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Non-sulfated cholecystokinin-8 increases enteric and hindbrain Fos-like immunoreactivity in male Sprague Dawley rats

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Abstract

Recently, we reported that non-sulfated cholecystokinin-8 (NS CCK-8) reduces food intake by cholecystokinin-B receptors (CCK-BR). To examine a possible site of action for this peptide, and based on the fact that both NS CCK-8 and CCK-BR are found centrally and peripherally, in the current study we hypothesized that NS CCK-8 increases Fos-like immunoreactivity (Fos-LI, a neuronal activation marker) in the dorsal vagal complex (DVC) of the hindbrain and the myenteric and submucosal plexuses of the small intestine. We found that intraperitoneal NS CCK-8 (0.5 nmol/kg) increases Fos-LI in the DVC, the myenteric and the submucosal plexuses of the duodenum and the myenteric plexus of the jejunum. The findings suggest, but does not prove, a potential role for the DVC and the enteric neurons in the feeding responses evoked by NS CCK-8.

Keywords

Non-sulfated CCK-8; Fos-like immunoreactivity; Enteric nervous system; Dorsal vagal complex; Food intake

1. Introduction

Cholecystokinin (CCK, see review (Sayegh, 2013)) is a gut brain peptide secreted by the enteroendocrine I cells of the small intestine and neurons of the peripheral and the central nervous systems. Based on the number of amino acids in the peptide chain CCK exists in multiple molecular forms e.g. CCK-8, CCK-33 and CCK-58. Furthermore, it has been shown that CCK-58 is the only endocrine form of CCK in rats (Reeve et al., 2003), while CCK-8 is still the most experimentally utilized one.

Prior to synthesis, the CCK prepropeptide undergoes proteolytic cleavage and yield two forms of CCK, a sulfated form at the seventh tyrosine residue from the carboxylic terminal (Asp-Tyr (SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH) and a non-sulfated form. However, each species secretes and utilizes both forms of CCK (Agersnap and Rehfeld, 2015).

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Sulfation of CCK alters the bioavailability of the peptide by changing its receptor binding strength, physiological responses, efficiency, circulating half-life and susceptibility to degradation (Bundgaard and Rehfeld, 2010; Rehfeld et al., 2007). For example, CCK evokes its physiological responses by activating two G-protein coupled receptors, CCK-A receptor (CCK-AR) or CCK₁R, distributed mainly in the gastrointestinal tract, and CCK-B receptor (CCK-BR) or CCK₂R, distributed mainly in the central nervous system. Sulfated CCK (S CCK) binds CCK-AR with 500–1000-fold higher affinity than non-sulfated CCK (NS CCK); however, S CCK and NS CCK bind CCK-BR with similar affinity (see (Huang et al., 1994) for review). In addition, S CCK and NS CCK differ in their physiological responses. For example, S CCK stimulates gallbladder contraction (Ivy, 1928) and exocrine pancreatic secretion (Harper and Raper, 1943) and inhibits gastric emptying (Debas et al., 1975) and food intake (Gibbs et al., 1973b) by activating CCK-AR, whereas NS CCK acts as a neurotransmitter (Dockray et al., 1978b; Rehfeld, 1978), stimulates insulin, glucagon, pancreatic polypeptide and somatostatin secretions (Rehfeld, 1971; Rehfeld et al., 1980) and acts as an autocrine growth factor in C-cell carcinomas/ thyroid and possibly Ewing's sarcoma (Rehfeld et al., 1990) through activating CCK-BR.

Recently, we have shown that NS CCK-8, similar to S CCK-8, also reduces meal size (MS), prolongs intermeal interval (IMI, time between first and second meal) length and increases satiety ratio (SR, IMI/MS or amount of food consumed per a unit of time) by activating CCK-BR (Dafalla et al., 2018) and through vagal and sympathetic pathways (Dafalla et al., in review). However, because both the peptide and the receptor are found peripherally and centrally, the current study hypothesized that NS CCK-8 increases Fos-like immunoreactivity (Fos-LI, a neuronal activation marker) in the dorsal vagal complex (DVC) of the hindbrain and the myenteric and submucosal plexuses of the small intestine. If proven to be the case, then the results may suggest, but will not confirm, a potential role for these neurons in the feeding responses evoked by NS CCK-8.

