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Characterization of the feeding inhibition and neural activation produced by dorsomedial hypothalamic cholecystokinin administration

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Abstract

Within the dorsomedial hypothalamus (DMH), cholecystokinin (CCK) has been proposed to modulate neuropeptide Y (NPY) signaling to affect food intake. However, the neural circuitry underlying the actions of this CCK-NPY signaling system in the controls of food intake has yet to be determined. We sought to characterize the feeding inhibition and brain neural activation produced by CCK administration into the DMH of rats. We determined the time course of feeding inhibitory effects of exogenous DMH CCK, assessed NPY gene expression in the DMH in response to DMH CCK administration, and characterized c-Fos activation in the entire brain induced by CCK injection into the DMH using c-Fos like immunohistochemistry. We found that parenchymal injection of CCK into the DMH decreased food intake during the entire 22 hour observation period, with a primary effect in the first 4 hours, and down-regulated NPY gene expression in the DMH. c-Fos immunohistochemistry revealed that DMH CCK increased the number of c-Fos positive cells in the paraventricular nucleus (PVN), arcuate nucleus, suprachiasmatic nucleus and retrochiasmatic area as well as in the contralateral DMH. This pattern of activity is different from that produced by peripherally administered CCK which is short acting and primarily activates neurons in the nucleus of the solitary tract and area postrema, as well as the PVN and DMH. Together, these data suggest that DMH CCK plays an important role in the control of food intake, and does so by activating different pathways from those activated by peripheral CCK.

Keywords

Neuropeptide Y (NPY); Cholecystokinin (CCK) 1 receptor; c-Fos immunoreactivity; Nucleus of the solitary tract; Food intake

Cholecystokinin (CCK) is a brain-gut peptide that plays an important role in the control of food intake (Bi and Moran, 2002). Peripheral CCK acts as a satiety signal to limit meal size. Peripheral administration of CCK reduces food intake in a dose-related manner across a range of experimental situations and in a variety of species (Gibbs et al., 1973; Anika et al., 1981; Gibbs et al., 1976; Kissileff et al., 1981), and the actions of CCK in food intake are specific to a reduction in meal size (West et al., 1984). The feeding inhibitory effects of exogenous CCK appear to mimic a physiological role for endogenous CCK. Administration of CCK receptor-

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specific antagonists results in an increase in food intake (Shillabeer and Davison, 1984; Reidelberger and O'Rourke, 1989; Silver et al., 1989; Moran et al., 1992), and this increase is manifested as an increase in meal size (Moran et al., 1993; Smith and Gibbs, 1994). Moreover, endogenous CCK is released from the duodenum and jejunum in response to the intra-luminal presence of nutrient-digestive products. Intraintestinal nutrient infusions reduce subsequent food intake, and such suppression is attenuated or blocked by CCK receptor-specific antagonists (Yox et al., 1992). The feeding inhibitory actions of both endogenously released and exogenously administered CCK are primarily mediated by CCK1 receptors on vagal afferent fibers (Moran et al., 1990; Moran et al., 1992), and some probably also through a nonvagal mechanism (Reidelberger, 1992).

In contrast to the well characterized satiety actions of peripheral CCK, a role for brain CCK in the control of food intake has been controversial. Initial work reported that continuous picomole infusions of CCK into the cerebral ventricles of sheep suppressed feeding (Della-Fera and Baile, 1979), but results in rodent models have been mixed. Although some studies have identified feeding inhibitory actions of central ventricular CCK administration, issues of dosage and access to peripheral sites have been raised (Crawley, 1985; Schick et al., 1986). Recently, Blevins et al. demonstrated that infusing small doses of CCK-8 into specific brain sites resulted in site-specific feeding inhibitory actions in the rat (Blevins et al., 2000b), and did so at a dose of CCK-8 when injected into the paraventricular nucleus (PVN) that did not increase plasma CCK-8 levels sufficiently to suppress feeding by a peripheral mechanism (Blevins et al., 2000a).

Data from Otsuka Long-Evans Tokushima fatty (OLETF) rats, that have a congenital CCK1 receptor deficiency and become hyperphagic and obese (Kawano et al., 1992; Moran et al., 1998), have suggested that both peripheral and brain CCK play roles in the controls of food intake. As well as having a peripheral CCK satiety deficit that results in increases in meal size (Moran et al., 1998), OLETF rats exhibit a deficit in responding to central CCK administration. While chronic intracerebroventricular infusion of CCK inhibited daily food intake in Long-Evans Tokushima Otsuka (LETO) control rats, such infusions did not decrease food intake in OLETF rats (Miyasaka et al., 1994).

Analysis of hypothalamic gene expression in OLETF rats revealed a primary deficit in the regulation of neuropeptide Y (NPY) gene expression in the dorsomedial hypothalamus (DMH). Pair-feeding OLETF rats to the intake of LETO controls completely prevented their obesity and normalized their alterations in NPY and proopiomelanocortin (POMC) gene expression in the arcuate nucleus (Arc). However, pair feeding resulted in significantly elevated NPY gene expression in the DMH of pair-fed, normal weight OLETF rats, and this elevation was also found in 5 week old pre-obese OLETF rats (Bi et al., 2001). These data have suggested that the dysregulation of NPY gene expression in the DMH resulting from CCK1 receptor deficiency may play an etiological role in the hyperphagia and obesity of OLETF rats (Bi et al., 2001).

Subsequent evidence supported the view that CCK plays a role in modulating DMH NPY signaling to affect food intake. Immunohistochemical studies have revealed that CCK1 receptors and NPY were co-localized in DMH neurons, and pharmacological data demonstrate that parenchymal administration of CCK into the DMH down-regulated NPY gene expression in the DMH and inhibited food intake in intact rats (Bi et al., 2004).

Although we have proposed a role for CCK acting through NPY in the DMH in the control of food intake, we have yet to identify the pathways underlying such an action. In the present experiments, we sought to characterize the feeding inhibition and the pattern of neural activation produced by injection of CCK into the DMH. We first examined the time course of

feeding inhibitory effects of DMH CCK administration. We also determined NPY gene expression in the DMH in response to DMH CCK administration and characterized the distribution of CCK1 receptors in the hypothalamus. Finally, we assessed brain patterns of c-Fos activation induced by DMH CCK administration to identify candidate brain sites that might mediate the actions of DMH CCK in the control of food intake.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 250–300 g purchased from Charles River Laboratories, Inc. (Wilmington, MA) served as subjects. Rats were individually housed in hanging wire mesh cages and maintained on a 12:12-h light-dark cycle in a temperature-controlled environment (22°C) with ad libitum access to water and feeding schedules as described in each experiment. All procedures were approved by the Institutional Animal Care and Use Committee at the Johns Hopkins University.

