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Cholecystokinin activates both A- and C-type vagal afferent neurons

Steven M. Simasko and Robert C. Ritter
Program in Neuroscience, Department of Veterinary and Comparative Anatomy, Pharmacology, and Physiology, College of Veterinary Medicine, Washington State University, Pullman, Washington 99164-6520

CCK is a peptide hormone released from endocrine I cells located in the upper small intestinal mucosa after entry of fats and proteins into the small intestines. CCK triggers gallbladder contraction (70), inhibits gastric emptying (15), stimulates pancreatic enzyme secretion (17, 74), modulates intestinal motility (20, 46, 54, 68), and contributes to the process of satiation (23, 59). These actions of CCK slow the delivery of food into the small intestine and promote nutrient digestion and absorption. Many of these effects of CCK are mediated by vagal afferent fibers destroyed by capsaicin (24, 31, 33, 41, 43, 47, 61).

Cell bodies for all visceral vagal afferent neurons reside within the nodose ganglia. These neurons have been classified as capsaicin-resistant (A-type) or capsaicin-sensitive (C-type) according to the conduction velocities of their axonal fiber (21). Study of these neurons has revealed that A-type neurons exhibit TTX-sensitive action potentials, whereas about half of the C-type neurons exhibit TTX-resistant action potentials (62). The TTX-sensitive Na⁺ current is faster and activates at relatively negative potentials compared with the TTX-resistant Na⁺ current, which is slower and activates at relatively depolarized potentials (7). An additional difference between these two neuron types is the expression of hyperpolarization-activated current (Ih) carried by monovalent cations. This current was first described in a study (62) that revealed a strong inward rectification on passage of Ih in A-type neurons but not in most C-type neurons. This strong inward rectification is a hallmark of Ih. A subsequent patch-clamp study in dissociated nodose neurons demonstrated that A-type vagal afferent neurons express a large and rapidly activating Ih, whereas C-type vagal afferent neurons express a small and slowly activating Ih (16). Finally, cultured C-type vagal afferent neurons from the nodose ganglia have been shown to respond to capsaicin (16). Thus, although the conduction velocity of the fiber cannot be measured directly, it is possible to use several criteria, Na⁺ current, Ih, and capsaicin sensitivity, to characterize nodose neurons along a continuum of traits, and in so doing to recognize a majority of them that behave as classically defined A- or C-type neurons.

Many CCK-mediated digestive effects appear to be mediated by capsaicin-sensitive vagal neurons. However, it is clear that some vagal afferents are not destroyed by capsaicin. For example, a significant proportion of gastric mechanoreceptive afferents survive capsaicin treatments (3, 44). In vivo extracellular recordings indicate that at least some gastric mechanoreceptive afferents also respond to CCK (13). However, other extracellular recording studies have suggested that CCK activates gastric mechanoreceptive units indirectly, by altering gastric tone (6). Consequently, it is not entirely clear whether CCK directly activates capsaicin-insensitive visceral vagal afferents. In the present study, we used electrophysiological methods to distinguish subpopulations of dissociated nodose neurons in acute culture from adult male rats. We then tested whether sensitivity to CCK was restricted to only C-type, capsaicin-sensitive neurons.

Address for reprint requests and other correspondence: S. M. Simasko, Program in Neuroscience, Dept. of Veterinary and Comparative Anatomy, College of Veterinary Medicine, Washington State Univ., Pullman, WA 99164-6520 (E-mail: simasko@vetmed.wsu.edu).

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We found that subpopulations of both A- and C-type neurons responded to CCK with a depolarization.

**MATERIALS AND METHODS**

**Dissociation and culture of vagal afferent neurons.** Vagal afferent neurons were obtained from the nodose ganglia of male Sprague-Dawley rats as previously described (58). Briefly, rats were anesthetized with ketamine 25 mg/100 g, xylazine 2.5 mg/100 g and nodose ganglia isolated under aseptic conditions. Ganglia were placed in HBSS, cleaned of connective tissue, and desheathed. Neurons were dissociated in ~3 ml of digestion buffer (1 mg/ml dispase II and 1 mg/ml collagenase type Ia in HBSS) for 90 min in an incubator at 37°C. Dispersed cells were washed with HEPES-buffered DMEM (HDMEM) and plated onto poly-t-lysine-coated coverslips (100 μg/ml poly-lysine for 30 min). The plated neurons were maintained in HDMEM supplemented with 10% fetal calf serum. All experimental procedures were conducted within 2 days of collection of the nodose ganglia.

