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Non-sulfated cholecystokinin-8 reduces meal size and prolongs the intermeal interval in male Sprague Dawley rats

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Abstract

The current study measured seven feeding responses by non-sulfated cholecystokinin-8 (NS CCK-8) in freely fed adult male Sprague Dawley rats. The peptide (0, 0.5, 1, 3, 5 and 10nmol/kg) was given intraperitoneally (ip) prior to the onset of the dark cycle, and first meal size (MS), second meal size, intermeal interval (IMI) length, satiety ratio (SR = IMI/MS), latency to first meal, duration of first meal, number of meals and 24-hour food intake were measured. We found that NS CCK-8 (0.5 and 1.0nmol/kg) reduced MS, prolonged IMI length and increased SR during the dark cycle. Furthermore, the specific CCK-B receptor antagonist L365, 260 (1 mg/kg, ip) attenuated these responses. These results support a possible role for NS CCK-8 in regulating food intake.

Keywords

Non-sulfated CCK; Sulfated CCK; Food intake; L365,260; Devazepide

1. Introduction

Cholecystokinin (CCK) is a gut brain peptide secreted by the enteroendocrine I cells of the intestine and the neurons of the peripheral and the central nervous systems (see (Rehfeld, 2017) for review). Prior to synthesis, the CCK prepropeptide undergoes proteolytic cleavage and posttranslational modifications yielding a sulfated form of CCK on the seventh tyrosine residue from the carboxylic terminal (Asp-Tyr (SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH) and a non-sulfated form (Beinfeld, 2003).

Sulfated CCK (S CCK) and non-sulfated CCK (NS CCK) differ in their physiological responses. For example, S CCK stimulates gallbladder contraction (Ivy & Oldberg, 1928), exocrine pancreatic secretion (Harper & Raper, 1943), inhibits gastric emptying (Debas et

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al., 1975) and reduces food intake (Gibbs et al., 1973). However, NS CCK functions as a neurotransmitter (Rehfeld, 1978; Dockray et al., 1978), stimulates insulin, glucagon, pancreatic polypeptide and somatostatin secretion (Rehfeld et al., 1980; Rehfeld, 1971) and acts as an autocrine growth factor in C-cell carcinomas/thyroid and possibly Ewing's sarcoma (Rehfeld et al., 1990).

Cholecystokinin activates 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 (for review see (Noble & Roques, 1999)). Sulfated CCK binds CCK-AR with 500–1000-fold higher affinity than NS CCK; however, S CCK and NS CCK bind CCK-BR with similar affinity (see (Wank, 1994) for review).

Our laboratory is interested in the regulation of the short-term control of food intake by gut peptides such as CCK. As mentioned earlier S CCK-8 reduces food intake (Gibbs et al., 1973), an action which is mediated by the CCK-AR (Corwin et al., 1991). On the other hand, reduction of food intake by NS CCK-8 has been investigated sporadically in rats and mice (Crawley et al., 1984). For example, Kadar et al. reported that NS CCK-8 given intraperitoneally (ip) and intracerebroventricularly (icv) failed to reduce cumulative food intake in 24hrs fasted, young adult (160–180 g), male Sprague Dawley rats maintained in a familiar environment (Kadar et al., 1985).

In the current study, we hypothesized that NS CCK-8 may have a role in regulating short-term control of food intake i.e. meal size (MS) and intermeal interval (IMI) length for the following reasons. (A) The major sources of peripheral NS CCK are the I cells and the neurons of the gut (Larsson & Rehfeld, 1978; Polak et al., 1976; Buffa et al., 1976). The gut is the chief organ that releases most of the satiety peptides that regulate MS and IMI length e.g., CCK, gastrin releasing peptide (GRP) and glucagon like peptide-1 (GLP-1) (Sayegh, 2013a; Sayegh, 2013b), which are released in response to the presence of food in the gut (Liddle et al., 1985). (B) The CCK—BR, which mediates the physiological responses evoked by NS CCK, is distributed in the gastrointestinal tract (Sayegh, 2013a; Reubi et al., 1997; Monstein et al., 1996; Mantyh et al., 1994) and central areas that control food intake e.g. paraventricular nucleus of the hypothalamus, locus coeruleus, area postrema (AP), nucleus tractus solitarius (NTS) and dorsal motor nucleus of the vagus (DMV) (Sugeta et al., 2015; Monnikes et al., 1997; O'Shea & Gundlach, 1993). (C) The vagus nerve, which comprises the major parasympathetic extrinsic innervation of the gut and communicates the satiety signals between the gut and the central feeding areas mentioned previously, also expresses CCK—BR (Dockray et al., 1981; Moriarty et al., 1997). (D) NS CCK stimulates the secretion of peptides that regulate food intake and glucose levels e.g. insulin and glucagon (Rehfeld et al., 1980; Rehfeld, 1971). As such, NS CCK may have a potential role in regulating MS and IMI length. The current study demonstrated that NS CCK-8 given ip prior to the onset of the dark cycle reduces MS and prolongs the IMI in free fed, adult male Sprague Dawley rats by activating the CCK-BR.

2. Materials and methods

The Tuskegee University Animal Care and Use Committee approved the animal protocol for this study. Adult, male, Sprague-Dawley rats weighing between 350 and 450 g ($n = 12$) were used in these experiments. The rats were housed in a controlled environment (12 h dark/12 h light cycle—lights off at 1700 h, 21.5 °C, with ad lib water and pelleted rodent chow (Teklad, WI).

3. The BioDAQ food and water intake monitor

The BioDAQ E2 food and water intake system reports food intake data as bouts, the smallest unit of episodic intake. The unique design allows for minimal food spillage and hoarding of pelleted diets. A tray at the bottom of the hopper captures crumbs to ensure an accurate measurement of food intake. The computerized data stream associated with each bout includes a time and date stamp marking the initiation of intake activity, the period of the activity, and the weight of food or water consumed. The period of a bout is variable and is defined by the animal's activity. According to the manufacturer, a meal was defined as the collection of continuous bouts and inter-bout intervals with no more than 5 s of inactivity in the hopper.

