CCK enhances response to gastric distension by acting on capsaicin-insensitive vagal afferents

E. H. E. M. van de Wall, P. Duffy, and R. C. Ritter

1University of Groningen, Haren, The Netherlands; and 2Programs in Neuroscience and Department of Veterinary and Comparative Anatomy, Pharmacology, and Physiology, Washington State University, Pullman, Washington

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van de Wall, E. H. E. M., P. Duffy, and R. C. Ritter. CCK enhances response to gastric distension by acting on capsaicin-insensitive vagal afferents. Am J Physiol Regul Integr Comp Physiol 289: R695–R703, 2005. First published May 19, 2005; doi:10.1152/ajpregu.00809.2004.—Capsaicin treatment destroys vagal afferent C fibers and markedly attenuates reduction of food intake and induction of hindbrain Fos expression by CCK. However, both anatomical and electrophysiological data indicate that some gastric vagal afferents are not destroyed by capsaicin. Because CCK enhances behavioral and electrophysiological responses to gastric distension in rats and people, we hypothesized that CCK might enhance the vagal afferent response to gastric distension via an action on capsaicin-insensitive vagal afferents. To test this hypothesis, we quantified expression of Fos-like immunoreactivity (Fos) in the dorsal vagal complex (DVC) of capsaicin-treated (Cap) and control rats (Veh), following gastric balloon distension alone and in combination with CCK injection. In Veh rats, intraperitoneal CCK significantly increased DVC Fos, especially in nucleus of the solitary tract (NTS), whereas in Cap rats, CCK did not significantly increase DVC Fos. In contrast to CCK, gastric distension did significantly increase Fos expression in the NTS of both Veh and Cap rats, although distension-induced Fos was attenuated in Cap rats. When CCK was administered during gastric distension, it significantly enhanced NTS Fos expression in response to distension in Cap rats. Furthermore, CCK’s enhancement of distension-induced Fos in Cap rats was reversed by the selective CCK-A receptor antagonist lorglumide. We conclude that CCK directly activates capsaicin-sensitive C-type vagal afferents. However, in capsaicin-resistant A-type afferents, CCK’s principal action may be facilitation of responses to gastric distension.

Fos; dorsal vagal complex; C fibers; A fibers

VAGAL AFFERENT NEURONS THAT detect gastric distension play an important role in the process of satiation for food and subsequent meal termination (for review, see Ref. 28). However, during ingestion of a normal meal, gastric distension does not occur in the absence of postgastric signals. Rather, gastric distension, intestinal nutrients, and gut hormones all are potential contributors to vagal afferent activation. Therefore, vagal afferents constitute a potential location for integration and modulation of these multiple meal-related signals. Indeed, interactions between gastric distension and other gastrointestinal stimuli have been reported. For example, the gut hormone CCK has been reported to sensitize gastric and duodenal mechanosensitive vagal afferents (7, 8). Likewise, CCK and gastric distension appear to interact to reduce food intake in a variety of species, including rats, monkeys, and humans (14, 15, 17, 18, 38). Thus there is compelling evidence for cooperation between gastric distension and postgastric signals, such as CCK, in vagal activation and reduction of food intake.

Capsaicin has been used successfully as a tool to study the involvement of primary C-type vagal afferents (31) in food intake. Although the vagus consists mainly of small unmyelinated C-type afferents (25), Berthoud et al. (3) reported that a substantial number of intraganglionic laminar endings (IGLEs) and vagal intramuscular arrays in the stomach survive capsaicin treatment, suggesting that at least some gastric mechanoreceptive afferents are A-type afferents. Results of other experiments that used capsaicin support the hypothesis that gastric distension and CCK acted via distinct populations of vagal afferents. For example, CCK-induced reduction of food intake is attenuated in capsaicin-treated (Cap) rats (29, 30, 41), whereas reduction of food intake in response to gastric loading is not attenuated (29). Many investigators have reported that CCK-induced expression of Fos immunoreactivity (Fos) in the hindbrain is attenuated by capsaicin treatment (10, 20, 34), whereas Berthoud et al. (3) reported that gastric balloon distension-induced Fos in the dorsal vagal complex is not significantly decreased in Cap rats. Finally, Ritter et al. (30) reported that hindbrain extracellular responses to near-celiac artery infusion of CCK were abolished in Cap rats, whereas responses to gastric distension were still present. These data suggest that CCK’s action may be limited to activation of capsaicin-sensitive C-type vagal afferents. They also suggest that at least some of the distension-sensitive gastric afferents are capsaicin-insensitive A-type neurons. If the modalities of CCK sensitivity and gastric distension sensitivity are confined to separate and distinct populations of afferents, then cooperation between these two satiation signals must be integrated in the brain.