The DVC is an important central satiety control location (Campos et al., 2012; Williams et al., 2009; Wright et al., 2011; Zheng et al., 2005), which through vagal and/or splanchnic pathways (Altschuler et al., 1993; Batten, 1995; Kanemasa et al., 1995; Leong and Ling, 1991; Li et al., 2002; Mantyh and Hunt, 1984; Sim and Joseph, 1991; Viltart et al., 2006), it receives input from satiety peptides secreted by the gastrointestinal tract e.g. CCK (Campos et al., 2012; Williams et al., 2009; Wright et al., 2011; Zheng et al., 2005). In addition, the DVC contains CCK-BR (Noble and Roques, 1999; Noble, 2007), which mediates reduction of food intake by NS CCK-8 (Dafalla et al., 2018), and it can be activated by this peptide (Blevins et al., 2004; Chang and Kapas, 1997; Cox et al., 1995; Della-Fera et al., 1990; Monnikes et al., 1997a; O'Shea and Gundlach, 1993).

The DVC, located in the caudal and dorsomedial parts of the medulla oblongata, consists mainly of three nuclei. The nucleus tractus solitarius (NTS) primarily integrates information coming from the gut through the vagus nerve (Altschuler et al., 1993). The area postrema (AP) is a circumventricular organ that is located outside the blood-brain barrier and protrudes into the fourth ventricle above the NTS. It too receives signals from the vagus nerve. The dorsal motor nucleus of the vagus (DMV) receives its input from the NTS and sends signals to the gut wall (Altschuler et al., 1993; Batten, 1995; Kanemasa et al., 1995;

Leong and Ling, 1991; Li et al., 2002; Mantyh and Hunt, 1984; Sim and Joseph, 1991; Viltart et al., 2006).

The ENS (see review (Sayegh et al., 2005)) is a component of the autonomic nervous system, which is distributed along the gastrointestinal tract and controls the various functions of the gut e.g. motility, secretion, absorption and digestion. This system consists of a myenteric plexus, located between the longitudinal and circular muscle layers of the gut wall, and a submucosal plexus, located under the submucosal layer. Furthermore, the ENS communicates with the central feeding areas of the DVC via the vagus nerve, which provides the parasympathetic innervation to the gut, and the splanchnic nerves, which provides the sympathetic innervation to the gut (Sayegh et al., 2005). In addition, the gut also contains and expresses CCK-BR (Sayegh, 2013).

Previously, it has been shown that S CCK-8 reduces food intake (Gibbs et al., 1973a) and activates the enteric neurons of the duodenum and the jejunum as well as the DVC (Gulley et al., 2005b; Luckman, 1992; Sayegh and Ritter, 2000). Recently, we have shown that NS CCK-8 also reduces food intake (Dafalla et al., 2018), but, it remains unknown if the peptide activates the DVC and/or the enteric neurons. In the current study, we hypothesized that, similar to S CCK-8, NS CCK-8 increases Fos-LI in the DVC and the enteric neurons of the small intestine. A positive result may suggest a potential role for these neurons in the feeding responses evoked by NS CCK-8. Our results show that the same dose of NS CCK-8 which reduced food intake in our recent work (0.5 nmol/kg, ip) (Dafalla et al., 2018) also increased Fos-LI in the DVC and the enteric neurons in this study.

2. Materials and methods

2.1. Animals

Tuskegee University Animal Care and Use Committee approved the animal protocol for this study. Adult male Sprague Dawley rats (460–520 g, $n = 18$) were divided into three groups, (a) S CCK-8-treated group ($n = 6$ rats), (b) NS CCK-8-treated group ($n = 6$ rats) and (c) saline-treated group ($n = 6$ rats). Rats were housed individually in clear plastic cages in a controlled environment, 12 h dark/12 h light cycle, lights were off at 1700 h, temperature was maintained at 21.5 °C and the rats were provided with ad libitum water and pelleted rodent chow (Teklad, WI).

To habituate the rats to the laboratory environment and the experimental design of the study, each rat was weighed, handled for 10min and received an intraperitoneal (ip) injection of saline daily at 0900. All injections were delivered in a volume of 0.5 ml.