4. Habituation and formation of base line food intake

To habituate the rats to the laboratory environment and experimental design, every day, and at the same time for a period of two weeks, each rat was weighed, handled for 10 min and received an ip injection of saline. All injections were made in a volume of 0.5 ml and were given 5–10 min prior to the onset of the dark cycle at the 1700 h. Before measuring food intake in response to CCK, a baseline for food intake was established for each rat by the following procedure. Animals were fasted from food but not water one hour prior to the onset of the dark cycle i.e., at the 1600 h. At the start of the dark cycle, the feeding gates were opened and rats have free access to rat chow and water. Every bout of food was recorded automatically, and the data were collected every 24 h. Data were analyzed to determine first MS, second MS, IMI length, satiety ratio (SR, IMI/first MS), duration of first meal, latency to first meal, number of meals and total food intake. This process formed the baseline for each rat. The baseline values for each rat were compared with the values of the saline treatment days. If these data did not match, then the data for that rat were not included in the statistical analysis. Only the data that matched the base line data were analyzed.

5. Experiment 1

5.1. Food intake

At 1600 h on Mondays, Wednesdays and Fridays, feeding gates were closed and all rats received ip injection of 0.5 ml saline 10 min prior to the onset of the dark cycle. On Tuesdays, Thursdays and Saturdays, the rats received ip injection of NS CCK-8 (0.5, 1, 3, 5 and 10 nmol/kg) 10 min prior to the onset of the dark cycle. Immediately following the injections, lights were shut off and the feeding gates were opened. Sundays were reserved for maintenance of the cages and the BioDAQ system. Data collection started immediately

following the opening of the feeding gates and were analyzed for each rat every 24 h to determine the feeding responses. All rats received all treatments (repeated measure).

6. Experiment 2

6.1. Antagonists

At 1600 h on Mondays, Wednesdays and Fridays the feeding gates were closed and all rats received ip injections of 0.5 ml saline (two injections per rat, 10 min apart). On Tuesdays, Thursdays and Saturdays the feeding gates were closed at 1600 h and all rats received two injections prior to the onset of the dark cycle. The first injection was given 10 min prior to the onset of the dark cycle. Rats received an ip injection of L365,260 (1 mg/kg), devazepide (1 mg/kg) or vehicle for the antagonists (dissolved in 0.1 M PBS), followed 10 min later by a second ip injection of NS CCK-8 (0.5 nmol/kg) or saline (as a vehicle for the peptide). The combination of treatments for this experiment were vehicle/saline (veh/sal) as a control for the antagonist and peptide respectively, vehicle/NS CCK-8 (veh/cck), devazepide/saline (dev/sal), devazepide/NS CCK-8 (dev/cck), L365,260/saline (l365/sal) and L365,260/NS CCK-8 (l365/cck).

Immediately following the second injection, lights were shut off and the feeding gates were opened. Data collection started immediately following the opening of the feeding gates and were analyzed for each rat every 24 h to determine the feeding responses.

7. Statistical analysis for both experiments

The data from the saline injections during the treatment days agreed with the baseline data prior to the start of the peptide injections (collected before the experiment started) and with the resting days. The saline treatments were averaged and considered to be the control for the NS CCK-8 treatments. Data were analyzed using a General Linear Model (GLM) and a post hoc test for repeated measures with one variable. This variable was dose of peptide for the food intake experiment and combination of treatments for the antagonist experiment (SYSTAT for Windows version 11.00). Results are displayed as mean $\pm$ SEM and data were considered significant when p was ≤ 0.05 .

8. Results

8.1. Experiment 1

8.1.1. First meal size—The analysis revealed a main effect of treatment ($F_{(5, 55)} = 2.46$, $p = .04$, $\eta^2 = 0.18$). Non-Sulfated CCK-8 (0.5 and 1 nmol/kg), reduced the size of the first meal compared to saline control ($p = .01$ and $p = .01$ respectively) (Fig. 1).

8.1.2. Intermeal interval length—The analysis revealed no main effect of treatment ($F_{(5, 55)} = 2.01$, $p = .09$, $\eta^2 = 0.2$). Non-sulfated CCK-8 (0.5 and 1 nmol/kg) significantly increased IMI length relative to saline control ($p = .03$ and $p = .02$ respectively) (Fig. 1).

8.1.3. Satiety ratio—The analysis revealed a main effect of treatment ($F_{(5, 55)} = 2.72$, $p = .03$, $\eta^2 = 0.2$). Non-Sulfated CCK-8 (0.5 and 1 nmol/kg) increased the SR relative to saline control ($p = .002$ and $p = .03$ respectively) (Fig. 1).

8.1.4. Second meal size, latency and duration of first meal—The analysis revealed no main effect of treatment on the size of the second meal, latency to the first meal and duration of the first meal ($p = .5$, $p = .1$ and $p = .15$ respectively) (Fig. 2).

8.1.5. Total intake—The analysis revealed no main effect of treatment ($F_{(5, 55)} = 1.2$, $p = .3$, $\eta^2 = 0.10$). Non-sulfated CCK-8 (0.5 nmol/kg) significantly reduced total food intake relative to saline control ($p = .004$) (Fig. 3).

8.1.6. Number of meals—The analysis revealed no main effect of treatment on the number of meals (Fig. 3).

8.2. Experiment 2

8.2.1. Antagonist

8.2.1.1. First meal size: The analysis revealed no main effect of treatment ($F_{(5, 55)} = 1.33$, $p = .27$, $\eta^2 = 0.11$) and post hoc analysis showed that veh/cck significantly reduced the size of the first meal relative to veh/sal ($p = .03$). In addition, the CCK-BR antagonist L365,260, (l365/cck) but not devazepide (dev/cck), significantly attenuated this response ($p = .05$) (Fig. 4).