Although it seems clear that CCK and mechanical stimuli can exert synergistic or cooperative effects on the control of food intake, the neural substrates for the interaction of distension and CCK are incompletely appreciated. Richards et al. (27) found that administration of the CCK-A receptor antagonist MK-329 blocked excitation of vagal afferents by CCK. However, this population of afferents was not excited by distension of the intestine. Furthermore, in afferents that were excited by distension, the CCK-A receptor antagonist did not attenuate distension-induced firing. In contrast to the Richards et al. result, Davison and Clarke (7) and others (4, 26) reported that afferents excited by gastric distension also fire in response to CCK. Unfortunately, these studies do not rule out the possibility that CCK-induced firing is secondary to CCK-induced changes in smooth muscle tone in the viscera. Thus,
Although CCK and gastric distension might coactivate mechanoreceptive vagal afferents, it is also possible that CCK-sensitive and mechanosensitive afferents represent distinct populations and that interaction between these modalities occurs entirely by synaptic convergence in the hindbrain.

Recently, however, Simasko and Ritter (40), working with primary cultures of vagal afferent neurons, reported that CCK excites subpopulations of both capsaicin-sensitive C-type afferents and capsaicin-insensitive A-type afferents. Simasko and Ritter did not attempt to determine whether CCK-sensitive A-type vagal afferents innervated the stomach. Nevertheless, because CCK enhances behavioral and in vivo responses to gastric distension in rats and people, we hypothesized that CCK might enhance the vagal afferent response to gastric distension via actions involving capsaicin-insensitive vagal afferents. To test this hypothesis, we quantified expression of c-Fos (Fos) in the dorsal vagal complex (DVC) of Cap and control [vehicle-treated (Veh)] rats following gastric balloon distension alone and in combination with CCK injection. We found that Fos expression after exogenous CCK administration alone was abolished in Cap rats, suggesting that it depended on C-type vagal afferents. We also found that CCK enhances Fos expression in response to gastric distension even in Cap rats, where CCK alone did not significantly increase vagal afferent activation. The effect of CCK, but not gastric distension, was reversed by administration of the selective CCK-A receptor antagonist lorglumide. Together with previous observations, our results suggest that CCK influences vagal afferent activity in two different ways. First, CCK directly activates capsaicin-sensitive C-type afferents. Second, it enhances the activity of distension-sensitive A-type afferents, which may not ordinarily be excited by CCK on its own.

**METHODS**

**Animals.** All experiments were approved by the Animal Care and Use Committee. Male Sprague-Dawley rats (Simonsen) weighing between 280 and 380 g were subjects for these experiments. The rats were housed individually in a climate-controlled room under a 12:12-h light-dark cycle (lights on at 7:00 AM). Experiments were performed between 0800 and 1200. Food and water were available ad libitum except that animals were food deprived overnight before capsaiacin treatment, prior to surgery, and prior to experiments.

**Capsaicin treatment.** Rats were treated with increasing doses of capsaiacin (90% grade; Sigma, St. Louis, MO) to destroy a specific population of vagal afferent sensory fibers as previously described by Yox and Ritter (45). Capsaicin was dissolved in a vehicle consisting of Tween 80 (10%), ethanol (10%), and 0.9% NaCl (80%). A series of three capsaiacin doses were injected intraperitoneally over a 36-h period. The first dose was 25 mg/kg, and the second and third doses were 50 mg/kg. Controls were injected intraperitoneally with the vehicle solution only, and the vehicle injections were made according to the same schedule and under the same conditions as capsaiacin injections. All injections of capsaiacin or vehicle were made under isoflurane inhalation anesthesia. Ten minutes before the first capsaiacin or vehicle injection of a series, rats received an 0.2-ml ip injection of atropine sulfate (15 mg/ml). Immediately after the first and/or second capsaiacin injections, rats exhibited cutaneous vasodilatation and respiratory arrest, as described by Ritter and Ladenheim (29). During respiratory arrest, the anesthesia was maintained while the rats were artificially ventilated until spontaneous breathing resumed, usually within 15–20 min. After resumption of spontaneous breathing, the rats were permitted to arouse from anesthesia over the ensuing 5–10 min. Vehicle injections never were associated with either vasodilatation or respiratory arrest. The rats were allowed 2 wk between capsaiacin or vehicle treatment and surgery to implant a stainless steel gastric cannula (see below). During this period, both Cap and Veh animals gained weight normally, and there was no significant difference gained between treatment groups at the time of surgery. Efficacy of the capsaiacin treatment was assessed by testing the corneal chemosensory response (30, 32). This test involved application of 1% NH4OH into the eye. Veh animals immediately exhibited two to five paw wipes of the affected eye, whereas Cap animals did not exhibit any response to the application of 1% NH4OH. Absence of the corneal chemosensory response indicated that the small, unmyelinated afferent fibers of the trigeminal nerve, and presumably all of the other small unmyelinated primary afferents, were successfully destroyed by capsaiacin. All of our Veh rats and none of our Cap animals responded to this test, confirming the efficacy of capsaiacin treatment and the inefficacy of the vehicle treatment.