DMH cannulation and habituation of DMH injection

Thirteen male Sprague-Dawley rats were implanted with unilateral indwelling DMH cannulae. Rats were adapted to a feeding schedule in which pelleted chow was removed from the cages 2 hours before lights off and returned to the cages just before dark onset. At the time of surgery, the rats were anesthetized with an intraperitoneal (ip) injection of a mixture of Ketamine (100 mg/kg) and Xylazine (20 mg/kg) and placed in a stereotaxic apparatus. A 26-gauge stainless-steel guide cannula (Plastics One, Wallingford, CT) was implanted into the DMH with coordinates: 3.1 mm caudal to bregma, 0.4 mm lateral to midline and 8.1 mm ventral to skull surface (Paxinos and Watson, 2005). A 33-gauge stainless-steel obturator was inserted into the cannula to maintain patency. Animals were allowed to recover for 7 days. Before injection of CCK into the DMH, animals were given pseudo-injections during the measurement of daily body weight, i.e., the obturator was removed from the cannula and then reinserted so that rats were adapted to the procedure of injections.

Injection of CCK-8 into the DMH and determination of food intake

After habituation, 13 cannulated rats were randomly divided into two groups. Just before lights off, one group of 6 animals was injected with 0.3 μ l of artificial cerebral-spinal fluid (aCSF: 147 mM Na⁺, 2.7 mM K⁺, 1.2 mM Ca⁺⁺, 0.85 mM Mg⁺⁺ and 153.8 mM Cl⁻) and the other group of 7 animals was injected with 500 pmol of CCK (sulfated CCK-8, Bachem, Torrance, CA) in 0.3 μ l aCSF, a dose that has been demonstrated to inhibit food intake at this site (Blevins et al., 2000b; Bi et al., 2004), but PVN administration of CCK-8 does not elevate plasma CCK-8 levels sufficiently to suppress feeding by a peripheral mechanism (Blevins et al., 2000a). All injections were made with a Gilmont's micrometer syringe attached to polyethylene tubing and a 33-gauge stainless-steel injector (Plastics One, Wallingford, CT). The tip of injector extended 1.0 mm past the tip of guide cannula. Injections were made over 10 s, and the injector remained in a place for additional 20 s before removal. Pelleted chow was returned to the cages immediately after injections. Food intake was measured at the time of 30 min, 1 h, 2 h, 4 h, and 22 h later. After 7-day recovery, all rats were given a second DMH injection with aCSF or 500 pmol of CCK-8 in counterbalanced order. Food intake was measured as following the first injections.

Determination of NPY gene expression in the DMH

After feeding tests, 13 DMH cannulated rats were weight matched and randomly divided into two groups: aCSF control (n = 6) and CCK-8 treatment (n = 7), for assessing the effects of injection of CCK-8 into the DMH on NPY mRNA expression in the DMH. Again, rats received either aCSF or CCK-8 injections as described above but food was not returned to the cages after DMH injections. Three hours following injections, rats were sacrificed with an overdose

of sodium pentobarbital, and brains were removed rapidly and frozen for subsequent analyses of NPY gene expression in the DMH.

Cryosectioning and verification of DMH cannulation

As previously described (Bi et al., 2004), 14- μ m coronal brain sections were cut with a cryostat, mounted on superfrost/plus slides (Fisher Scientific), and postfixed with 4% paraformaldehyde. The site of DMH injection in rats was anatomically examined via cresyl violet staining after processing brain sections. Data from rats with incorrect cannula placements were excluded from subsequent statistical analyses. The number of rats (*n*) presented in the Results section represented the final number of animals with correct cannula placements used for data analyses.

In situ hybridization

As previously described (Bi et al., 2005), ^{35} S-labeled antisense riboprobe of NPY was transcribed from rat NPY precursor cDNA by using in vitro transcription systems (Promega, Madison, WI), and purified by Quick Spin RNA Columns (Roche, Indianapolis, IN). Sections ranging from 3.0–3.5 mm posterior to bregma (Paxinos and Watson, 2005) were selected, anatomically matched among animals and used for the determination of NPY mRNA levels in the DMH. Briefly, sections were treated with acetic anhydride and incubated in hybridization buffer containing 50% formamide, 0.3 M NaCl, 10 mM Tris•Cl, pH 8.0, 1 mM EDTA, pH 8.0, 1X Denhardt's solution (Eppendorf), 10% dextran sulfate, 10 mM DTT, 500 μ g/ml yeast tRNA and 10^8 cpm/ml of ^{35} S-UTP at 55°C overnight. After hybridization, sections were washed three times with 2 X SSC, treated with 20 μ g/ml RNase A (SIGMA) at 37 °C for 30 min, and then rinsed in 2 X SSC twice at 55 °C and washed twice in 0.1 X SSC at 55 °C for 15 min. Slides were dehydrated in gradient ethanol, air-dried and exposed with BMR-2 film (Kodak) for 1–3 days. After film autoradiography, slides were dipped in Amersham LM1 emulsion (GE Healthcare Life Sciences), exposed at 4°C for 10 days, and developed in Kodak D-19 developer and Kodak fixer (Kodak) according to the manufacturer's protocols. Sections were examined under dark-field microscope.

Quantitative analysis of the in situ hybridization data was done with NIH Scion image software (National Institutes of Health) as previously described (Bi et al., 2005). Briefly, autoradiographic images on film were first scanned by EPSON Professional Scanner (EPSON) and saved via a computer for subsequent analyses with Scion image software using autoradiographic ^{14} C micro scales (Amersham) as a standard. NPY mRNA levels were determined by a mean of the product of hybridization area $\times$ density (background density was subtracted) in each animal. Data from each group were normalized to vehicle aCSF treated controls as 100 %, and all data are presented as mean $\pm$ SEM.

CCK binding assay

Autoradiographic CCK binding assay was conducted in a separate group of rats as previous described (Bi et al., 2004). Briefly, rat coronal sections (20 μ m) over the DMH regions from 3.0–3.5 mm posterior to bregma (Paxinos and Watson, 2005) were cut via a cryostat, and mounted on cold gelatin-coated slides. To differentiate between CCK1 and CCK2 receptors, we compared the ability of the CCK1 receptor antagonist devazepide and the CCK2 receptor agonist desulfated CCK-8 (dCCK) to displace the binding site of [^{125} I]-labeled CCK-8. If the binding was inhibited by devazepide but not by dCCK, the binding site was occurring to CCK1 receptors. In contrast, if the binding was inhibited by dCCK but not by devazepide, the binding site was occurring to CCK2 receptors. Thus, following pre-incubation in 50 mM Tris-HCl buffer (pH 7.4) containing 0.5% bovine serum albumin (BSA) for 20 min at 24°C, slides were incubated in the standard binding buffer containing 50 pM [^{125} I]-Bolton Hunter labeled CCK-8 (PerkinElmer Life Sciences, Inc., Boston, MA) for 2 hours at 24°C, either alone or in the

presence of 10 nM devazepide, 100 nM dCCK or 100 μ M sulfated CCK-8. After incubation, slides were washed in ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 0.5% BSA 6 times for 10 min each. Washed slides were completely air-dried and exposed with BMR-2 film (Kodak) for 5–7 days.