8.2.1.2. Intermeal interval length: The analysis revealed no main effect of treatment ($F_{(5, 55)} = 1.6$, $p = .18$, $\eta^2 = 0.13$) and the post hoc analysis showed that veh/cck increased the IMI relative to veh/sal ($p = .03$). The CCK-BR antagonist L365,260 (l365/cck), but not the CCK-AR antagonist devazepide (dev/cck), significantly attenuated this response ($p = .04$) (Fig. 4).

8.2.1.3. Satiety ratio: The analysis revealed no main effect of treatment ($F_{(5, 55)} = 2.4$, $p = .06$, $\eta^2 = 0.28$) and the post hoc analysis showed that veh/cck increased the SR relative to veh/sal ($p = .05$) (Fig. 4). Non-sulfated CCK-8 (veh/cck) increased the SR but neither L365,260 (l365/cck) nor devazepide (dev/cck) affected this response.

8.2.1.4. Second meal size: The analysis revealed no main effect of treatment ($F_{(5, 55)} = 0.96$, $p = .45$, $\eta^2 = 0.08$). Vehicle/cck failed to reduce the size of the second meal relative to veh/sal (Fig. 5) and there was no effect of L365,260 (l365/cck) or devazepide (dev/cck) on this response.

8.2.1.5. Latency to the first meal: The analysis revealed no main effect of treatment ($F_{(5, 55)} = 3.1$, $p = .2$, $\eta^2 = 0.21$). Vehicle/cck failed to increase latency to the first meal relative to veh/sal (Fig. 5) and there was no effect of L365,260 (l365/cck) or devazepide (dev/cck) on this response.

8.2.1.6. Duration of the firstmeal: The analysis revealed no main effect of treatment ($F_{(5, 55)} = 1.04$, $p = .4$, $\eta^2 = 0.09$). Vehicle/cck failed to reduce the duration of the first meal relative to veh/sal (Fig. 5) and there was no effect of L365,260 (l365/cck) or devazepide (dev/cck) on this response.

8.2.1.7. Total food intake: The analysis revealed no main effect of treatment ($F_{(5, 55)} = 0.9$, $p = .5$, $\eta^2 = 0.08$). Vehicle/cck failed to reduce total food intake relative to veh/sal (Fig. 6) and there was no effect of L365,260 (l365/cck) or devazepide (dev/cck) on this response.

8.2.1.8. Number of meals: The analysis revealed no main effect of treatment ($F_{(5, 55)} = 1.4$, $p = .35$, $\eta^2 = 0.27$). Vehicle/cck failed to reduce the number of meals relative to veh/sal (Fig. 6) and there was no effect of L365,260 (l365/cck) or devazepide (dev/cck) on this response.

Discussion

The current study found that NS CCK-8 given ip reduces food intake in free fed rats by suppressing MS and prolonging IMI length. In addition, the specific CCK-BR antagonist L365, 260 attenuated these responses.

A role for NS CCK-8 in regulating the short-term control of food intake i.e. MS and IMI length is plausible because one of the main peripheral sources for the peptide is the endocrine I cells of the gut and the peptide is secreted in response to the presence of food in the gut (see review (Rehfeld, 2017)). The CCK-BR, which mediates the physiological responses evoked by NS CCK, is expressed on the vagus nerve (Moriarty et al., 1997), the main innervation of the gut that communicate with the central feeding areas, as well as on the nodose ganglia, hypothalamus, AP, NTS and DMV (Monnikes et al., 1997; Broberger et al., 2001; Monnikes et al., 1997). Furthermore, Clerc et al. reported significant increase in food intake and body weight in CCK-BR deficient mice (Clerc et al., 2007). Therefore, a role for NS CCK-8 in the short-term control of food intake is plausible. The results of the current study support this possibility.

In the literature, one study examined the effect of NS CCK-8 on food intake in rats (Kadar et al., 1985). They found that NS CCK-8 given ip in young adult albino male rats maintained in a familiar, but not novel, environment failed to reduce 30 min food intake. However, there are fundamental differences between their study and the current one. First, the previous study utilized young rats weighing 160–180 g while we used adult rats weighing 350–450 g. It is known that the neuronal circuits of the gastrointestinal tract, namely the enteric nervous system (ENS) (Schafer et al., 1999; Fausone-Pellegrini et al., 1996; Belai et al., 1995), and the vagus nerve (Tolosa et al., 2005; Powley et al., 2001; Kuehl-Kovarik & Jacobson, 1996; MacLean, 1985; Kerr, 1975), as well the central ones (Gutkind et al., 1988), continue to develop in young animals. These circuits participate in carrying the peripheral satiety signals e.g. NS CCK-8 from the gut to the central satiety areas e.g. NTS, DMV and AP. Therefore, failure of NS CCK-8 to reduce food intake in young rats is possibly due to underdevelopment of such circuits. This has been documented previously in rat and human by S CCK-8 (Anika, 1983; Balasko et al., 2013; Weller, 2006; Voigt et al., 1996; Salorio et

al., 1994) as well as other peptides (Akimoto & Miyasaka, 2010). They have shown that the effect of S CCK-8 on food intake in young rats has been attenuated compared to adults.

Second, (Kadar et al., 1985) injected NS CCK-8 in 24 hrs fasted rats whereas in the current work the peptide was injected in free fed rats. Injecting NS CCK-8 in free fed rats reveals the satiety effects of the peptide under physiological conditions. For example, it has been shown that fasting (over 96 h) lowers plasma and duodenal levels of S CCK (Zheng et al., 1987; Koop et al., 1987), and reduces CCK receptor affinity (Chowdhury & Rayford, 2001). In addition, other reports found that fasting attenuates the satiety effects of peptides such as S CCK-8, simmondsin and oleoylethanolamide in rats (Gallmann et al., 2006; Flo et al., 2000; Gaetani et al., 2003). To explain these results one study found that leptin deficiency induced by fasting attenuates 30 minutes food intake suppression by CCK, an effect that was reversed by leptin replacement (McMinn et al., 2000).