**Surgery.** Each rat was implanted with stainless steel gastric cannula under isoflurane anesthesia as previously described by Yox and Ritter (45). Briefly, a ventral midline laparotomy incision was made, and the stomach was located and isolated. A pericorporeal incision was made in the nonglandular part of the stomach at the greater curvature, using an 18-gauge hypodermic needle. This incision was stretched slightly, and a gastric cannula was inserted through the incision. A tobacco-pouch stitch was sewn through the gastric serosa and around the cannula. A patch of surgical polypropylene mesh was also placed around the fistula to promote connective tissue growth between the gastric serosa and the parietal peritoneum. The exterior end of the cannula was exteriorized through the left ventral paramedian incision, and the cannula was occluded with a stainless steel screw except during experimental procedures or habituation training. Rats were allowed to recover from cannula implantation surgery for at least 10 days before training or experimental procedures.

**Experimental procedures.** Rats were habituated to the experimental setting and procedure daily for a minimum of 1 wk before the experiment to reduce background induction of Fos due to stress or novelty. Briefly, each rat was handled, the fistula screw was removed, and the rat was connected to tubing used for inflation of a gastric balloon during experiments. The rat was then placed in the experimental chamber for 40 min. After at least 1 wk of habituation, rats were food deprived overnight before the experiment. In the morning, each rat was subjected to one of the following conditions: control treatment, gastric distension alone, CCK alone, gastric distension in combination with intraperitoneal CCK octapetide injection (4 μg/kg, Peptides International, Louisville, KY, dissolved in 0.9% NaCl and injected intraperitoneally), distension + CCK + intraperitoneal lorglumide or distension + saline + intraperitoneal lorglumide. The CCK-A antagonist lorglumide (Sigma) was dissolved in 0.9% NaCl and administered at a dose of 300 μg/kg ip. Gastric distension was produced by inserting a modified no. 10 Foley catheter through the open gastric cannula and inflating the catheter tip balloon with either 2 or 5 ml of water at 37°C. Balloon inflation was commenced immediately after saline or CCK injection and sustained for 90 min, at which time the animal was anesthetized and perfused. For the purpose of data reduction and statistical analyses, we established nine experimental groups of vehicle- and Cap rats as follows: 1) control: intraperitoneal saline injection and balloon tubing attached without inflation (n = 9 Veh and n = 9 Cap rats); 2) CCK: intraperitoneal CCK injection and balloon tubing attached without inflation (n = 7 Veh and n = 8 Cap rats); 3) gastric distension (2 ml): intraperitoneal saline injection with balloon inflated to 2 ml (n = 7 Veh and n = 9 Cap rats); 4) CCK-gastric distension (2 ml): intraperitoneal CCK injection with balloon inflated to 2 ml (n = 4 Veh and n = 6 Cap rats); 5) gastric distension (5 ml): intraperitoneal saline injection with balloon inflated to 5 ml (n = 6 Veh and n = 6 Cap rats); 6) CCK-gastric distension (5 ml): intraperitoneal CCK injection with balloon inflated to 5 ml (n = 3 Veh and n = 3 Cap rats); 7) lorglumide-CCK: intraperitoneal lorglumide, intraperitoneal CCK, and CCK-A antagonist lorglumide.
and balloon tubing attached without inflation (n = 7 Cap rats); 8)
lorglumide-gastric distension (2 ml): intraperitoneal lorglumide, intra-
peritoneal saline, and balloon inflated to 2 ml (n = 8 Cap rats); and 9)
lorglumide-CCK-gastric distension (2 ml): intraperitoneal lorglumide, intra-
peritoneal CCK, and balloon inflated to 2 ml (n = 7 Cap rats).