2.2. Experimental procedure

The immunohistochemistry protocol used in this experiment has been utilized in our laboratory (Gulley et al., 2005a; Sayegh and Ritter, 2003; Washington et al., 2011; Webb et al., 2005). Briefly, at the time of the experiment, rats were fasted from food, but not water, beginning at 1700 h on the day prior to the experiment. The following morning, the three groups of rats received either S CCK-8 (100 nmol/kg), NS CCK-8 (0.5 nmol/kg) (Bachem,

CA) or saline vehicle ip. Our recent study (Dafalla et al., 2018) demonstrated that NS CCK-8 (0.5 nmol/kg) produces significant reduction in food intake relative to saline vehicle.

Ninety minutes following the injection, which is the optimum time for Fos expression according to our previous work (Gulley et al., 2005a; Sayegh and Ritter, 2003; Washington et al., 2011; Webb et al., 2005), all rats were sacrificed with an overdose of sodium pentobarbital (10 mg/kg, ip) and perfused transcardially. The perfusion took place in two stages, first, with 500 ml of Krebs solution to collect the intestine, and second, with 500 ml of 4% formaldehyde solution dissolved in 0.1 M of phosphate buffered saline (PBS) to collect and fix the brains in situ.

The small intestines were exposed and collected through a ventral midline celiotomy incision. Five-centimeter segments of small intestine were collected between 5 and 10 cm aborad from the pylorus (duodenum), between 20 and 25 cm aborad from the pylorus (jejunum) and between 5 and 10 cm orad from the cecum (ileum). The lumens of the removed segments were rinsed with Krebs solution and placed in Sylgard-coated Petri dishes (World Precision and prepared Instruments, FL), opened along the mesenteric attachment, stretched and pinned with the mucosal side up, and stored overnight in Zamboni fixative at 4 °C. On the next day, the tissues were unpinned and cleared three times in 100% dimethyl sulfoxide (DMSO), 10 min each time, followed by three 10-min rinses with 0.1 M PBS, pH 7.4. We collected approximately five, 1 cm², whole mounts from each intestinal level, duodenum, jejunum, ileum, of each animal. The whole mounts of the intestinal wall with adhering myenteric and submucosal plexuses from each intestinal level were prepared using a dissecting microscope. The mucosa was removed by gentle scraping with a dull scalpel blade, turned to the opposite side and the remaining circular muscle layers were removed by gentle peeling using a jeweler's forceps. The inner circular muscle layer of the same sections was peeled from that outer longitudinal muscle layer with the adhering myenteric plexuses on it using a jeweler's forceps. All whole mounts from each intestinal level of every animal were processed for immunohistochemical detection of Fos-LI.

The brains were collected and post-fixed in 4% formaldehyde for 2 h at 4 °C. Then, they were placed in a 25% sucrose solution, on a shaker, overnight at room temperature. On the following day, 40, 40 µm sections of each brainstem were collected at — 20 °C, blocked with 50% ethyl alcohol for 30 min to reduce the production of free radicals and improve background staining, and processed to detect Fos-LI, as described previously (Gulley et al., 2005a; Sayegh and Ritter, 2003; Washington et al., 2011; Webb et al., 2005). The 40 sections included the following levels of the DVC according to the rat brain atlas of Paxinos and Watson, AP (— 4.5 mm caudal to the interaural plane), NTS (— 4.5 and —4.8 mm caudal to the interaural plane) and DMV (— 4.5 and —4.8 mm caudal to the interaural plane). Sections were taken at multiple levels of the NTS and DMV to insure sampling from sites receiving intestinal vagal afferent innervation.

The intestinal whole mounts and the brainstem sections were incubated for 24 h at room temperature in a primary antiserum raised in rabbit against a peptide consisting of amino acids 4–17 of human Fos (Oncogene, Ab-5, San Diego, CA [1:12,000 dilution]). The same

antibodies, both primary and secondary, were used for the whole mounts and the brainstem sections.

On the next day, the whole mounts and the brainstem sections were rinsed with 0.01 M tris phosphate buffer solution (TPBS) and incubated overnight in a biotinylated donkey anti-rabbit serum (Jackson Immuno Research Laboratories, PA [1:500 dilution]). Then, the tissues were incubated for 3 h in avidin conjugated to horseradish peroxidase, washed with 0.01 M TPBS, and reacted with glucose oxidase and diami-nobenzidine (DAB, Sigma, MO) intensified with nickel.