Therefore, unlike the previous work, the conditions of the current work provided a more suitable experimental environment to reveal the satiety effects of NS CCK-8 in rats.

Third, Kader et al. measured cumulative 30-min food intake during the light cycle whereas the current work determined seven feeding behaviors by NS CCK-8 including the two components that comprise short-term control of intake, MS and IMI length (Sayegh, 2013a; Sayegh, 2013b; Strubbe & Woods, 2004). This study represents a more detailed analysis of the feeding responses evoked by NS CCK-8 in the rat. Here, we measured the min-to-min feeding behavior of the animal by an automated system during the dark and light cycles. Rats not only are nocturnal feeders (Kraly et al., 1980), but also it has been suggested that CCK is more sensitive during the dark cycle, possibly due to CCK/ leptin interaction, which is more intense during the night (Merino et al., 2008). Therefore, the current study represents a more physiologically relevant analysis of the role of NS CCK-8 in the short-term control of food intake in rats.

Finally, the current study showed that NS CCK-8 reduces food intake by activating CCK-BR. In the literature, it has been shown that food intake reduction by S CCK-8 is mediated by CCK-AR (Corp et al., 1997; Moran & Bi, 2006). The CCK-BR may have a role in controlling food intake by NS CCK. For example, CCK-BR knockout mice showed increased gastric emptying and overexpression of ghrelin receptors, which may suggest an inhibitory role for CCK-BR in feeding (Miyasaka et al., 2004). Furthermore, Clerc et al., reported that CCK-BR knockout mice showed significant increase in body weight and food intake. In addition, intracerebroventricular injection of gastrin into control mice showed that hypothalamic CCK-BR mediates inhibition of food intake (Clerc et al., 2007). This receptor is widely expressed in the gut (Noble & Roques, 1999), vagus nerve, brain stem (AP, NTS, and DVC) and hypothalamic areas that control feeding (Moriarty et al., 1997; Broberger et al., 2001). The current study demonstrated that NS CCK-8 reduces food intake by reducing meal size and prolonging the IMI through activating CCK-BR receptors. On the other hand, earlier studies have shown that CCK-BR antagonist is not involved in reduction of food intake by S CCK (Corwin et al., 1991). Furthermore, the current finding that L365,260, a specific and potent CCK-BR antagonist, failed to alter food intake under this experimental design provides further evidence that NS CCK-8 has a role in the short-term control of food

intake. Non-sulfated CCK-8 reduces food intake by activating CCK-BR and not CCK-AR. The current work did not test other CCK-BR antagonists.

Reduction of food intake by NS CCK-8 may be regulated at a central or a peripheral site because CCK-BR is expressed both centrally (Broberger et al., 2001) and peripherally (Reubi et al., 1997; Monstein et al., 1996). However, although a central site of action, regulating reduction of food intake by NS CCK-8 is plausible; three findings support a peripheral site of action for this peptide. First, NS CCK-8 does not cross the blood brain barrier (Sugeta et al., 2015; Hagino et al., 1989). Second, we have shown that subdiaphragmatic vagotomy and celiacomesenteric ganglionectomy, individually, attenuated reduction of food intake by ip NS CCK-8 (Dafalla et al., 2018, in review). The vagus and the splanchnic nerves form the neuronal connections between the gut and the brain (Altschuler et al., 1993; Quinson et al., 2001; Isomura et al., 1985). Third, NS CCK-8 increased Fos-like immunoreactivity (Fos-LI, metabolic neuronal activation marker) in the enteric neurons and in the feeding areas of the dorsal vagal complex of the hindbrain (Dafalla et al., 2018, in review). As such, the data suggest that NS CCK-8 reduces food intake by a peripheral site of action. Further studies are required to determine the specific peripheral site of action that regulates reduction of food intake by NS CCK-8. For example, through intra-arterial infusions of various gut satiety peptides our laboratory demonstrated that the gastrointestinal tract contains sites of action regulating food intake reduction by S CCK-8, sulfated CCK-58, glucagon like peptide-1 and gastrin releasing peptide-29 (Sayegh et al., 2015; Williams et al., 2016; Washington et al., 2016; Washington et al., 2014). Similar experiments are needed to demonstrate a peripheral site of action regulating reduction of food intake by NS CCK-8.

Finally, a central site of action for NS CCK-8 is plausible for other physiological responses evoked by this peptide e.g. anxiety (Derrien et al., 1994; Costall et al., 1991; Singh et al., 1991) and stimulation of gastric acid secretion (Prinz et al., 1993; Sandvik & Waldum, 1991). However, as mentioned earlier the current study is the first systematic study in the literature that shows a satiety effect by NS CCK-8.

Furthermore, the insulintropic and the glucagonotropic effect of NS CCK-8 (Hermansen, 1984; Jensen et al., 1981) may have contributed to the reduction of food intake by this peptide shown in the current study. It is known that insulin (Schwartz et al., 1991; Woods & Gibbs, 1989) and glucagon (Geary, 1990) reduce food intake (Anika et al., 1980; Vanderweele, 1982). In addition, both peptides interact with CCK (Vanderweele, 1982; Fehmann et al., 1990). As such, this possibility is valid; however, it requires further testing.