Nine minutes after injection of NaCl or CCK, the rats were deeply anesthetized (100 mg/kg pentobarbital sodium) and intracar-
dially perfused with 0.1 M sodium phosphate buffer, pH 7.4, followed
by 4% formaldehyde in 0.1 M sodium phosphate buffer, pH 7.4. Brains
were removed and postfixed for 5 h in 4% formaldehyde. Subsequently, the brains were stored in 25% sucrose overnight to

cryoprotect them and reduce freezing artifact. Thirty-micrometer sections were cut from the hindbrain in a microtome. The sections
were incubated for 24 h at room temperature in primary antiserum (1:50 000) raised in rabbit against a synthetic peptide representing
amino acids 4–17 of human Fos. After washing and subsequent
overnight incubation in a biotinylated donkey anti-rabbit serum (1:
50 000), sections were incubated in avidin conjugated to horseradish
peroxidase. Thereafter, the sections were washed and processed to
devise horseradish peroxidase activity using diaminobenzidine inten-
sified with nickel as previously described by Ritter and Dinh (32).

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Subsequently, the brains were stored in 25% sucrose overnight to

Table 1. Effect of distension alone and in combination with CCK on expression of Fos immunoreactivity in the DVC

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group</th>
<th>NTS</th>
<th>AP</th>
<th>AsP</th>
<th>DMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Vehicle</td>
<td>8.0±1.8</td>
<td>7.1±1.5</td>
<td>3.1±0.7</td>
<td>0.9±0.4</td>
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<td></td>
<td>Capsaicin</td>
<td>5.3±1.7</td>
<td>7.4±1.3</td>
<td>3.9±1.0</td>
<td>0.7±0.2</td>
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<td>CCK</td>
<td>Vehicle</td>
<td>91.8±13.5†</td>
<td>46.6±8.6‡</td>
<td>24.1±6.0*</td>
<td>8.6±1.6</td>
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<tr>
<td></td>
<td>Capsaicin</td>
<td>19.6±5.6¹</td>
<td>13.0±3.1¹</td>
<td>9.1±2.2*</td>
<td>1.9±0.5*</td>
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<tr>
<td>2-ml Distension</td>
<td>Vehicle</td>
<td>86.4±16.4†</td>
<td>38.1±10.5*</td>
<td>19.6±7.4</td>
<td>13.2±1.7</td>
</tr>
<tr>
<td></td>
<td>Capsaicin</td>
<td>29.8±7.9,¹</td>
<td>24.2±2.6*</td>
<td>13.4±5.3</td>
<td>2.7±0.8</td>
</tr>
<tr>
<td>2-ml Distension + CCK</td>
<td>Vehicle</td>
<td>127.7±53.4</td>
<td>50.9±16.2†</td>
<td>30.4±8.6†</td>
<td>19.6±7.6</td>
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<tr>
<td></td>
<td>Capsaicin</td>
<td>76.4±1.6,¹@</td>
<td>39.0±6.8‡</td>
<td>24.4±4.5‡</td>
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</tr>
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<td>5-ml Distension</td>
<td>Vehicle</td>
<td>144.5±19.0†</td>
<td>45.9±9.4‡</td>
<td>38.2±6.8</td>
<td>20.9±3.0</td>
</tr>
<tr>
<td></td>
<td>Capsaicin</td>
<td>102.7±6.8‡</td>
<td>34.8±9.0‡</td>
<td>22.4±5.0‡</td>
<td>15.2±3.4</td>
</tr>
<tr>
<td>5-ml Distension + CCK</td>
<td>Vehicle</td>
<td>254.7±41.2‡</td>
<td>100.5±15.1‡</td>
<td>50.7±7.5‡</td>
<td>34.3±5.3‡</td>
</tr>
<tr>
<td></td>
<td>Capsaicin</td>
<td>182.6±12.8,¹@</td>
<td>67.7±3.1,§</td>
<td>49.7±5.6,@</td>
<td>22.9±4.1¹</td>
</tr>
</tbody>
</table>

Values are means ± SE. DVC, dorsal vagal complex; NTS, nucleus of the solitary tract; AP, area postrema; AsP, area subpostrema. Significantly different from control treatment (*P < 0.05, †P < 0.01, ‡P < 0.001); significantly different from vehicle-treated rats (*P < 0.05, †P < 0.01); significantly different from distension (S < 0.05, ‡P < 0.01). See text for different P values.
Fig. 1. Photomicrographs of the dorsal hindbrain showing the dorsal vagal complex (DVC) at the level of the rostral area postrema (AP) in vehicle-treated (Veh) rats (left) and capsaicin-treated (Cap) rats (right) that received control treatment, CCK (4 μg/kg), or 2- or 5-ml distension. Control treatment did not induce significant c-Fos (Fos) in any of the areas that we counted in Cap or Veh rats. CCK induced an increase in Fos in the nucleus of the solitary tract (NTS) and AP of Veh animals, but this effect was attenuated in Cap rats. Gastric distension (2 or 5 ml) induced significant increases in the amount of Fos expression in the DVC of Veh and Cap rats. However, 2-ml distension induced significantly less Fos in Cap animals compared with Veh animals. Scale bar = 200 μm.