Injection of CCK into the DMH and c-Fos immunohistochemistry

Twenty-eight additional male Sprague-Dawley rats were implanted with unilateral indwelling DMH cannulae as described above. After postoperative recovery and habituation to the injection procedure, rats were randomly divided into two groups (n=14): one group received 0.3 μ l of aCSF and the other received 500 pmol of CCK-8 in 0.3 μ l aCSF. All DMH injections were made as described above, but rats were not allowed access to chow after DMH injection. Ninety minutes following injections, rats were anesthetized with Euthasol (pentobarbital sodium and phenytoin, Delmarva Laboratories, Midlothian, VA) and perfused transcardially with phosphate buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in PBS. Brains were removed and stored in 25% sucrose containing 4% paraformaldehyde at 4°C for subsequent c-Fos immunoreactivity determinations.

In the initial step, 3 animals per group were examined for the determination of regions where c-Fos activation was potentially induced by DMH CCK injection. Coronal sections were taken through the entire brains ranging from 5.64 mm anterior to 15.96 mm posterior to bregma (Paxinos and Watson, 2005) at 40 μ m via a cryostat, and a series of sections (one in five throughout the brain) was collected in PBS and processed with our standard c-Fos-like immunohistochemistry (Emond et al., 1999).

Since the initial determination of c-Fos immunoreactivity revealed that c-Fos positive cells produced by DMH CCK injection were exclusively localized to hypothalamic areas, subsequent quantitative c-Fos immunoreactivity was only determined in brain regions over the hypothalamus in the remaining animals (11 rats per group). Forty μ m coronal sections extending from 0.48 mm anterior to bregma to 4.36 mm posterior to bregma (Paxinos and Watson, 2005) were cut, and every other section was collected in PBS for the determination of c-Fos immunoreactivity. The other set of sections was stained with cresyl violet for verifying the site of DMH injection. Data from rats with incorrect cannula placements were excluded from subsequent statistical analyses as described above.

As previously described (Emond et al., 1999), c-Fos positive cells were identified using Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA). Briefly, sections were incubated in 0.3% hydrogen peroxide for 60 min, rinsed in PBS and pre-absorbed with normal goat blocking serum in PBS containing 0.1% Triton X-100 for 30 min. They were then incubated in rabbit anti-c-Fos antiserum (1:10,000 dilution for forebrain and 1:20,000 dilution for brainstem respectively, Oncogene Science, San Diego, CA) for 16–18 h at 4°C. After 3 washes in PBS containing 0.1% Triton X-100, sections were incubated in biotinylated goat anti-rabbit IgG for 60 min, washed 3 times in PBS containing 0.1% Triton X-100, followed by incubation in ABC reagent (avidin DH:biotinylated horseradish peroxidase H complex) for 30 min. Finally, sections were stained by incubation with 3, 3'-diaminobenzidine (DAB) chromagen and nickel enhancer (DAB substrate kit for peroxidase, Vector Laboratories, Burlingame, CA). They were then mounted onto gelatin-coated slides, air dried, dehydrated in an ascending series of ethanol and cover-slipped.

The number of c-Fos positive cells was quantified in the following areas: the suprachiasmatic nucleus (SCh), the retrochiasmatic area (RCh), the supraoptic nucleus (SON), the PVN, the DMH, the Arc, the medial eminence (ME), the ventromedial hypothalamus (VMH), and the lateral hypothalamus (LH), as well as the central nucleus of amygdala (CeA). Images of sections were captured by digital camera (Retiga 2000R, QImaging, Burnaby, Canada)

attached to Zeiss Axio Imager (Carl Zeiss MicroImaging, Inc., Thornwood, NY). The area of interest was outlined based on cellular morphology and c-Fos positive cells were automatically counted by the imaging program (IPLab, Scanalytics, Fairfax, VA) by setting minimum and maximum optical density levels. Cell counts of c-Fos immunoreactivity were made separately in the ipsilateral (iDMH) and contralateral (cDMH) to the site of DMH CCK injection. Data for c-Fos activation in all other areas were bilaterally assessed, and were presented as the total number of c-Fos positive cells per section.

Peripheral administration of CCK and determination of food intake

Ten male Sprague-Dawley rats served as subjects with a feeding schedule in which pelleted chow was removed from the cages 2 hours before lights off and returned to the cages just before dark onset. Rats were allowed to habituate the feeding schedule and ip injection with 0.9% saline for 7 days. At the time of feeding determination, 5 rats received CCK-8 (3.2 nmol/kg, ip) and other 5 animals received 0.9% saline (ip) just before lights off. Pelleted chow was returned to the cages immediately after injections. Food intake was measured at the time of 30 min, 1 h, 2 h, 4 h, and 22 h later. After 2-day recovery, all rats were given a second injection with saline or CCK-8 (3.2 nmol/kg, ip) in counterbalanced order. Food intake was measured as following the first injections.

Peripheral administration of CCK and c-Fos immunohistochemistry

After feeding tests, c-Fos immunohistochemistry was conducted in these animals. Rats were randomly assigned into two groups. One group of 5 rats received CCK-8 (3.2 nmol/kg, ip) and the other group of 5 animals received saline (ip) administration, but rats were not allowed to access to chow after injections. Ninety minutes following injections, rats were anesthetized with Euthasol and perfused transcardially with PBS (pH 7.4) followed by 4% paraformaldehyde in PBS as described above. Brains were removed and stored in 25% sucrose containing 4% paraformaldehyde at 4°C. Forty mm coronal sections through the hindbrain region of the nucleus of the solitary tract (NTS) and area postrema (AP) [from 13.6 mm to 14.3 mm posterior to Bregma (Paxinos and Watson, 2005)] were cut via a cryostat, and every other section was used for c-Fos immunoreactivity determinations as following the same procedure as above.

Ten additional male Sprague-Dawley rats were used for determinations of peripheral CCK-induced c-Fos activation in the hypothalamus and CeA. Following the same procedures of peripheral CCK injection and paraformaldehyde fixation as above, forty mm coronal sections through the hypothalamic region [from 0.48 mm anterior to bregma to 4.36 mm posterior to bregma (Paxinos and Watson, 2005)] were cut via a cryostat, and every other section was used for c-Fos immunoreactivity determinations as described above.

Statistical analysis

Data for food intake were analyzed by two-way repeated measures ANOVA and followed by planned *t* comparisons. Data for gene expression and c-Fos activation were analyzed by using Student's *t* test. $P < 0.05$ was taken to be a statistically significant difference.