**Electrophysiological measurements.** Standard whole cell patch-clamp techniques were used to obtain both current clamp and voltage-clamp recordings. An Axon Instruments (Union City, CA) 200A patch-clamp amplifier was used. Voltage-clamp protocols were controlled, and current responses collected with Axon Instruments software (pCLAMP 6.0). Illustrated current traces are not corrected for leak current and the first millisecond of current after the voltage jump, which is contaminated by residual uncompensated capacity current, has been removed for clarity. For current-clamp recordings, the voltage signals were recorded onto videotape and monitored with a strip-chart recorder (model TA240; Gould Instruments Systems, Valley View, OH). Voltage responses to peptide and drug applications were read directly from the strip-chart records. For illustrations, the VCR records were converted to digital files at a frequency of 1,000 Hz.

All manipulations and experiments were performed at room temperature (20–22°C) in a physiological saline (in mM: 140 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 6 glucose, and 10 HEPES with the pH adjusted to 7.4 with NaOH). Coverslips on which neurons were growing were washed in physiological saline and placed in a chamber (~0.5 ml volume) through which solutions were perfused by gravity (~3 ml/min). Changes in bathing solutions were made by switching in 3 ml/min). All experimental procedures were conducted within 2 days of collection of the nodose ganglia.

**Sensitivity of the fast Na⁺ current to TTX.** Sensitivity of the fast Na⁺ current to TTX (1.0 μM) was tested by applying the depolarizing voltage pulse series described above to the neurons followed by bath application of TTX and repeat of the voltage-pulse protocol. TTX was then washed from the neurons and capsaicin sensitivity was determined by application of capsaicin (10 nM) while applying voltage ramps to the neurons (ramps from ~80 to ~40 mV, 250 ms in duration, from a holding potential of ~60 mV, repeated every 5 s). At least three control ramps were obtained before capsaicin application and the protocol continued for at least 3 min after capsaicin application. We chose to use a single capsaicin concentration of 10 nM, because nanomolar capsaicin concentrations appear to reliably and selectively activate neurons that express the vanilloid receptor 1 (VR1), which historically has been associated with C-type neurons (12, 27). Although higher capsaicin concentrations also may activate VR1 receptors, we have observed that neurons often do not recover after application of higher concentrations. Finally, a recent report by Bielefeldt (5) indicates that high micromolar capsaicin concentrations may actually inhibit some visceral afferent neurons, perhaps via actions on sodium or potassium channels (5). The IC₅₀ for this novel capsaicin effect was 8–40 μM. Therefore, the 10 nM capsaicin concentration we used should have avoided the high dose effects reported by Bielefeldt (5) while permitting reliable activation of neurons expressing VR1 receptors.

**Classification of neurons.** To ensure that only healthy neurons were included in our analysis, we required that each neuron exhibit a resting membrane potential negative to −40 mV and an input resistance of 100 MΩ or greater, and express a fast Na⁺ current that was at least 2.0 nA at its peak. The fast Na⁺ currents frequently exceeded 5 nA, which would saturate the amplifier. We did not attempt to obtain an accurate measurement of the peak Na⁺ current, because we focused on the threshold voltage for activation and the sensitivity of the currents to TTX, which we could observe despite not capturing the maximal current responses.
Results from the voltage-clamp measurements were used to classify a neuron as A- or C-type according to a numerical scoring system. Three criteria used in scoring the neurons were: 1) characteristics of the fast Na\(^+\) current, 2) characteristics of the I\(_h\) current, and 3) responses to capsaicin. For each criterion, a value ranging from 0.0 (C-type characteristics) to 1.0 (A-type characteristics) was assigned (details of assigning values are described in RESULTS). These values were then summed (maximum score of 3.0), and if the neuron scored <1.0, it was categorized as C type; if it scored 1.0, it was categorized as A and C type, and if it scored between 1.0 and 2.0, it was categorized as mixed. If for some reason one of the criteria were not obtained, the neuron would be classified on the basis of the two remaining criteria (<1/3 of total points, C type; >2/3 total points, A type; between 1/3 and 2/3 of total points, mixed).