2.3. Counting procedure

With the slide labels concealed, two examiners counted Fos-positive neurons in the enteric neurons. For each animal, at each intestinal level (duodenum, jejunum, ileum) and for each plexus (myenteric, submucosal) twenty, non-overlapping, 100X microscopic fields images were captured by using Q-Imaging software and Fos-positive neurons were counted and averaged. All the numbers for the six rats were tabulated, analyzed, averaged then graphed. The criteria for the positive neurons included those with only black, rounded, regular nuclei. All neurons with large, irregular, faintly stained shapes were considered cell bodies and were not counted. The agreement rate between the two examiners exceeded 98%.

In the brainstem, Fos-positive neurons in the AP, NTS, and DMV according to the rat brain atlas of Paxinos and Watson were counted by an automated computer software (ImagePro Plus, Media Cybernetics). From the brain of each rat we cut 40 sections to make sure that we did not miss the areas of interest, however, only two sections from each rat were counted. The sections that matched the atlas. One section contains AP, left and right NTS and left and right DMV at — 4.5 mm caudal to the interaural plane. The second section contains the left and right NTS and the left and right DMV at —4.8 mm caudal to the interaural plane. Areas (AP, NTS and DMV) were encircled using ImagePro Plus (ImagePro Plus, Media Cybernetics) and an examiner set a certain color density criteria for Fos-positive nuclei. The counts of the six rats were collected, tabulated, analyzed, averaged and graphed.

2.4. Statistical analysis

The counts of Fos-positive neurons in the DVC were compared using a two-way Analysis of Variance (ANOVA) with treatment and level as the independent variables. The counts of Fos-Positive neurons in the intestine were compared using a three-way ANOVA with treatment, level and layer as the independent variables. The tests were followed by a Tukey test for multiple comparisons and the results were displayed as mean $\pm$ SEM. Counts were considered significant if p was < 0.05 .

3. Results

3.1. Effect of NS CCK-8 on activation of the dorsal vagal\ complex

There was a significant effect of treatment ($DF = 2$, $36F = 69.542$, $p < 0.001$), section ($DF = 4$, $F = 3.544$, $p = 0.007$) and treatment/ section interaction ($DF = 8$, $F = 2.348$, $p = 0.018$). Non-sulfated CCK-8 and S CCK-8 increased Fos-LI relative to saline in the AP, NTS and

DMV (— 4.5 mm caudal to interaural plane) ($p < 0.001$ each) and in the NTS and DMV (— 4.8 mm caudal to interaural plane) ($p < 0.001$ each) (Figs. 1 and 2).

3.2. Effect of NS CCK-8 on activation of the enteric neurons

There was a significant effect of the treatment ($DF = 2$, $F = 113.432$, $p < 0.001$), section ($DF = 2$, $F = 9.876$, $p < 0.001$), layer ($DF = 1$, $F = 21.445$, $p < 0.001$), treatment/section interaction ($DF = 4$, $F = 46.562$, $p < 0.001$), treatment/layer interaction ($DF = 2$, $F = 9.661$, $p < 0.001$) and treatment/section/layer interaction ($DF = 4$, $F = 16.069$, $p < 0.001$). In the myenteric and submucosal plexuses of the duodenum, NS CCK-8 and S CCK-8 increased Fos-LI relative to saline ($p < 0.001$ each). In the myenteric plexus of the jejunum, NS CCK-8 and S CCK-8 increased Fos-LI relative to saline ($p = 0.008$ and $p = 0.002$ respectively). In the submucosal plexus of the jejunum, NS CCK-8 and CCK-8 failed to increase Fos-LI relative to 0.095 each (Figs. 3 and 4). saline ($p = 0.082$ and $p = 0.10$ respectively). In the myenteric and submucosal plexuses of the ileum, NS CCK-8 and S CCK-8 failed to increase Fos-LI relative to saline vehicle ($p = 0.071$, 0.075 , 0.121 and 0.095 each) (Figs. 3 and 4).

4. Discussion

Recently (Dafalla et al., 2018), we have shown that NS CCK-8 (0.5 nmol/kg, ip) reduces food intake in adult male Sprague Dawley rats by activating the CCK-BR. The current work demonstrated that the same dose of the peptide increases Fos-LI, a neuronal activation marker, in the DVC of the hindbrain and the enteric neurons of the small intestine. This result may suggest a potential role for these locations in reduction of food intake by NS CCK-8.

