic NTS neurons (1, 5). However, we cannot rule out direct action of circulating CCK on NTS neurons through both presynaptic and postsynaptic mechanisms in the present experiments. Such mechanisms are suggested from medulla slice preparations in rats and mice (1, 3) and from an inability of vagal deafferentation to completely inhibit the food intake suppressing effects of higher doses of CCK (20).

Fig. 9. Absence of colocalization of pERK in GAD67- and nNOS-expressing NTS neurons in mice or rats administered CCK. Merged confocal image stacks (15 × 1.5 μm) showing pERK (red) and GAD67-GFP (A and B), or nNOS (C and D, green). Scale bar = 200 μm for A and B; scale bar = 100 μm for C and D.

Fig. 10. Absence of colocalization of pERK and leptin receptor in NTS neurons in mice administered CCK (5 μg/kg ip). A and B: merged confocal image stacks (15 × 1.5 μm) showing pERK (red) and ObRb-GFP (green). C: merged confocal image stack (15 × 1.5 μm) showing pERK (red) with transmitted light image showing pSTAT3 (black cell nuclei on green background). Arrow indicates a rare double-labeled neuron. D: merged image of confocal stack (15 × 1.5 μm) showing ObRb-GFP (green) with transmitted light image showing pSTAT3 (red cell nuclei). Scale bar = 50 μm for A and C; scale bar = 25 μm for B and D.
The roughly 20% of TH neurons displaying CCK-induced pERK1/2 immunoreactivity in our study is similar to the proportion of TH neurons that expressed c-Fos induced by gastric balloon distension (40) or ingestion of a satiating meal (29).

POMC-containing neurons in the NTS have been implicated in mediating satiating effects of CCK (10, 18). Peripheral CCK injections have been shown to induce c-Fos immunoreactivity in POMC neurons in the NTS, and administration of the melanocortin receptor antagonist SHU9119 into the fourth ventricle attenuated CCK-induced inhibition of food intake (10). In addition, overexpression of the POMC gene in the NTS has been shown to reduce food intake, body weight, and visceral adiposity in a rat model of adult-onset obesity (18).

Our finding of a small but significant number of POMC neurons exhibiting CCK-induced ERK-phosphorylation suggests that satiating effects of CCK may be partly mediated by ERK signaling in POMC neurons.

It has been shown previously that leptin and CCK have synergistic effects on food intake (4) and on the expression of c-Fos in the NTS (38), and we hypothesized that CCK would induce pERK 1/2 in leptin-responsive neurons in the NTS. However, pERK 1/2 was not induced in either ObRb-GFP-positive neurons or in neurons that expressed pSTAT3 after injections of leptin. These findings suggest that leptin and CCK activate separate populations of neurons in the NTS and that synergistic effects occur downstream of these neurons. Alternatively, synergistic effects may occur through another intracellular signaling cascade. In support of this suggestion, it has been demonstrated that both leptin and CCK can activate several signaling cascades, including p38 and JNK (13, 39).

Since leptin and CCK act synergistically to induce the expression of c-Fos in NTS neurons, it is likely that CCK, either through vagal afferents or directly, activates these alternate signaling pathways in leptin-responsive neurons, leading to the induction of c-Fos.

Leptin-responsive neurons were identified by the use of GFP-reporter gene and by expression of pSTAT3 induced by leptin injections (9). Consistent with a previous report (17), the number of GFP-expressing neurons in the current study was only a fraction of neurons that expressed pSTAT3 following leptin injections. Although the reason for this difference between the numbers of pSTAT3 and GFP-positive neurons is not clear, the expression levels of ObRb and consequently GFP in the brain stem may be very low. Alternatively, leptin may be inducing expression of pSTAT3 in some neurons indirectly, through release of another cytokine capable of activating the STAT3 pathway.

Although not every leptin-responsive neuron was labeled with GFP, every GFP-containing neuron also expressed pSTAT3 following leptin injections. This observation suggests that every neuron containing GFP responds to leptin and contains a functional leptin receptor.

**Perspectives and Significance**

A key role for the hindbrain in the control of food intake has long been recognized, but the specific neural pathways and signaling mechanisms remain ill defined. Clearly, changes in feedback from preabsorptive and postabsorptive mechanoreceptors and chemosensors to the caudal brain stem eventually lead to changes in oromotor and autonomic outflow, either resulting in readiness to eat more or to stop eating. While considerable progress has been made identifying the sensory and motor limbs of these basic reflex arcs, much less is known about how sensory information is processed and leads to meaningful motor action. Specifically applied to satiety, we do not know how feedback from the gut is integrated with input from the gustatory system and information from higher brain areas, eventually leading to meal termination and a period of not eating. These integrative processes are likely performed by signaling steps both within specific NTS neurons and between neurons constituting the extended circuitry. Our previous work has demonstrated that the cAMP → ERK → CREB signaling cascade in NTS neurons is involved in the integration of at least two important determinants of meal size, CCK, and MC4 receptor melanocortin signaling (6). Here, we show that CCK-induced ERK signaling preferentially takes place in NTS neurons expressing certain transmitters and peptides. These findings corroborate other work implicating catecholamine and POMC expressing NTS neurons in mediating food satiation. However, because only a relatively small portion of neurons exhibiting CCK-induced activation of the ERK pathway is of these two phenotypes, significant questions remain. What is the neurochemical phenotype of the majority of activated neurons,
and is this phenotype important for satiety? Would a more natural and complete activation of feedback from the gut such as after a full meal activate a larger portion of catecholamine and POMC neurons? What is the role of other intracellular signaling pathways in satiety? Further identification of the rules and mechanisms of signal integration in the solitary nucleus will be important for understanding the process of food satiation, with implications for the development of appetite suppressant and antiobesity drugs.

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REFERENCES