Fig. 2. Quantification of Fos expression averaged for all levels of the NTS for Cap and Veh rats. Bars represent average number of Fos-positive neurons summed for all of the NTS levels that were analyzed. Animals received control treatment, CCK (4 μg/kg), 2-ml distension, or 5-ml distension. Gastric distension-induced Fos was significant in Cap and Veh rats at both distension levels (2 and 5 ml). However, 2-ml-induced Fos was significantly less in Cap rats compared with Veh rats. CCK did not induce significant Fos in Cap rats but did in Veh controls. CCK significantly enhanced distension-induced Fos in Cap but not in Veh rats. Significantly different from (control) treatment in Veh rats, F_{5,40} = 15.6 (**P < 0.01) and ***P < 0.001); significantly different from control treatment in Cap rats, F_{5,40} = 83.9 (##P < 0.01 and ###P < 0.001); significantly different between Cap and Veh rats (&P < 0.05); significantly different between treatments within Cap rats (@P < 0.001).

Fig. 3. Photomicrographs illustrating Fos expression in the NTS just rostral to the AP in Veh and Cap rats that received 2-ml gastric distension alone or in combination with CCK (4 μg/kg). CCK significantly enhanced the Fos expression in the NTS in response to 2-ml gastric distension in Cap but not in Veh rats. Scale bar = 200 μm.
tion in the NTS ($F_{17,219} = 9.3$, $P < 0.001$). All experimental treatments resulted in a significant increase in Fos, compared with controls, at all levels in Veh ($P < 0.001–0.04$), except that after 2-ml distension there was no significant increase in Fos expression in the NTS at $3.8$ mm IA. Furthermore, all of the experimental treatments produced a similar pattern of Fos expression, with the least Fos just caudal to the AP at $5.3$ mm IA and the most Fos at the rostral end of the AP ($4.68$ mm IA) and rostral to the AP at $4.3$ mm IA.

Nevertheless, there were differences in the amount of Fos expression between the various levels that we analyzed. For example, distension-induced Fos in the NTS was greatest at the rostral end of the AP ($4.68$ mm IA), where both 2- and 5-ml distensions induced significantly more Fos expression than occurred at other levels of the NTS ($P < 0.001–0.04$). Also, at this level, 5-ml distension induced significantly more Fos compared with 2-ml distension, ($P < 0.05$), although this effect was not significant when Fos expression for all levels of the NTS was averaged. Although gastric distension with 2 ml induced less Fos expression than 5 ml, it still resulted in an overall increase in Fos throughout the NTS, with the pattern of activation being similar to that produced by 5-ml distensions. In all cases, the lowest levels of Fos expression were just caudal to the AP ($5.3$ mm IA). Furthermore, all of the experimental treatments produced a similar pattern of Fos expression, with the least Fos just caudal to the AP at $-5.3$ mm IA and the most Fos at the rostral end of the AP ($-4.68$ mm IA) and rostral to the AP at $-4.3$ mm IA.

Nevertheless, there were differences in the amount of Fos expression between the various levels that we analyzed. For example, distension-induced Fos in the NTS was greatest at the rostral end of the AP ($-4.68$ mm IA), where both 2- and 5-ml distensions induced significantly more Fos expression than occurred at other levels of the NTS ($P < 0.001–0.04$). Also, at this level, 5-ml distension induced significantly more Fos compared with 2-ml distension, ($P < 0.05$), although this effect was not significant when Fos expression for all levels of the NTS was averaged. Although gastric distension with 2 ml induced less Fos expression than 5 ml, it still resulted in an overall increase in Fos throughout the NTS, with the pattern of activation being similar to that produced by 5-ml distensions. In all cases, the lowest levels of Fos expression were just caudal to the AP ($-5.3$ mm IA). CCK induced similar amounts of Fos expression at the various levels of the NTS as with 2-ml gastric distension. Furthermore, when CCK was given in combination with distension, Fos expression was enhanced at all levels, but the effect was greatest at the levels rostral to the AP ($P = 0.001$ at $-4.5$ mm IA; $P < 0.01$ at $-3.8$ mm).