Activation of the DVC and the enteric neurons as manifested by increased Fos-LI in response to other satiety gut peptides e.g., S CCK-8 (Gulley et al., 2005a; Sayegh and Ritter, 2000, 2003; Sullivan et al., 2007), glucagon like peptide-1 (GLP-1) (Washington et al., 2010), gastrin releasing peptide-29 (GRP-29) (Washington and Sayegh, 2011) and peptide tyrosine tyrosine (PYY) (Newman et al., 2017) has been documented prior to this report. The current study demonstrated that NS CCK-8 also increases Fos-LI in these neurons.

At least two reasons suggest that activation of the ENS and the DVC by NS CCK-8 is plausible. First, the peptide is secreted by both peripheral and central neurons (Rehfeld and Agersnap, 2012; Rehfeld, 2017). Second, the ENS and the DVC contain and express CCK-BR (Aras and Ekstrom, 2006; Babic et al., 2009; Beinfeld, 1992; Berthoud and Patterson, 1996; Lateef et al., 2012; Li et al., 1999; Millington et al., 1992; Monnikes et al., 1997b; Patterson et al., 2002; Sayegh, 2013; Schwartz et al., 1994), the receptor which mediates the various physiological responses evoked by NS CCK-8 e.g. neurotransmission (Dockray et al., 1978a; Larsson and Rehfeld, 1978), stimulation of insulin, glucagon, pancreatic polypeptide and somatostatin secretion (Hornnes et al., 1980; Rehfeld, 1971) and reduction of food intake (Dafalla et al., 2018). Therefore, activation of the ENS and DVC by NS CCK-8 is plausible.

Furthermore, activation of the ENS and the DVC by NS CCK-8, along with our current finding that the same dose of the peptide (0.5 nmol/kg ip) reduces food intake (Dafalla et al., 2018) may suggest, but does not prove, a potential role for these neurons in the satiety effects evoked by NS CCK-8. It is plausible that NS CCK-8 activates the enteric neurons, which through the vagus and the splanchnic nerves activate the DVC to reduce feeding. Multiple lines of evidence support, but again does not prove, this claim.

First, as mentioned above the ENS and the DVC secrete NS CCK-8, contain, and express CCK-BR, the receptor that mediates reduction of food intake by the peptide. Second, the ENS communicates with the DVC through the vagus and the splanchnic nerves (Altschuler et al., 1993; Berthoud et al., 1990; Hagbom et al., 2011; Holmes, 2012; Love et al., 2007; Patterson et al., 2003; Yuan and Yang, 2002), which also contain CCK-BR and can be activated by NS CCK (Branchereau et al., 1992; Campos et al., 2013; Corp et al., 1993; Hokfelt et al., 2001; Kasimay et al., 2005; Long, 1993; Urban et al., 1996; Valverde et al., 1995; Widdop et al., 1994; Williams et al., 2009; Zhou et al., 1993). Third, severing the sympathetic and the parasympathetic connections between the ENS and the DVC, by performing celiaco-mesenteric ganglionectomy and subdiaphragmatic vagotomy respectively, attenuated reduction of food intake by NS CCK-8 (Dafalla et al., in review). Therefore, collectively the data above suggest that activation of the ENS and the DVC neurons by NS CCK-8 may have a role in the reduction of food intake by the peptide. This claim, however, requires further testing.

Additionally, research is also required to determine the specific mode/site of action for NS CCK-8. One potential site is a peripheral/ local/gastrointestinal site. One line of evidence pointing at this possibility is that two non-sulfated forms of CCK, CCK-8 and CCK-4, does not cross the blood brain barrier (Hagino et al., 1989; Sugeta et al., 2015). Therefore, the actions of NS CCK-8, possibly including reduction of food intake, may be peripheral/local i.e., gastrointestinal. Others (Calingasan et al., 1992; Cox et al., 1996; Smith et al., 1988) and we (Mhalhal et al., 2017b; Newman et al., 2017; Sayegh et al., 2015; Washington et al., 2016b) have shown that the gastrointestinal tract is a potential site of action for reduction of food intake by S CCK-8, GRP-29 and GLP-1, (Mhalhal et al., 2017a, 2018; Washington et al., 2014), because intra-arterial infusions of S CCK-8 (0.05 nmol/kg) in the cranial mesenteric artery, which supplies the small intestine, but not ip injections, reduced food intake (Mhalhal et al., 2017a,b; Sayegh et al., 2015; Washington et al., 2016a,b). In addition, interrupting the enteric neurons utilizing a duodenal myotomy procedure also attenuated reduction of food intake by the same peptide (Lateef et al., 2012). Similar experiments are needed to determine the site of action for NS CCK-8. The current study demonstrated that NS CCK-8 increases Fos-LI in the ENS and the DVC of adult male rats.