RESULTS

Effects of DMH CCK on food intake and NPY gene expression

Ten out of thirteen rats had anatomically correct DMH cannula placements. Parenchymal injection of CCK into the DMH of these rats decreased food intake during the entire 22 hour observation period (Fig. 1). Two-way repeated measures ANOVA demonstrated main effects of DMH CCK administration [$F(1,9)=86.554, P<0.001$] and time [$F(4,36)=625.754, P<0.001$],

as well as a significant interaction between DMH CCK and time [$F(4,36)=9.434$, $P<0.001$]. Planned t comparisons revealed that DMH CCK administration significantly decreased food intake at all examined time points as compared with vehicle controls ($P<0.05$, Fig. 1). The magnitude of feeding inhibitory effect was time dependent. DMH CCK administration resulted in a 51% reduction in the first 30 min as compared with vehicle treated rats ($P<0.05$, Fig. 1). The feeding inhibition of DMH CCK was long lasting with a 38.4% suppression maintained at 4 hours ($P<0.05$, Fig. 1). Compensation for this reduction did not occur within the next 18 hours. Food intake remained significantly reduced at 22 hours in DMH CCK-treated rats relative to vehicle controls ($P<0.05$, Fig. 1).

In situ hybridization determination revealed that NPY gene expression was significantly down-regulated by parenchymal injection of CCK into the DMH. Exogenous DMH CCK resulted in a 46% reduction in NPY mRNA levels in the ipsilateral compact subregion of the DMH (Fig. 2B and 2C) relative to vehicle controls (Fig. 2A and 2C, $P<0.05$).

CCK1 receptors in the DMH

Autoradiographs of CCK binding assay revealed that both the VMH and DMH contained CCK binding activity (Fig 3A). CCK2 receptor binding sites were heavily concentrated within the VMH (Fig 3A and B), and binding at this site was displaced by the addition of d-CCK (Fig 3C). In contrast, CCK1 receptor binding sites were restricted to the compact subregion of the DMH (Fig 3A) as indicated by the displacement of this binding by devazepide (Fig 3B) but not by d-CCK (Fig 3C).

Characterization of c-Fos activation induced by DMH CCK

Examination of c-Fos immunoreactivity throughout the entire brain revealed that DMH CCK-induced c-Fos activation was exclusively localized to the hypothalamus. The positive sites included the cDMH, PVN and Arc (Fig. 4), as well as the Sch and RCh (Fig. 5), but not the SON, VMH, LH and ME (Fig. 6). Although the number of c-Fos positive cells were higher in the iDMH of CCK-treated rats (248 ± 87 , $n=7$) relative to that of vehicle treated controls (189 ± 40 , $n=6$), the difference did not reach statistically significant levels ($P>0.05$). However, DMH CCK administration induced c-Fos activation in the cDMH (Fig. 4B) and produced a 5-fold increase in the number of c-Fos positive cells as compared with vehicle controls (Fig. 6, $P<0.05$). This c-Fos activation was widely distributed in the cDMH. c-Fos immunoreactivity was detected in all three subregions, i.e., the dorsal, ventral and compact part of the cDMH (Fig. 4B). Within the PVN, DMH CCK-induced c-Fos activation was primarily located in the medial parvocellular part of the PVN (PVNp, Fig. 4D) with a 4-fold increase relative to vehicle controls (Fig. 6, $P<0.05$). Very few CCK induced c-Fos positive neurons were detected in the lateral magnocellular part of the PVN (PVNm, Fig. 4D). DMH CCK significantly increased c-Fos immunoreactivity in the Arc (Fig. 6, $P<0.05$), with a majority of c-Fos positive cells in the medial part (72% of c-Fos positive cells in medial part and only 28% in the lateral part, Fig. 4F). Exogenous DMH CCK also increased the number of c-Fos positive cells in the Sch with a 3.8-fold increase and in the RCh with a 5.3-fold increase relative to vehicle controls (Fig. 5 and Fig. 6, $P<0.05$).

In addition, we specifically assessed whether administration of CCK into the DMH affected c-Fos immunoreactivity in the CeA and NTS. DMH CCK did not result in a significant increase in the number of c-Fos positive cells in the CeA as compared with vehicle controls (Fig. 6, $P>0.05$). As well, administration of CCK into the DMH did not increase c-Fos immunoreactivity in the NTS. We did not detect any c-Fos positive cells in the NTS and AP of DMH CCK-treated rats (Fig. 7B) or vehicle controls (Fig. 7A).

Effects of peripheral CCK on food intake and c-Fos activation

Peripheral CCK produced a short-term feeding inhibitory effect. As shown in Fig. 7E, peripheral administration of CCK resulted in a 34% reduction in food intake at the first 30 min time point, followed by a rapid compensation within the next 30 min. Thereafter food intake remained normal (Fig. 7E).

c-Fos immunohistochemistry revealed that peripheral CCK primarily activated neurons in the NTS and AP. Peripheral administration of CCK significantly increased c-Fos immunoreactivity in the NTS and AP (Fig. 7D) and resulted in about 4-fold increases in the number of c-Fos positive cells in the NTS and the AP relative to vehicle controls (Fig 7F, $P < 0.05$). Peripheral administration of CCK also induced c-Fos activation in the PVN and DMH. The number of c-Fos positive cells in the PVN and DMH was significantly increased by 2.0 and 2.3-fold, respectively, compared with vehicle controls (Fig. 8, $P < 0.05$). However, peripheral administration of CCK did not affect c-Fos immunoreactivity in other hypothalamic areas including the Sch, RCh, SON, Arc, VMH, LH and ME (Fig. 8, $P > 0.05$). c-Fos immunohistochemistry also did not detect CCK-induced c-Fos activation in the CeA (Fig. 8).

DISCUSSION

The current data demonstrate that DMH CCK has a long lasting effect on food intake. Injection of CCK into the DMH resulted in a rapid decrease in food intake, and inhibited food intake for 4 hours, with no compensation for at least the next 18 hours. DMH CCK decreased NPY gene expression in the DMH. In response to DMH CCK administration, c-Fos immunoreactivity was increased in various hypothalamic areas including the cDMH, PVN, Arc, Sch and RCh. DMH CCK did not increase the number of c-Fos positive neurons in the SON, VMH, LH and ME and did not induce c-Fos in the CeA or caudal hindbrain NTS and AP. In contrast, peripheral CCK only produced the short-term feeding inhibitory effect. Peripheral CCK primarily increased c-Fos immunoreactivity in the NTS and AP, as well as the PVN and DMH. Thus, these data indicate that DMH CCK affects food intake through a mechanism different from that engaged by peripheral CCK in the hindbrain. The CCK induced decrease in DMH NPY mRNA expression suggests that decreased NPY activity may underlie the feeding inhibitory effect of DMH CCK. The results also suggest that DMH CCK activates neurons in multiple hypothalamic areas and the neurons in these areas may, or in part, contribute to the actions of DMH CCK in the control of food intake. No c-Fos activation in the NTS in response to CCK administration into the DMH indicates that although the NTS serves as an important site of peripheral CCK satiety actions, DMH CCK does not appear to activate neurons in the NTS to affect food intake.