In Cap rats, CCK did not significantly increase average NTS Fos expression. However, analyses of individual NTS levels revealed that CCK induced very small, but significant, increases in Fos expression in the NTS at the caudal and rostral end of the AP ($P < 0.01$). Nevertheless, the amount of Fos expressed at these levels of the NTS in Cap rats was significantly less ($65–87\%$) compared with CCK-induced Fos in Veh rats ($F_{1,13} = 67.9$, $P < 0.001$). It appeared that the 5-ml gastric distension-induced Fos expression at the caudal end of the AP
Combination of 2-ml distension with CCK resulted in an enhancement of Fos expression at NTS levels caudal and rostral to the AP \( (P < 0.05) \). Enhancement of the 5-ml distension-induced Fos by CCK resulted in an increased Fos at all measured levels of the NTS \( (P < 0.001–0.05) \). However, the largest effect was in the NTS at the level just rostral to the AP \( (-4.3 \text{ mm IA}) \), where Fos expression is significantly higher compared with all other levels \( (P < 0.001) \).

**Effect of CCK or distension on Fos expression at different anteroposterior DVC levels: AP.** Repeated-measure ANOVAs revealed a significant difference in Fos expression between the caudal end of the AP and the rostral end of the AP in both Cap and Veh rats \( (F_{1.63} = 96.5, P < 0.001) \) (see Fig. 7). As in the NTS, there was a significant level \( \times \) treatment interaction in the AP \( (F_{5.63} = 7.6, P < 0.001) \). Significant effects of treatments were mostly seen in the rostral AP \( (-4.68 \text{ mm IA}) \) in both Cap and Veh rats. CCK produced a significant increase in Fos expression in the rostral AP \( (P = 0.001) \), and capsaicin treatment abolished this CCK-induced increase in Fos \( (P < 0.01) \).

**DISCUSSION**

Our results indicate that CCK increased gastric distension-induced Fos expression by DVC neurons in Cap rats, in which C-type vagal afferents had been destroyed. In Cap rats, expression of DVC Fos in response to CCK alone was markedly attenuated and did not reach statistical significance. Therefore, our data suggest that, in addition to directly activating C-type vagal afferents, CCK enhances distension-induced vagal responses by capsaicin-insensitive A-type afferents.

Several previous reports have suggested that responses to CCK and gastric distension might involve distinct populations

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**Table 2. Effect of lorglumide in combination with distension and CCK or distension alone on Fos expression in the DVC**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NTS</th>
<th>AP</th>
<th>AsP</th>
<th>DMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lorglumide + CCK + tubing</td>
<td>1.9±0.5</td>
<td>8.9±3.0</td>
<td>2.5±0.9</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>CCK</td>
<td>19.6±5.6</td>
<td>13.0±3.1</td>
<td>9.1±2.2</td>
<td>1.9±0.6</td>
</tr>
<tr>
<td>2-ml Distension + CCK</td>
<td>29.8±7.8†</td>
<td>24.2±2.5*</td>
<td>13.4±5.3</td>
<td>2.7±0.8</td>
</tr>
<tr>
<td>2-ml Distension</td>
<td>76.4±1.66‡</td>
<td>39.0±6.8‡</td>
<td>24.4±5.1‡</td>
<td>8.6±1.1‡$</td>
</tr>
<tr>
<td>Lorglumide + 2-ml distension</td>
<td>36.7±6.1</td>
<td>21.4±3.9</td>
<td>9.1±2.4</td>
<td>2.6±1.3</td>
</tr>
<tr>
<td>Lorglumide + CCK + 2-ml distension</td>
<td>35.9±8.9</td>
<td>23.6±6.1</td>
<td>12.4±3.0</td>
<td>1.7±1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Significantly different from control treatment \( (†P < 0.01 \text{ and } ‡P < 0.001) \); significantly different from distension \( (@P < 0.05 \text{, } @P < 0.01) \); significantly different from distension + CCK \( (*P < 0.01) \). See text for different \( F \) values.
of vagal afferent neurons. For example, Ritter et al. (30), recording extracellularly from the DVC in rats, found that changes in neuronal firing in response to exogenous CCK were abolished in Cap rats, although responses to gastric distension were not diminished. Likewise, several investigators have found that DVC Fos expression after injection of CCK was abolished or attenuated by capsaicin, whereas Fos expression in response to gastric distension was not diminished (11, 21, 34). These results are consistent with the anatomical observations of Berthoud et al. (3), who found that 70% of gastric vagal IGLEs survive capsaicin treatment, whereas virtually all intestinal IGLEs are destroyed by the neurotoxin (3, 12). Together, these results suggest that Fos responses to CCK are mediated largely by capsaicin-sensitive C-type vagal afferents, whereas responses to gastric distension are mediated, at least in part, by capsaicin-insensitive A-type afferents.