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References

- Akimoto S, Miyasaka K, 2010 Age-associated changes of appetite-regulating peptides. *Geriatr Gerontol Int* 10 (Suppl. 1), S107–S119. [PubMed: 20590826]
- Altschuler SM, et al., 1993 The central organization of the vagus nerve innervating the colon of the rat. *Gastroenterology* 104 (2), 502–509. [PubMed: 8425692]

- Anika SM, 1983 Ontogeny of cholecystokinin satiety in rats. *Eur. J. Pharmacol* 89 (3–4), 211–215. [PubMed: 6873157]
- Anika SM, Houtp TR, Houtp KA, 1980 Insulin as a satiety hormone. *Physiol. Behav* 25 (1), 21–23. [PubMed: 6997904]
- Balasko M, et al., 2013 Age and nutritional state influence the effects of cholecystokinin on energy balance. *Exp. Gerontol* 48 (11), 1180–1188. [PubMed: 23876629]
- Beinfeld MC, 2003 Biosynthesis and processing of pro CCK: recent progress and future challenges. *Life Sci.* 72 (7), 747–757. [PubMed: 12479974]
- Belai A, Wheeler H, Burnstock G, 1995 Innervation of the rat gastrointestinal sphincters: changes during development and aging. *Int. J. Dev. Neurosci* 13 (2), 81–95. [PubMed: 7639099]
- Broberger C, et al., 2001 Expression and regulation of cholecystokinin and cholecystokinin receptors in rat nodose and dorsal root ganglia. *Brain Res.* 903 (1–2), 128–140. [PubMed: 11382396]
- Buffa R, Solcia E, Go VL, 1976 Immunohistochemical identification of the cholecystokinin cell in the intestinal mucosa. *Gastroenterology* 70 (4), 528–532. [PubMed: 767195]
- Chowdhury P, Rayford PL, 2001 Effect of food restriction on plasma cholecystokinin levels and exocrine pancreatic function in rats. *Ann. Clin. Lab. Sci* 31 (4), 376–382. [PubMed: 11688849]
- Clerc P, et al., 2007 Involvement of cholecystokinin 2 receptor in food intake regulation: hyperphagia and increased fat deposition in cholecystokinin 2 receptor-deficient mice. *Endocrinology* 148 (3), 1039–1049. [PubMed: 17122076]
- Corp ES, et al., 1997 The effect of centrally administered CCK-receptor antagonists on food intake in rats. *Physiol. Behav* 61 (6), 823–827. [PubMed: 9177553]
- Corwin RL, Gibbs J, Smith GP, 1991 Increased food intake after type a but not type B cholecystokinin receptor blockade. *Physiol. Behav* 50 (1), 255–258. [PubMed: 1946726]
- Costall B, et al., 1991 Anxiolytic effects of CCK-B antagonists. *Neuropeptides* 19 (Suppl), 65–73.
- Crawley JN, St-Pierre S, Gaudreau P, 1984 Analysis of the behavioral activity of C- and N-terminal fragments of cholecystokinin octapeptide. *J. Pharmacol. Exp. Ther* 230 (2), 438–444. [PubMed: 6086888]
- Debas HT, Farooq O, Grossman MI, 1975 Inhibition of gastric emptying is a physiological action of cholecystokinin. *Gastroenterology* 68 (5 Pt 1), 1211–1217. [PubMed: 1126597]
- Derrien M, et al., 1994 Heterogeneity of CCK-B receptors involved in animal models of anxiety. *Pharmacol. Biochem. Behav* 49 (1), 133–141. [PubMed: 7816864]
- Dockray GJ, et al., 1978 Isolation, structure and biological activity of two cholecystokinin octapeptides from sheep brain. *Nature* 274 (5672), 711–713. [PubMed: 79141]
- Dockray GJ, et al., 1981 Transport of cholecystokinin-octapeptide-like immunoreactivity toward the gut in afferent vagal fibres in cat and dog. *J. Physiol* 314, 501–511. [PubMed: 6273537]
- Faussone-Pellegrini MS, Matini P, Stach W, 1996 Differentiation of enteric plexuses and interstitial cells of Cajal in the rat gut during pre- and postnatal life. *Acta Anat. (Basel)* 155 (2), 113–125. [PubMed: 8828709]
- Fehmann HC, et al., 1990 Interaction of glucagon-like peptide-1 (7–36)amide and cholecystokinin-8 in the endocrine and exocrine rat pancreas. *Pancreas* 5 (3), 361–365. [PubMed: 1693001]
- Flo G, et al., 2000 The vagus nerve is involved in the anorexic effect of simmondsin in the rat. *Appetite* 34 (2), 147–151. [PubMed: 10744903]
- Gaetani S, Oveisi F, Piomelli D, 2003 Modulation of meal pattern in the rat by the anorexic lipid mediator oleoylethanolamide. *Neuropsychopharmacology* 28 (7), 1311–1316. [PubMed: 12700681]
- Gallmann E, et al., 2006 Effect of intraperitoneal CCK-8 on food intake and brain orexin-A after 48 h of fasting in the rat. *Regul. Pept* 133 (1–3), 139–146. [PubMed: 16271404]
- Geary N, 1990 Pancreatic glucagon signals postprandial satiety. *Neurosci. Biobehav. Rev* 14 (3), 323–338. [PubMed: 2234610]
- Gibbs J, Young RC, Smith GP, 1973 Cholecystokinin decreases food intake in rats. *J. Comp. Physiol. Psychol* 84 (3), 488–495. [PubMed: 4745816]
- Gutkind JS, et al., 1988 Increased concentration of angiotensin II binding sites in selected brain areas of spontaneously hypertensive rats. *J. Hypertens* 6 (1), 79–84. [PubMed: 3351297]