DMH CCK has been shown to reduce food intake. Blevins et al. have reported that parenchymal injection of CCK into the DMH produced a large feeding inhibition when assessing brain regions where CCK suppresses feeding in rats (Blevins et al., 2000b). We have also demonstrated that exogenous DMH CCK inhibited food intake at a 30 min time point (Bi et al., 2004). The current study extends these previous findings and demonstrates a long lasting effect of DMH CCK on food intake. We found that exogenous DMH CCK produced a feeding inhibition for 4 hours, with no compensatory feeding response for at least the next 18 hours, resulting in an overall reduction in daily food intake.

Local alterations in NPY gene expression in response to DMH CCK administration are consistent with the previous findings. We have demonstrated that while pair feeding normalized the obesity of OLETF rats lacking CCK1 receptors, pair feeding resulted in a large increase in NPY gene expression in the DMH, similar to levels found in young preobese OLETF rats (Bi et al., 2001). We have suggested that the elevation of NPY gene expression in the DMH may be a direct result of a CCK signaling deficit due to CCK1 receptor deficiency

and serves as a major contributing factor to the hyperphagia and obesity of OLETF rats (Bi and Moran, 2002). In support of this view, we have demonstrated that NPY-containing neurons in the DMH contain CCK1 receptors and that local CCK administration decreased NPY mRNA levels in the DMH and inhibited food intake in intact rats (Bi et al., 2004). In the current study, we localized the DMH CCK induced downregulation of NPY gene expression to the compact subregion of the DMH. Moreover, consistent with the finding that NPY and CCK1 receptors were co-localized in DMH neurons, the autoradiographic CCK binding assay demonstrates that CCK1 receptor binding sites precisely localized to the compact subregion of the DMH where NPY gene expression is altered by DMH CCK. Together, these data suggest that the actions of DMH CCK in food intake may be mediated by NPY signaling pathway in the DMH through interacting with local CCK1 receptors.

Although lesions of the DMH have long been known to affect food intake and body weight (Bernardis and Bellinger, 1987), the neural circuitry and/or the projections of the DMH contributing to its role in energy homeostasis have yet to be characterized. Li and colleagues have reported that NPY neurons in the DMH activated during lactation project to the PVN, but these lactation-induced NPY expressing neurons were not localized to the compact subregion of the DMH (Smith, 1993, Li et al., 1998). The current data demonstrate that DMH CCK decreased NPY gene expression in the compact subregion of the DMH and induced c-Fos activation in multiple hypothalamic areas including the cDMH, PVN, Arc, Sch and RCh. These results provide the first evidence indicating that the neurons in these hypothalamic areas may mediate the actions produced by DMH CCK and its reduced DMH NPY signaling. Whether the neurons in these hypothalamic areas are activated directly or indirectly by DMH CCK and/or its altered NPY signaling remains to be determined.

The PVN serves as a main feeding output area of the hypothalamus and plays an important role in energy homeostasis. Lesions of the PVN result in the hyperphagia and obesity of rats (Leibowitz et al., 1981). The PVN contains numerous neuropeptides and receptors that are related to energy balance control, such as corticotropin-releasing factor (CRF) (Bloom et al., 1982), oxytocin (Swaab et al., 1975), and thyrotropin-releasing hormone (TRH) (Lechan and Jackson, 1982), as well as NPY5 and NPY1 receptors (Parker and Herzog, 1999). The current data demonstrating that DMH CCK induced c-Fos activation in the PVN suggest that neuronal signaling in the PVN may underlie the actions of DMH CCK in the control of food intake. We demonstrate that within the PVN, the neurons activated by DMH CCK are primarily localized to the pPVN but less to the mPVN. These results are consistent with the axonal projections of the DMH. Thompson et al. reported that the PVN was a primary hypothalamic output of the DMH, and neurons in the DMH project heavily to the pPVN while sending very few axons to magnocellular neurons (Thompson et al., 1996). Together, these data suggest that the projection of the DMH to the pPVN may be a downstream site mediating the effects of DMH CCK and/or CCK-NPY signaling in the controls of food intake.

A role for the Arc in energy balance is also well characterized. There are two primary populations of feeding related neurons in the Arc. In the rat, orexigenic peptides NPY and the endogenous melanocortin receptor antagonist agouti-related protein (AgRP) are co-localized in medial neurons (Broberger et al., 1998; Hahn et al., 1998), and proopiomelanocortin (POMC), a precursor of the anorexigenic peptide α -melanocyte-stimulating hormone, is expressed in more lateral neurons. Both neuronal populations contain leptin receptors (Mercer et al., 1996; Cheung et al., 1997). Leptin, a hormone produced in the adipose tissue, down-regulates NPY and AgRP gene expression and up-regulates POMC gene expression to affect food intake and body weight (Schwartz et al., 2000). Alterations in the activity of these signaling pathways have been shown to produce significant disruptions in energy balance (Huszar et al., 1997; Shutter et al., 1997; Zhang et al., 1994; Tartaglia et al., 1995). The current results demonstrate that DMH CCK increases c-Fos immunoreactivity in the Arc, and that a

majority of CCK-induced c-Fos positive cells is localized to the medial part of the Arc where NPY/AgRP expressing neurons have been identified. We have demonstrated that DMH CCK administration also decreased NPY gene expression in the Arc (Bi et al., 2004). The current data may provide the basis for such actions. Thus, DMH CCK activates the neurons in the Arc that may regulate Arc NPY signaling to control food intake.