Although there is clear evidence that at least some gastric distension-responsive vagal afferents are capsaicin resistant, other reports indicate that some responses to gastric distension are mediated by capsaicin-sensitive C-type afferents. In the experiments reported here, we found that Fos expression in response to a 5-ml gastric balloon distension was not diminished in Cap rats. However, we also found that Fos expression after a 2-ml balloon distension, although not abolished, was significantly reduced by capsaicin treatment. Our results are consistent with those of Mazda et al. (16) who recently reported that capsaicin treatment abolished DVC Fos expression following 3-ml gastric distensions. On the other hand, Berthoud et al. (3) reported that DVC Fos expression following gastric distension is not diminished by capsaicin treatment. However, Berthoud et al. distended the stomach by inflating a balloon with 18 ml of saline. Hence, the level of distension in their study markedly exceeded that of Mazda et al. and our own and is likely to have been noxious in nature. Although the Berthoud et al. report did not provide a detailed analysis of the distribution of distension-induced Fos within the NTS following high levels of distension, a recent report by Sabbatini et al. (33) suggested that potentially noxious stimulation, produced by higher levels of gastric distension, induces a different distribution of NTS Fos than lower levels of distension. They reported that 7 ml of distension, administered as rhythmic patterns of inflations and deflations, induced an increase in Fos at caudal levels of the NTS (−5.6 and −4.8 from the intramural line). In our study, we found little Fos expression at this caudal level. Although increasing our distension level from 2 to 5 ml did induce a general increase in NTS Fos, the pattern of Fos expression did not change, and expression at the more caudal of the five NTS levels that we examined did not increase. Thus our experimental procedures and the pattern of Fos expression that we observed are consistent with the induction of Fos via nonnociceptive pathways. Furthermore, our results, together with those of others, suggest that both capsaicin-sensitive and capsaicin-insensitive afferents participate in induction of DVC Fos by gastric distension. However, it appears that, in the absence of CCK, vagal afferent responses to gastric distension are mediated mainly by C-type capsaicin-sensitive afferents. Furthermore, this C-fiber-mediated response occurs at lower levels of gastric distension than those of capsaicin-insensitive afferents.

Previous studies that used extracellular recordings support the hypothesis that CCK and gastric distension interact in the activation of vagal afferents. For example, Ewart and Wingate (8) reported that CCK modulated extracellularly recorded responses to gastric distension by neurons in the DVC. Davison and Clarke (7) reported that some vagal afferent fibers that responded to gastric distension could also be activated by injections of exogenous CCK. Schwartz et al. (37) found that close arterial infusion or intraperitoneal injection of CCK produced dose-dependent increases in the activity in gastric vagal mechanoreceptive afferent fibers. These extracellular recording studies demonstrated that cooperation exists between gastric distension and CCK in the activation of vagal afferents. However, like our Fos immunohistochemistry, in vivo recordings cannot be used to specify the anatomical site(s) at which interaction occurs. For example, recordings made from distension- and CCK-activated neurons in the hindbrain cannot differentiate between whether cooactivation of these neurons was the result of convergence of distinct populations of distension-sensitive and CCK-sensitive afferents or responses of a single afferent population that responds to both CCK and gastric distension. Likewise, recordings of CCK- and gastric distension-induced activation of teased vagal afferent fibers cannot rule out the possibility that responses to CCK are mediated by CCK actions on smooth muscle that is innervated by vagal mechanoreceptors. Indeed, several investigators have reported such indirect CCK effects in recordings from vagal afferents innervating the stomach (35, 36) or small intestine (42). Solely on the basis of our present results, we cannot specify whether CCK-induced enhancement of NTS Fos is due to a direct action of CCK on vagal afferents or whether CCK’s action is indirect. Nevertheless, our data clearly indicate that, in Cap rats, CCK contributes to elevated expression of DVC Fos mainly in the presence of gastric distension. Regardless of the site of CCK action, our data indicate that at least some distension-CCK interactions are mediated by capsaicin-insensitive afferents.

Although in vivo recordings and Fos immunohistochemical results cannot be used to localize the site of gastric distension-CCK interactions, recently published data have indicated that CCK can directly influence the activity of capsaicin-insensitive vagal afferents. Simasko and Ritter (40) reported the results of patch-clamp electrophysiological recordings from vagal afferents dissociated from adult rat nodose ganglia. They found that CCK induced depolarizations in capsaicin-sensitive C-type afferents and also in capsaicin-resistant A-type afferents. These responses were abolished by lorglumide, a CCK-1 receptor antagonist. Simasko and Ritter did not establish the innervation targets of the vagal afferents from which they recorded. Nevertheless, their results are consistent with the hypothesis that CCK can act directly on A-type vagal afferents in vivo and in doing so may enhance their responses to other stimuli, such as gastric distension. The relative CCK sensitivity of A-type and C-type vagal afferents in the absence of distension has not been systematically studied. Nevertheless, it is probable that A-type vagal afferents exhibit some degree of response to exogenous CCK in the absence of distension in vivo. Such A-type neuronal responses might account for the slight but nonsignificant increase in NTS Fos that we observed after CCK treatment alone. In addition, sensitivity of capsaicin-resistant A-type vagal afferents also may contribute to residual reductions of food intake observed in Cap rats following high CCK doses. Nonetheless, it is clear that expression of Fos in Cap rats was
markedly enhanced by gastric distension, suggesting that the coincidence of CCK with distension is much more effective for activating this afferent population.