- Hagino Y, Moroji T, Iizuka R, 1989 A behavioural pharmacological study on intracerebroventricularly administered CCK-8 related peptides in mice. *Neuropeptides* 13 (2), 107–113. [PubMed: 2739882]
- Harper AA, Raper HS, 1943 Pancreozymin, a stimulant of the secretion of pancreatic enzymes in extracts of the small intestine. *J. Physiol* 102 (1), 115–125. [PubMed: 16991584]
- Hermansen K, 1984 Effects of cholecystokinin (CCK)-4, nonsulfated CCK-8, and sulfated CCK-8 on pancreatic somatostatin, insulin, and glucagon secretion in the dog: studies in vitro. *Endocrinology* 114 (5), 1770–1775. [PubMed: 6143659]
- Isomura G, et al., 1985 Constitution of the greater splanchnic nerve in the rat. *Anat. Anz* 159 (1–5), 159–171. [PubMed: 4096395]
- Ivy AC, Oldberg EA, 1928 A hormone mechanism for gallbladder contraction and evacuation. *Am. J. Phys* 86, 599–613.
- Jensen SL, et al., 1981 Effect of sulfation of CCK-8 on its stimulation of the endocrine and exocrine secretion from the isolated perfused porcine pancreas. *Digestion* 22 (6), 305–309. [PubMed: 6277714]
- Kadar T, et al., 1985 Depression of rat feeding in familiar and novel environment by sulfated and nonsulfated cholecystokinin octapeptide. *Physiol. Behav* 34 (3), 395–400. [PubMed: 4011720]
- Kerr FW, 1975 Neuroplasticity of primary afferents in the neo-natal cat and some results of early deafferentation of the trigeminal spinal nucleus. *J. Comp. Neurol* 163 (3), 305–327. [PubMed: 1176642]
- Koop I, et al., 1987 Effect of food deprivation on the function of the intestinal cholecystokinin-producing cell in the rat. *Digestion* 38 (2), 114–123. [PubMed: 3440506]
- Kraly FS, Cushin BJ, Smith GP, 1980 Nocturnal hyperphagia in the rat is characterized by decreased postprandial satiety. *J. Comp. Physiol. Psychol* 94 (2), 375–387. [PubMed: 7365007]
- Kuehl-Kovarik MC, Jacobson CD, 1996 Ontogeny of cholecystokinin binding sites in the hindbrain of the laboratory rat. *Brain Behav. Evol* 47 (5), 246–256. [PubMed: 8724646]
- Larsson LI, Rehfeld JF, 1978 Distribution of gastrin and CCK cells in the rat gastrointestinal tract. Evidence for the occurrence of three distinct cell types storing COOH-terminal gastrin immunoreactivity. *Histochemistry* 58 (1–2), 23–31. [PubMed: 365836]
- Liddle RA, et al., 1985 Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction. *J. Clin. Invest* 75 (4), 1144–1152. [PubMed: 2580857]
- MacLean DB, 1985 Abrogation of peripheral cholecystokinin-satiety in the capsaicin treated rat. *Regul. Pept* 11 (4), 321–333. [PubMed: 2413505]
- Mantyh CR, Pappas TN, Vigna SR, 1994 Localization of cholecystokinin A and cholecystokinin B/gastrin receptors in the canine upper gastrointestinal tract. *Gastroenterology* 107 (4), 1019–1030. [PubMed: 7926455]
- McMinn JE, et al., 2000 Leptin deficiency induced by fasting impairs the satiety response to cholecystokinin. *Endocrinology* 141 (12), 4442–4448. [PubMed: 11108253]
- Merino B, et al., 2008 Leptin-mediated hypothalamic pathway of cholecystokinin (CCK-8) to regulate body weight in free-feeding rats. *Endocrinology* 149 (4), 1994–2000. [PubMed: 18096657]
- Miyasaka K, et al., 2004 Enhanced gastric emptying of a liquid gastric load in mice lacking cholecystokinin-B receptor: a study of CCK-A,B, and AB receptor gene knockout mice. *J. Gastroenterol* 39 (4), 319–323. [PubMed: 15168241]
- Monnikes H, Lauer G, Arnold R, 1997 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.* 770 (1–2), 277–288. [PubMed: 9372230]
- Monnikes H, et al., 1997 Pathways of Fos expression in locus coeruleus, dorsal vagal complex, and PVN in response to intestinal lipid. *Am. J. Phys* 273 (6 Pt 2), R2059–R2071.
- Monstein HJ, et al., 1996 Cholecystokinin-a and cholecystokinin-B/gastrin receptor mRNA expression in the gastrointestinal tract and pancreas of the rat and man. A polymerase chain reaction study. *Scand. J. Gastroenterol* 31 (4), 383–390. [PubMed: 8726308]
- Moran TH, Bi S, 2006 Hyperphagia and obesity in OLETF rats lacking CCK-1 receptors. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci* 361 (1471), 1211–1218. [PubMed: 16815799]