The mediation of feeding inhibitory effects of DMH CCK appears to differ from those of peripheral CCK. Although data from the findings of peripheral CCK-induced c-Fos activation in the PVN and DMH in the current and previous studies suggest that the PVN and DMH contribute to the effects of peripheral CCK on food intake (Monnikes et al., 1997; Rinaman, 2003; Kobelt et al., 2006), various evidence has demonstrated an important role for the NTS, the brain site of vagal afferent terminations, in the mediation of peripheral CCK satiety actions. Thus, the actions of peripheral CCK in the controls of food intake are mediated, at least in part, through its actions on the vagal afferents that terminate the NTS. Abdominal vagotomy blocks (Smith et al., 1981) and specific vagal afferent disconnections or chemical destruction of vagal afferents significantly attenuate the feeding inhibitory actions of CCK (Ritter and Ladenheim, 1985). Celiac arterial administration of CCK activates vagal afferent fibers (Schwartz et al., 1991) and nutrient-induced vagal afferent activation can be blocked by local administration of CCK receptor antagonists (Eastwood et al., 1998). Moreover, lesions of the NTS block or attenuate the feeding inhibitory effects of CCK (Crawley and Schwaber, 1984; Edwards et al., 1986). Electrophysiological studies have demonstrated that peripheral administered CCK induces activation of neurons within the NTS (Raybould et al., 1988). c-Fos immunohistochemistry has revealed increased c-Fos immunoreactivity in the NTS in response to peripheral CCK injection and this elevation has been proposed to contribute to the satiety action of peripheral CCK (Fraser and Davison, 1992; Rinaman et al., 1993; Rinaman, 2003). Consistent with this view, we currently demonstrate peripheral CCK reduced food intake and induced c-Fos activation in the NTS. In contrast to such actions of peripheral CCK, the present study finds that although DMH CCK decreases food intake, c-Fos activation is not detected in the NTS of DMH CCK treated rats. Together, these data suggest that the pathways for actions of DMH and peripheral CCK in the control of food intake differ: while peripheral CCK stimulates NTS neural signaling to inhibit food intake, DMH CCK does not activate neurons in the NTS to control food intake.

It is worth addressing that although peripherally administered CCK has been shown to activate the neurons in the SON and CeA (Renaud et al., 1987; Hamamura et al., 1991; Rinaman, 2003), we do not find such activations in the rats receiving peripheral CCK. The differences between previous findings and ours are likely due to the dose of CCK used in the experiments. In contrast to a large dose of CCK (10–50 µg/kg) used in others studies (Renaud et al., 1987; Hamamura et al., 1991; Rinaman, 2003), we have conducted the current experiment with a relative small dose of CCK (3.2 nmol/kg, or 3.7 mg/kg). Moreover, although the DMH contains CCK cell bodies (Innis et al., 1979), the current data do not identify the source of endogenous DMH CCK that may play a role in modulating DMH NPY and controlling food intake.

In summary, the present results demonstrate an action of DMH CCK in the control of food intake. In contrast to the short term effects of peripheral CCK on food intake, DMH CCK has long lasting feeding inhibitory actions. DMH CCK-induced reductions in NPY gene expression in the DMH appear to mediate the actions of DMH CCK. DMH CCK and/or its-induced reductions in DMH NPY signaling activate neurons within the multiple hypothalamic areas including the cDMH, the PVN, the Arc, the Sch, and the RCh. In contrast to peripheral CCK, DMH CCK does not activate neurons in the NTS. The actions of DMH CCK appear to be primarily mediated through hypothalamic neural pathways.

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References

- Anika SM, Houtpt TR, Houtpt KA. Cholecystokinin and satiety in pigs. *Am J Physiol* 1981;240:R310–318. [PubMed: 6263113]
- Bernardis LL, Bellinger LL. The dorsomedial hypothalamic nucleus revisited: 1986 update. *Brain Res* 1987;434:321–381. [PubMed: 3300862]
- Bi S, Ladenheim EE, Schwartz GJ, Moran TH. A role for NPY overexpression in the dorsomedial hypothalamus in hyperphagia and obesity of OLETF rats. *Am J Physiol Regul Integr Comp Physiol* 2001;281:R254–260. [PubMed: 11404301]
- Bi S, Moran TH. Actions of CCK in the controls of food intake and body weight: lessons from the CCK-A receptor deficient OLETF rat. *Neuropeptides* 2002;36:171–181. [PubMed: 12359507]
- Bi S, Scott KA, Hyun J, Ladenheim EE, Moran TH. Running wheel activity prevents hyperphagia and obesity in Otsuka long-evans Tokushima Fatty rats: role of hypothalamic signaling. *Endocrinology* 2005;146:1676–1685. [PubMed: 15625240]
- Bi S, Scott KA, Kopin AS, Moran TH. Differential roles for cholecystokinin receptors in energy balance in rats and mice. *Endocrinology* 2004;145:3873–3880. [PubMed: 15123537]
- Blevins JE, Hamel FG, Fairbairn E, Stanley BG, Reidelberger RD. Effects of paraventricular nucleus injection of CCK-8 on plasma CCK-8 levels in rats. *Brain Res* 2000a;860:11–20. [PubMed: 10727619]
- Blevins JE, Stanley BG, Reidelberger RD. Brain regions where cholecystokinin suppresses feeding in rats. *Brain Res* 2000b;860:1–10. [PubMed: 10727618]
- Bloom FE, Battenberg EL, Rivier J, Vale W. Corticotropin releasing factor (CRF): immunoreactive neurones and fibers in rat hypothalamus. *Regul Pept* 1982;4:43–48. [PubMed: 6750704]
- Broberger C, De Lecea L, Sutcliffe JG, Hokfelt T. Hypocretin/orexin- and melanin-concentrating hormone-expressing cells form distinct populations in the rodent lateral hypothalamus: relationship to the neuropeptide Y and agouti gene-related protein systems. *J Comp Neurol* 1998;402:460–474. [PubMed: 9862321]
- Cheung CC, Clifton DK, Steiner RA. Proopiomelanocortin neurons are direct targets for leptin in the hypothalamus. *Endocrinology* 1997;138:4489–4492. [PubMed: 9322969]
- Crawley JN. Clarification of the behavioral functions of peripheral and central cholecystokinin: two separate peptide pools. *Peptides* 1985;6(Suppl 2):129–136. [PubMed: 3001671]
- Crawley JN, Schwaber JS. Abolition of the behavioral effects of cholecystokinin following bilateral radiofrequency lesions of the parvocellular subdivision of the nucleus tractus solitarius. *Brain Res* 1984;295:289–299. [PubMed: 6713189]
- Della-Fera MA, Baile CA. Cholecystokinin octapeptide: continuous picomole injections into the cerebral ventricles of sheep suppress feeding. *Science* 1979;206:471–473. [PubMed: 504989]
- Eastwood C, Maubach K, Kikup AJ, Grundy D. The role of endogenous cholecystokinin in the sensory transduction of liminual nutrient signaling in the rat jejunum. *Neurosci Lett* 1998;254:145–9. [PubMed: 10214978]
- Edwards GL, Ladenheim EE, Ritter RC. Dorsomedial hindbrain participation in cholecystokinin-induced satiety. *Am J Physiol* 1986;251:R971–977. [PubMed: 3777222]
- Emond M, Schwartz GJ, Ladenheim EE, Moran TH. Central leptin modulates behavioral and neural responsivity to CCK. *Am J Physiol* 1999;276:R1545–1549. [PubMed: 10233050]
- Fraser KA, Davison JS. Cholecystokinin-induced c-fos expression in the rat brain stem is influenced by vagal nerve integrity. *Exp Physiol* 1992;77:225–228. [PubMed: 1543589]
- Gibbs J, Falasco JD, McHugh PR. Cholecystokinin-decreased food intake in rhesus monkeys. *Am J Physiol* 1976;230:15–18. [PubMed: 814821]
- Gibbs J, Young RC, Smith GP. Cholecystokinin decreases food intake in rats. *J Comp Physiol Psychol* 1973;84:488–495. [PubMed: 4745816]
- Hahn TM, Breininger JF, Baskin DG, Schwartz MW. Coexpression of AgRP and NPY in fasting-activated hypothalamic neurons. *Nat Neurosci* 1998;1:271–272. [PubMed: 10195157]