In contrast to some reports, which indicated that capsaicin treatment did not diminish distension-induced Fos in the DVC, we found that Fos expression in response to 2-ml distensions, but not 5-ml distensions, was diminished in Cap rats. Thus it appears that 2-ml distension may activate mechanoreceptors with lower thresholds compared with 5-ml distension. It may be that low-threshold mechanoreceptors are capsaicin sensitive but higher-threshold mechanoreceptors survive capsaicin treatment. Possibly, higher-volume distensions activate more high-threshold mechanoreceptors. Activation of these higher threshold afferents might converge to activate many of the same DVC neurons activated by C-type afferents. Such convergence of mechanoreceptive inputs from A- and C-type afferents could explain why we did not observe large enhancements of distension-induced Fos by CCK in the control rats.

Interaction with CCK and distension and food intake. Reports from several research groups have indicated that the interaction of CCK and gastric distension is behaviorally significant. For example, McHugh and Moran (17) reported that in monkeys a saline preload was necessary to decrease food intake after a low dose of CCK. This result suggests that, in monkeys, CCK’s effect might be manifested mainly through the interaction of CCK with mechanoreceptive stimulation. Similarly, in humans, Muurahainen et al. (22a) demonstrated that intake of a test meal in humans was significantly lower when CCK was given after a 500-g but not a 100-g soup preload. In a more recent study, Kissileff et al. (14) investigated the effects of a nonnutritive increase in gastric volume on food intake after administration of CCK or saline. They demonstrated that a low-dose intravenous infusion of CCK combined with a subthreshold gastric distension resulted in a significant reduction in intake of a liquid meal compared with saline infusion and no distension. These results support a role for the interaction of CCK and gastric distension in the control of food intake in humans and other primates.

Lorglumide completely reversed the enhancement of distension-induced Fos expression by CCK in Cap rats but did not diminish expression of Fos induced by distension alone. This result indicates that the enhancement of distension-induced Fos expression by CCK occurs through the CCK-A receptor. This result is consistent with previous reports (5, 9) and indicates that lorglumide does not nonspecifically reduce the response of vagal afferents to distension. However, the result also indicates that CCK-A receptors must be occupied by an agonist to allow the maximal response to distension.

The AP also receives synaptic input from gastric vagal afferent fibers (39). Indeed, distension significantly enhanced Fos in the AP, especially the rostral portions of the AP. Yet there was no relation between degree of gastric distension and the amount of increase in AP Fos. CCK at the dose that we used increased Fos expression in the AP only when it was coadministered with 5 ml of gastric distension, suggesting that the vagal afferent innervation to the AP includes capsaicin-insensitive A-type afferents.

Distension-sensitive afferent fibers have been demonstrated in spinal nerves innervating the gastrointestinal tract as well as in the vagi (43). Signals from spinal afferents probably reach the NTS via the spinosolitary tract (19, 22, 43). It might be that high-volume distension activates these spinal pathways as well as activating vagal afferents. However, we believe that our 2- and 5-ml distensions are within the range of physiological distension and therefore are not noxious. For instance, rats frequently consume liquid meals of 9 ml and more after an overnight fast (6). Moreover, Traub et al. (43) showed that noxious distension (80 mmHg, comparable with 18 ml of balloon distension) induced Fos in the DVC via splanchnic as well as vagal afferents. However, even with this very large distensile stimulus, the contribution of the splanchnic afferents to total distension-induced Fos was small compared with vagal contribution. Therefore, although we cannot entirely exclude the contribution of spinal afferents in distension-induced Fos in the hindbrain, a significant spinal contribution seems unlikely, especially at the levels of distension that we used.

In conclusion, our results confirm previous reports that induction of DVC Fos by exogenous CCK depends on capsaicin-sensitive primary C-type afferents. However, our results also indicate that capsaicin-insensitive afferents respond to CCK and contribute to the activation of hindbrain neurons during gastric distension. Together with recent patch-clamp data from cultured vagal afferents, our data suggest that CCK enhances the response of A-type vagal afferents to gastric distension. Cooperation between CCK and gastric distension in the activation of capsaicin-insensitive primary afferent neurons could play an important role in control of food intake by these two visceral signals.

REFERENCES

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