- Moriarty P, et al., 1997 Characterization of cholecystokininA and cholecystokininB receptors expressed by vagal afferent neurons. *Neuroscience* 79 (3), 905–913. [PubMed: 9219953]
- Noble F, Roques BP, 1999 CCK-B receptor: chemistry, molecular biology, biochemistry and pharmacology. *Prog. Neurobiol* 58 (4), 349–379. [PubMed: 10368033]
- O'Shea RD, Gundlach AL, 1993 Regulation of cholecystokinin receptors in the hypothalamus of the rat: reciprocal changes in magnocellular nuclei induced by food deprivation and dehydration. *J. Neuroendocrinol* 5 (6), 697–704. [PubMed: 8680444]
- Polak JM, et al., 1976 Proceedings: simultaneous differentiation of gastrin, CCK, *EG and VIP cells*. *Gut* 17 (5), 392–393.
- Powley TL, et al., 2001 Gastrointestinal projection maps of the vagus nerve are specified permanently in the perinatal period. *Brain Res. Dev. Brain Res* 129 (1), 57–72. [PubMed: 11454413]
- Prinz C, et al., 1993 Histamine secretion from rat enterochromaffinlike cells. *Gastroenterology* 105 (2), 449–461. [PubMed: 7687574]
- Quinson N, et al., 2001 Locations and innervation of cell bodies of sympathetic neurons projecting to the gastrointestinal tract in the rat. *Arch. Histol. Cytol* 64 (3), 281–294. [PubMed: 11575424]
- Rehfeld JF, 1971 Effect of gastrin and its C-terminal tetrapeptide on insulin secretion in man. *Acta Endocrinol.* 66 (1), 169–176. [PubMed: 5107421]
- Rehfeld JF, 1978 Immunochemical studies on cholecystokinin. II. Distribution and molecular heterogeneity in the central nervous system and small intestine of man and hog. *J. Biol. Chem* 253 (11), 4022–4030. [PubMed: 649618]
- Rehfeld JF, 2017 Cholecystokinin-from local Gut hormone to ubiquitous messenger. *Front. Endocrinol (Lausanne)* 8, 47.
- Rehfeld JF, et al., 1980 Neural regulation of pancreatic hormone secretion by the C-terminal tetrapeptide of CCK. *Nature* 284 (5751), 33–38. [PubMed: 6101907]
- Rehfeld JF, et al., 1990 Non-sulphated cholecystokinin in human medullary thyroid carcinomas. *J. Endocrinol* 124 (3), 501–506. [PubMed: 2332719]
- Reubi JC, et al., 1997 Localization of cholecystokinin A and cholecystokinin B-gastrin receptors in the human stomach. *Gastroenterology* 112 (4), 1197–1205. [PubMed: 9098003]
- Salorio CF, et al., 1994 Age-dependent effects of CCK and devazepide in male and female rats. *Physiol. Behav* 56 (4), 645–648. [PubMed: 7800726]
- Sandvik AK, Waldum HL, 1991 CCK-B (gastrin) receptor regulates gastric histamine release and acid secretion. *Am. J. Phys* 260 (6 Pt 1), G925–G928.
- Sayegh AI, 2013a The role of cholecystokinin receptors in the short-term control of food intake. *Prog. Mol. Biol. Transl. Sci* 114, 277–316. [PubMed: 23317788]
- Sayegh AI, 2013b The role of bombesin and bombesin-related peptides in the shortterm control of food intake. *Prog. Mol. Biol. Transl. Sci* 114, 343–370. [PubMed: 23317790]
- Sayegh AI, et al., 2015 Celiac and the cranial mesenteric arteries supply gastrointestinal sites that regulate meal size and intermeal interval length via cholecystokinin-58 in male rats. *Horm. Behav* 67, 48–53. [PubMed: 25479193]
- Schafer KH, Hansgen A, Mestres P, 1999 Morphological changes of the myenteric plexus during early postnatal development of the rat. *Anat. Rec* 256 (1), 20–28. [PubMed: 10456982]
- Schwartz MW, et al., 1991 Central insulin administration reduces neuropeptide Y mRNA expression in the arcuate nucleus of food-deprived lean (Fa/Fa) but not obese (fa/fa) Zucker rats. *Endocrinology* 128 (5), 2645–2647. [PubMed: 2019270]
- Singh L, et al., 1991 Evidence for an involvement of the brain cholecystokinin B receptor in anxiety. *Proc. Natl. Acad. Sci. U. S. A* 88 (4), 1130–1133. [PubMed: 1996314]
- Strubbe JH and Woods SC, The timing of meals. *Psychol. Rev*, 2004 111(1): p. 128–41. [PubMed: 14756590]
- Sugeta S, et al., 2015 Presynaptically mediated effects of cholecystokinin-8 on the excitability of area postrema neurons in rat brain slices. *Brain Res.* 1618, 83–90. [PubMed: 26005131]
- Tolosa JN, et al., 2005 Ontogeny of retrograde labeled chemoafferent neurons in the newborn rat nodose-petrosal ganglion complex: an ex vivo preparation. *Neurosci. Lett* 384 (1–2), 48–53. [PubMed: 15896903]

- Vanderweele DA, 1982 CCK, endogenous insulin condition and satiety in free-fed rats. *Physiol. Behav* 29 (5), 961–964. [PubMed: 6760223]
- Voigt JP, et al., 1996 Effects of cholecystokinin octapeptide (CCK-8) on food intake in adult and aged rats under different feeding conditions. *Peptides* 17 (8), 1313–1315. [PubMed: 8971925]
- Wank SA, Pisegna JR, de Weerth A, 1994 Cholecystokinin receptor family. Molecular cloning, structure, and functional expression in rat, guinea pig, and human. *Ann. N. Y. Acad. Sci* 713, 49–66. [PubMed: 8185215]
- Washington MC, Aglan AH, Sayegh AI, 2014 The stomach and/or upper duodenum contain sites of action that control meal size and intermeal interval length by exogenous rat gastrin releasing peptide. *Peptides* 55, 41–46. [PubMed: 24556509]
- Washington MC, Mhalhal TR, Sayegh AI, 2016 Cholecystokinin-33, but not cholecystokinin-8 shows gastrointestinal site specificity in regulating feeding behaviors in male rats. *Horm. Behav* 85, 36–42. [PubMed: 27497934]
- Weller A, 2006 The ontogeny of postingestive inhibitory stimuli: examining the role of CCK. *Dev. Psychobiol* 48 (5), 368–379. [PubMed: 16770766]
- Williams KE, et al., 2016 Exogenous glucagon-like peptide-1 acts in sites supplied by the cranial mesenteric artery to reduce meal size and prolong the intermeal interval in rats. *Appetite* 96, 254–259. [PubMed: 26431682]
- Woods SC, Gibbs J, 1989 The regulation of food intake by peptides. *Ann. N. Y. Acad. Sci* 575, 236–243. [PubMed: 2699189]
- Zheng B, Eng J, Yalow RS, 1987 Brain/gut peptides in fed and fasted rats. *Endocrinology* 120 (2), 714–717. [PubMed: 3803300]

Fig. 1. Effect of non-sulfated cholecystokinin-8 on first meal size, intermeal interval length and satiety ratio during the light and dark cycles.