- Hamamura M, Leng G, Emson PC, Kiyama H. Electrical activation and *c-fos* mRNA expression in rat neurosecretory neurones after systemic administration of cholecystokinin. *J Physiol* 1991;444:51–63. [PubMed: 1822561]
- Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, Lee F. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 1997;88:131–141. [PubMed: 9019399]
- Innis RB, Correa FM, Uhl GR, Schneider B, Snyder SH. Cholecystokinin octapeptide-like immunoreactivity: histochemical localization in rat brain. *Proc Natl Acad Sci U S A* 1979;76:521–525. [PubMed: 284371]
- Kawano K, Hirashima T, Mori S, Saitoh Y, Kurosumi M, Natori T. Spontaneous long-term hyperglycemic rat with diabetic complications. Otsuka Long-Evans Tokushima Fatty (OLETF) strain. *Diabetes* 1992;41:1422–1428. [PubMed: 1397718]
- Kissileff HR, Pi-Sunyer FX, Thornton J, Smith GP. C-terminal octapeptide of cholecystokinin decreases food intake in man. *Am J Clin Nutr* 1981;34:154–160. [PubMed: 6259918]
- Kobelt P, Paulitsch S, Goebel M, Stengel A, Schmidtman M, van der Voort IR, Tebbe JJ, Veh RW, Klapp BF, Wiedenmann B, Tache Y, Monnikes H. Peripheral injection of CCK-8S induces Fos expression in the dorsomedial hypothalamic nucleus in rats. *Brain Res* 2006;1117:109–117. [PubMed: 17005163]
- Lechan RM, Jackson IM. Immunohistochemical localization of thyrotropin-releasing hormone in the rat hypothalamus and pituitary. *Endocrinology* 1982;111:55–65. [PubMed: 6806077]
- Leibowitz SF, Hammer NJ, Chang K. Hypothalamic paraventricular nucleus lesions produce overeating and obesity in the rat. *Physiol Behav* 1981;27:1031–1040. [PubMed: 7335803]
- Li C, Chen P, Smith MS. Neuropeptide Y (NPY) neurons in the arcuate nucleus (ARH) and dorsomedial nucleus (DMH), areas activated during lactation, project to the paraventricular nucleus of the hypothalamus (PVH). *Regul Pept* 1998;75–76. 93–100.
- Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, Morgan PJ, Trayhurn P. Coexpression of leptin receptor and preproneuropeptide Y mRNA in arcuate nucleus of mouse hypothalamus. *J Neuroendocrinol* 1996;8:733–735. [PubMed: 8910801]
- Miyasaka K, Kanai S, Ohta M, Kawanami T, Kono A, Funakoshi A. Lack of satiety effect of cholecystokinin (CCK) in a new rat model not expressing the CCK-A receptor gene. *Neurosci Lett* 1994;180:143–146. [PubMed: 7700567]
- Monnikes H, Lauer G, Arnold R. Peripheral administration of cholecystokinin activates *c-fos* expression in the locus coeruleus/subcoeruleus nucleus, dorsal vagal complex and paraventricular nucleus via capsaicin-sensitive vagal afferents and CCK-A receptors in the rat. *Brain Res* 1997;770:277–288. [PubMed: 9372230]
- Moran TH, Ameglio PJ, Peyton HJ, Schwartz GJ, McHugh PR. Blockade of type A, but not type B, CCK receptors postpones satiety in rhesus monkeys. *Am J Physiol* 1993;265:R620–624. [PubMed: 8214156]
- Moran TH, Ameglio PJ, Schwartz GJ, McHugh PR. Blockade of type A, not type B, CCK receptors attenuates satiety actions of exogenous and endogenous CCK. *Am J Physiol* 1992;262:R46–50. [PubMed: 1733339]
- Moran TH, Katz LF, Plata-Salaman CR, Schwartz GJ. Disordered food intake and obesity in rats lacking cholecystokinin A receptors. *Am J Physiol* 1998;274:R618–625. [PubMed: 9530226]
- Moran TH, Norgren R, Crosby RJ, McHugh PR. Central and peripheral vagal transport of cholecystokinin binding sites occurs in afferent fibers. *Brain Res* 1990;526:95–102. [PubMed: 2078822]
- Parker RM, Herzog H. Regional distribution of Y-receptor subtype mRNAs in rat brain. *Eur J Neurosci* 1999;11:1431–1448. [PubMed: 10103138]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. 5. Elsevier Academic Press; San Diego, California: 2005.
- Raybould HE, Gayton RJ, Dockray GJ. Mechanisms of action of peripherally administered cholecystokinin octapeptide on brain stem neurons in the rat. *J Neurosci* 1988;8:3018–3024. [PubMed: 3411366]
- Reidelberger RD. Abdominal vagal mediation of the satiety effects of exogenous and endogenous cholecystokinin in rats. *Am J Physiol* 1992;263:R1354–1358. [PubMed: 1481948]