4.1. Effect of non-sulfated cholecystokinin-8 on Fos-like immunoreactivity in the dorsal vagal complex

Non-sulfated cholecystokinin-8 (NS CCK-8, 0.5 nmol/kg, white bars), sulfated CCK-8 (S CCK-8, 100 ug/kg, gray bars) or saline (black bars) were given intraperitoneally (ip) to overnight food deprived male Sprague Dawley rats (n = 6 rats per group) and the neurons that expressed Fos-like immunoreactivity (Fos-LI, a marker for neuronal activation) were

quantified. Two sections from each rat were counted, one contains the area postrema (AP), the nucleus tractus solitaries (NTS) and the dorsal motor nucleus of the vagus (DMV) at — 4.5 mm caudal to the interaural plane according to the brain atlas of Paxinos and Watson, and the other contains the NTS and the DMV at — 4.8 mm caudal to the interaural plane. The counts from the six animals were analyzed, averaged and graphed. At —4.5 mm caudal to the interaural plane NS CCK-8 and S CCK-8 increased Fos-LI in the AP, NTS and DMV relative to saline vehicle. In addition, at —4.8 mm caudal to interaural plane both peptides increased it in the NTS and the DMV relative to saline vehicle. *indicates significance ($p < 0.05$).

4.2. Fos-like immunoreactivity in the dorsal vagal complex in response to non-sulfated cholecystokinin

Photomicrographs of the dorsal vagal complex in rats injected with saline (A, D), non-sulfated cholecystokinin-8 (NS CCK-8) (B, E) and sulfated cholecystokinin-8 (S CCK-8) (C, F) in the area postrema (AP), nucleus tractus solitaries (NTS) and dorsal motor nucleus of the vagus (DMV) at —4.5 mm caudal to interaural plane (A, B and C) and in the NTS and DMV at — 4.8 mm caudal to the interaural plane (D, E and F). Non-sulfated and S CCK-8 increased Fos-like immunoreactivity (B, C, E, F) relative to saline (A, D). Fos-positive nuclei appear as dark pinpoint dots. cc: central canal, Scale bar =100 μ m

4.3. Effect of non-sulfated cholecystokinin on Fos-like immunoreactivity in the small intestine

Non-sulfated cholecystokinin-8 (NS CCK-8, 0.5 nmol/kg, white bars), sulfated CCK-8 (100 μ g/kg, gray bars) or saline (black bars) were given intraperitoneally (ip) to overnight food deprived male Sprague Dawley rats ($n = 6$ rats per group). For each animal, at each intestinal level (duodenum, jejunum, ileum) and for each plexus (myenteric, submucosal) twenty, non-overlapping, 100X microscopic fields images were captured by using Q-Imaging software and Fos-positive neurons were counted and averaged. All the numbers for the six rats were analyzed, averaged and graphed. Non-sulfated CCK-8 and S CCK-8 increased Fos-LI in the myenteric and submucosal plexuses of the duodenum and the myenteric plexus of the jejunum relative to saline vehicle. * indicates significance ($p < 0.05$).

4.4. Fos-like immunoreactivity in the enteric nervous system of the small intestine in response to non-sulfated cholecystokinin

Photomicrographs of myenteric and submucosal plexuses of the small intestine from rats injected with saline (A-F), non-sulfated cholecystokinin-8 (NS CCK-8) (G-L) and sulfated cholecystokinin-8 (S CCK-8) (M-R). Non-sulfated CCK-8 and S CCK-8 increased Fos-like immunoreactivity (Fos-LI) relative to saline in the myenteric (G and M) and submucosal plexuses (H and N) of the duodenum and the myenteric plexus of the jejunum (I and O). Fos-positive nuclei appear as dark pinpoint dots. Scale bar =100 μ m.