Intraperitoneal injections of non-sulfated cholecystokinin-8 (NS CCK-8, 0.5, 1, 3, 5 and 10 nmol/kg) were given prior to the onset of the dark cycle to nearly free fed rats ($n = 12$) and first meal size (MS), length of intermeal interval (IMI) and satiety ratio ($SR = IMI/MS$) were measured. In the dark cycle, NS CCK-8 (0.5 and 1 nmol/kg) reduced MS ($p = .01$ and $p = .01$), increased IMI length ($p = .03$ and $p = .02$) and increased SR relative to saline control ($p = .002$ and $p = .03$). *denotes significance relative to saline control, $p < .05$.

Fig. 2. Effect of non-sulfated cholecystokinin-8 on second meal size, latency to first meal and duration of first meal during the light and dark cycles.

Intraperitoneal injections of non-sulfated cholecystokinin-8 (NS CCK-8, 0.5, 1, 3, 5 and 10 nmol/kg) were given prior to the onset of the dark cycle in nearly free fed rats ($n = 12$) and second meal size, latency to first meal and duration of first meal were measured. Non-sulfated CCK-8 failed to affect these responses relative to saline vehicle during both cycles.

Fig. 3. Effect of non-sulfated cholecystinin-8 on total intake and number of meals during the light and dark cycles.

Intraperitoneal injections of NS CCK-8 (0, 0.5, 1, 3, 5 and 10 nmol/kg) were given prior to the onset of the dark cycle to nearly free fed rats ($n = 12$) and total (24-h) intake and number of meals were determined. Non-sulfated CCK-8 (0.5 nmol/kg) reduced total intake relative to saline control ($p = .004$) but failed to affect the number of meals. *denotes significance relative to saline control ($p < .05$).

Fig. 4. Effect of cholecystokinin A and B receptors blockade on first meal size reduction, intermeal interval prolongation and increased satiety ratio in response to non-sulfated cholecystokinin-8 during the light and dark cycles.

Prior to the onset of the dark cycle, each free fed adult male rat ($n = 12$) received two intraperitoneal (ip) injections, 10 min apart. The first injection was L365,260, an antagonist for the cholecystokinin (CCK) B receptor (CCK-BR, 1 mg/kg), devazepide, a CCK-AR (1 mg/kg) or a vehicle (0.1 M phosphate buffered saline). The second injection consisted of non-sulfated CCK-8 (0.5 nmol/kg) or saline vehicle. Following the second injection, first meal size (MS), intermeal interval (IMI) and satiety ratio (SR, IMI/MS) were measured. Veh/cck significantly reduced the first MS relative to veh/sal ($p = .03$) and l365/cck attenuated this effect ($p = .05$), veh/cck increased the IMI relative to veh/sal ($p = .03$) and l365/cck attenuated this effect ($p = .04$) and veh/cck increased the SR relative to veh/sal (p

= .05). * denotes significant difference relative to veh/sal control, † denotes significance difference relative to l365/cck, $p < .05$).

Abbreviations: vehicle/saline (veh/sal), vehicle/non-sulfated cholecystokinin-8 (veh/cck), devazepide/saline (dev/sal), devazepide/non-sulfated cholecystokinin-8 (dev/cck), L365,260/saline (l365/sal) and L365,260/non-sulfated cholecystokinin-8 (l365/cck)

Fig. 5. Effect of cholecystokinin A and B receptors blockade on second meal size, latency to first meal and duration of first meal in response to non-sulfated cholecystokinin-8 during the light and dark cycles.

Prior to the onset of the dark cycle, each free fed adult male rat ($n = 12$) received two intraperitoneal (ip) injections, ten minutes apart. The first injection was L365,260, an antagonist for the cholecystokinin (CCK) B receptor (CCK-BR, 1 mg/kg), devazepide, a CCK-AR (1 mg/kg) or a vehicle. The second injection consisted of non-sulfated CCK-8 (0.5 nmol/kg) or saline vehicle. Following the second injection second meal size, latency to first meal and duration of first meal were measured. Veh/cck failed to reduce the second meal size, increase the latency to the first meal and duration of the first meal relative to veh/sal ($p = .3$).

Abbreviations: vehicle/saline (veh/sal), vehicle/non-sulfated cholecystokinin-8 (veh/cck), devazepide/saline (dev/sal), devazepide/ non-sulfated cholecystokinin-8 (dev/cck), L365,260/saline (l365/sal) and L365,260/ non-sulfated cholecystokinin-8 (l365/cck)

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Fig. 6. Effect of cholecystokinin A and B receptors blockade on total food intake and the number of meals in response to non-sulfated cholecystokinin-8 during the light and dark cycles.

Prior to the onset of the dark cycle, each free fed adult male rat ($n = 12$) received two intraperitoneal (ip) injections, ten minutes apart. The first injection was L365,260, an antagonist for the cholecystokinin (CCK) B receptor (CCK-BR, 1 mg/kg), devazepide, a CCK-AR (1 mg/kg) or a vehicle. The second injection consisted of non-sulfated CCK-8 (0.5 nmol/kg) or saline vehicle. Following the second injection total food intake and number of meals were measured. Veh/cck failed to reduce the total food intake and number of meals relative to veh/sal ($p = .1$).

Abbreviations: vehicle/saline (veh/sal), vehicle/non-sulfated cholecystokinin-8 (veh/cck), devazepide/saline (dev/sal), devazepide/ non-sulfated cholecystokinin-8 (dev/cck), L365,260/saline (l365/sal) and L365,260/ non-sulfated cholecystokinin-8 (l365/cck)