- Reidelberger RD, O'Rourke MF. Potent cholecystokinin antagonist L 364718 stimulates food intake in rats. *Am J Physiol* 1989;257:R1512–1518. [PubMed: 2481407]
- Renaud LP, Tang M, McCann MJ, Stricker EM, Verbalis JG. Cholecystokinin and gastric distension activate oxytocinergic cells in rat hypothalamus. *Am J Physiol* 1987;253:R661–665. [PubMed: 3661761]
- Rinaman L. Hindbrain noradrenergic lesions attenuate anorexia and alter central cFos expression in rats after gastric viscerosensory stimulation. *J Neurosci* 2003;23:10084–10092. [PubMed: 14602823]
- Rinaman L, Verbalis JG, Stricker EM, Hoffman GE. Distribution and neurochemical phenotypes of caudal medullary neurons activated to express cFos following peripheral administration of cholecystokinin. *J Comp Neurol* 1993;338:475–490. [PubMed: 8132858]
- Ritter RC, Ladenheim EE. Capsaicin pretreatment attenuates suppression of food intake by cholecystokinin. *Am J Physiol* 1985;248:R501–4. [PubMed: 3985191]
- Schick RR, Yaksh TL, Go VL. Intracerebroventricular injections of cholecystokinin octapeptide suppress feeding in rats--pharmacological characterization of this action. *Regul Pept* 1986;14:277–291. [PubMed: 3749526]
- Schwartz GJ, McHugh PR, Moran TH. Integration of vagal afferent responses to gastric load and CCK in rats. *Am J Physiol* 1991;261:R64–9. [PubMed: 1858957]
- Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000;404:661–671. [PubMed: 10766253]
- Shillabeer G, Davison JS. The cholecystokinin antagonist, proglumide, increases food intake in the rat. *Regul Pept* 1984;8:171–176. [PubMed: 6463283]
- Shutter JR, Graham M, Kinsey AC, Scully S, Luthy R, Stark KL. Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. *Genes Dev* 1997;11:593–602. [PubMed: 9119224]
- Silver AJ, Flood JF, Song AM, Morley JE. Evidence for a physiological role for CCK in the regulation of food intake in mice. *Am J Physiol* 1989;256:R646–652. [PubMed: 2923253]
- Smith GP, Gibbs J. Satiating effect of cholecystokinin. *Ann N Y Acad Sci* 1994;713:236–241. [PubMed: 8185165]
- Smith MS. Lactation alters neuropeptide-Y and proopiomelanocortin gene expression in the arcuate nucleus of the rat. *Endocrinology* 1993;133:1258–1265. [PubMed: 8365368]
- Smith GP, Jerome C, Cushman BJ, Eterno R, Simansky KJ. Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. *Science* 1981;213:1036–7. [PubMed: 7268408]
- Swaab DF, Nijveldt F, Pool CW. Distribution of oxytocin and vasopressin in the rat supraoptic and paraventricular nucleus. *J Endocrinol* 1975;67:461–462. [PubMed: 1206330]
- Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Wool EA, Monroe CA, Tepper RI. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 1995;83:1263–1271. [PubMed: 8548812]
- Thompson RH, Canteras NS, Swanson LW. Organization of projections from the dorsomedial nucleus of the hypothalamus: a PHA-L study in the rat. *J Comp Neurol* 1996;376:143–173. [PubMed: 8946289]
- West DB, Fey D, Woods SC. Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats. *Am J Physiol* 1984;246:R776–787. [PubMed: 6326618]
- Yox DP, Brenner L, Ritter RC. CCK-receptor antagonists attenuate suppression of sham feeding by intestinal nutrients. *Am J Physiol* 1992;262:R554–561. [PubMed: 1373576]
- Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;372:425–432. [PubMed: 7984236]

Figure 1.

Effect of dorsomedial hypothalamic (DMH) CCK on food intake. Parenchymal administration of 500 pmol CCK into the DMH decreased food intake during the entire 22 hour observation period as compared with vehicle controls. CCK: CCK treated group; VEH: vehicle aCSF treated group. Values are means $\pm$ SEM ($n = 10$ per group). *, $P < 0.05$ compared with vehicle controls, by two-way repeated measures ANOVA and planned t comparisons.

Figure 2. Effect of DMH CCK on NPY gene expression in the DMH. A–B: Representative micrographs of in situ hybridization with ^{35}S -labeled antisense riboprobe of NPY showing NPY gene expression in the compact subregion of the DMH in vehicle controls (A) and its expression visibly suppressed in the ipsilateral DMH of CCK-treated rats (B). C. NPY mRNA levels were significantly decreased in the DMH of CCK-treated rats (CCK) relative to vehicle controls (VEH). Values are means $\pm$ SEM ($n = 5$ per group). *, $P < 0.05$ compared with vehicle controls.

Figure 3.

Autoradiographs of 50 pM [^{125} I]-Bolton Hunter labeled CCK-8 binding in the rat brain with 4 conditions: (A) alone, or with (B) 10 nM devazepide, (C) 100 nM desulfated CCK-8 (dCCK) or (D) 100 μ M sulfated CCK-8 (sCCK). Within the hypothalamus (3.3 mm posterior to bregma (Paxinos and Watson, 2005), 10 nM devazepide blocked ^{125} I-CCK-8 binding in the DMH but not in the ventromedial hypothalamus (VMH) (B), whereas 100 nM dCCK blocked ^{125} I-CCK-8 binding in the VMH but not in the DMH (C). sCCK blocked all ^{125} I-CCK-8 binding activity (D).

Figure 4.

DMH CCK produced c-Fos activation in the DMH, PVN and Arc. A–B: Relative to vehicle controls (A), administration of 500 pmol CCK into the DMH induced c-Fos activation in the contralateral DMH (cDMH) and these induced c-Fos positive cells were distributed in all three subregions of the cDMH, i.e., the dorsal (DMHd), ventral (DMHv) and compact part (DMHc) (B). C–D: As compared with controls (C), DMH CCK induced c-Fos activation in the PVN and these induced c-Fos positive cells were primarily localized to the medial parvocellular part of the PVN (PVNp) but less to the lateral magnocellular part of the PVN (PVNm) (D). E–F: Relative to controls (E), DMH CCK induced c-Fos activation in the Arc with a majority of c-Fos positive cells in the medial part (F). 3v: the third ventricle.

Figure 5. DMH CCK produced c-Fos activation in the SCh and RCh. Relative to the control SCh (A) and RCh (C), DMH CCK induced c-Fos activation in the SCh (B) and RCh (D).

Figure 6.

Effects of CCK injection into the DMH on c-Fos immunoreactivity. Administration of 500 pmol CCK into the DMH increased the number of c-Fos positive cells in the SCh, RCh, PVN, cDMH and Arc as compared with vehicle controls. c-Fos immunoreactivity in the SON, VMH, LH, ME or CeA did not differ between CCK and VEH groups. CCK: CCK-treated group; VEH: vehicle-treated group. Values are means $\pm$ SEM ($n = 6-8$). *, $P < 0.05$ compared with vehicle controls.

Figure 7.

c-Fos activation in the NTS produced by peripheral CCK but not DMH CCK. c-Fos immunoreactivity was not detectable in the NTS and AP in both vehicle controls (A) and rats receiving DMH CCK administration (B), whereas peripheral CCK produced c-Fos activation in the NTS and AP (D) as compared with vehicle treated rats (C). E: Peripheral administration of CCK inhibited food intake at a 30 min time point relative to their baseline ($n = 10$ per group). F: Account of c-Fos positive cells in the NTS and AP was significantly increased in CCK-treated rats (CCK, $n = 5$) relative to vehicle controls (VEH, $n = 5$). Values are means $\pm$ SEM. *, $P < 0.05$ compared with vehicle controls.

Figure 8.

Effects of peripheral CCK on c-Fos immunoreactivity in the hypothalamus. Peripheral administration of CCK significantly increased the number of c-Fos positive cells in the PVN and DMH, but not the SCh, RCh, SON, Arc, VMH, LH, ME or CeA as compared with vehicle controls. Values are means $\pm$ SEM ($n = 5$ per group). *, $P < 0.05$ compared with vehicle controls.