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HIGHLIGHTS

- Non-sulfated cholecystokinin-8 (NS CCK-8) reduced food intake.
- NS CCK-8 increased Fos-LI immunoreactivity (Fos-LI) in the hindbrain.
- NS CCK-8 increased Fos-LI in the myenteric neurons.
- NS CCK-8 increased Fos-LI in the submucosal neurons.
- These neurons may have a role in reduction of food intake by NS CCK-8.

Fig. 1.

Effect of non-sulfated cholecystinin-8 on Fos-like immunoreactivity in the dorsal vagal complex. Non-sulfated cholecystinin-8 (NS CCK-8, 0.5 nmol/kg, white bars), sulfated CCK-8 (S CCK-8, 100ug/kg, gray bars) or saline (black bars) were given intraperitoneally (ip) to overnight food deprived male Sprague Dawley rats (n = 6 rats per group) and the neurons that expressed Fos-like immunoreactivity (Fos-LI, a marker for neuronal activation) were quantified. Two sections from each rat were counted, one contains the area postrema (AP), the nucleus tractus solitaries (NTS) and the dorsal motor nucleus of the vagus (DMV) at — 4.5 mm caudal to the interaural plane according to the brain atlas or Paxinos and Watson, and the other contains the NTS and the DMV at — 4.8 mm caudal to the interaural plane. The counts from the six animals were analyzed, averaged and graphed. At — 4.5 mm caudal to the interaural plane NS CCK-8 and S CCK-8 increased Fos-LI in the AP, NTS and DMV relative to saline vehicle. In addition, at — 4.8 mm caudal to interaural plane both peptides increased it in the NTS and the DMV relative to saline vehicle. * indicates significance ($p < 0.05$).

Fig. 2.

Fos-like immunoreactivity in the dorsal vagal complex in response to non-sulfated cholecystokinin. Photomicrographs of the dorsal vagal complex in rats injected with saline (A, D), non-sulfated cholecystokinin-8 (NS CCK-8) (B, E) and sulfated cholecystokinin-8 (S CCK-8) (C, F) in the area postrema (AP), nucleus tractus solitarius (NTS) and dorsal motor nucleus of the vagus (DMV) at — 4.5 mm caudal to interaural plane (A, B and C) and in the NTS and DMV at — 4.8 mm caudal to the interaural plane (D, E and F). Non-sulfated and S CCK-8 increased Fos-like immunoreactivity (B, C, E, F) relative to saline (A, D). Fos-positive nuclei appear as dark pinpoint dots. cc: central canal, Scale bar = 100 μ m.

Fig. 3.

Effect of non-sulfated cholecystokinin on Fos-like immunoreactivity in the small intestine. Non-sulfated cholecystokinin-8 (NS CCK-8, 0.5 nmol/kg, white bars), sulfated CCK-8 (100 ug/kg, gray bars) or saline (black bars) were given intraperitoneally (ip) to overnight food deprived male Sprague Dawley rats ($n = 6$ rats per group). For each animal, at each intestinal level (duodenum, jejunum, ileum) and for each plexus (myenteric, submucosal) twenty, non-overlapping, 100X microscopic fields images were captured by using Q-Imaging software and Fos-positive neurons were counted and averaged. All the numbers for the six rats were analyzed, averaged and graphed. Non-sulfated CCK-8 and S CCK-8 increased Fos-LI in the myenteric and submucosal plexuses of the duodenum and the myenteric plexus of the jejunum relative to saline vehicle. * indicates significance ($p < 0.05$).

Fig. 4.

Fos-like immunoreactivity in the enteric nervous system of the small intestine in response to non-sulfated cholecystokinin. Photomicrographs of myenteric and submucosal plexuses of the small intestine from rats injected with saline (A-F), non-sulfated cholecystokinin-8 (NS CCK-8) (G-L) and sulfated cholecystokinin-8 (S CCK-8) (M-R). Non-sulfated CCK-8 and S CCK-8 increased Fos-like immunoreactivity (Fos-LI) relative to saline in the myenteric (G and M) and submucosal plexuses (H and N) of the duodenum and the myenteric plexus of the jejunum (I and O). Fos-positive nuclei appear as dark pinpoint dots. Scale bar = 100 μ m.