**Statistical analysis.** We used \( \chi^2 \) analysis to determine whether differences in frequencies of observed responses in C-type vs. A-type neurons were statistically significant \((P < 0.05)\). We used t-tests to determine whether any measured characteristic of C- vs. A-type neurons or CCK-responsive vs. CCK-nonresponsive neurons, were statistically significant \((P < 0.05)\).

**Chemicals.** In all experiments, the sulfated form of CCK-8 was used. Culture media and fetal calf serum were obtained from Life Technologies (Grand Island, NY). Dispase II was obtained from Boehringer-Mannheim (Indianapolis, IN). All other chemicals were obtained from Sigma (St. Louis, MO).

**RESULTS**

**Characterization of Na\(^+\) currents.** Although previous studies have shown that A-type vagal afferent neurons tend to express a low voltage-activated, TTX-sensitive Na\(^+\) current, whereas C-type vagal afferent neurons tend to express a high voltage-activated, TTX-insensitive Na\(^+\) current, these studies also revealed that most vagal afferent neurons express a mixture of these two currents (7, 62). Because we were primarily interested in using the characteristics of the Na\(^+\) current to classify neurons, we devised an objective scoring system to rate the degree to which a cell expressed one type of Na\(^+\) current vs. the other (the higher the score, the more A-type-like the current). Threshold voltage for activation was assigned 0.0 to 0.2 points, and sensitivity to TTX was assigned 0.0 to 0.8 points. Activation voltage was assigned to the potential at which the Na\(^+\) current first exceeded 1.0 nA. The neuron would score 0.2 points if its Na\(^+\) current activated at voltages less than or equal to −30 mV, 0.1 if the Na\(^+\) current activated between −25 and −15 mV, and 0.0 if the Na\(^+\) current required voltages of −10 mV or more positive to activate. If application of TTX reduced peak Na\(^+\) current to <200 pA at both the activation voltage and 0 mV, the neuron scored 0.8 points (neuron expressed primarily TTX-sensitive Na\(^+\) current); if TTX reduced Na\(^+\) current to below 200 pA at the activation voltage but it failed to reduce Na\(^+\) current to <2.0 nA at 0 mV, the neuron scored 0.4 points (neuron expressed both TTX-sensitive and -insensitive Na\(^+\) current); and if TTX failed to reduce Na\(^+\) current to <1.0 nA at the activation voltage and 2.0 nA at 0 mV, the neuron scored 0.0 points (neuron expressed primarily TTX-insensitive Na\(^+\) current).

We found that some neurons were easily classified as expressing just one of the two Na\(^+\) current. For example, Fig. 1A illustrates a Na\(^+\) current that activated between −40 and −35 mV (low-voltage activated). This current was abolished by TTX both near the threshold voltage for activation and at a strong depolarizing
FIG. 2. Whole cell patch-clamp recordings in cultured nodose neurons in response to hyperpolarizing voltage pulses. Traces above the current recordings illustrate voltage protocol. Time to one-half peak current (t_{1/2}) is indicated on traces in response to −110 mV pulse. A: large and fast-activating cytopolarization-activated current (I_h) characteristic of an A-type neuron (same neuron as that illustrated in Fig. 1A). B: small and slowly activating I_h characteristic of a C-type neuron (same neuron as that illustrated in Fig. 1B). C: neuron expressing an I_h that had mixed characteristics (same neuron as that illustrated in Fig. 1C).

voltage. These characteristics clearly indicate that this neuron had an A-type-typical Na⁺ current. On the other hand, Fig. 1B illustrates a Na⁺ current that required a depolarization between −15 and −10 mV to activate (high-voltage activated), and it was completely resistant to a high concentration of TTX at both the threshold voltage for activation and at 0 mV. These characteristics clearly indicate that this neuron had a C-type-typical Na⁺ current. In contrast to the Na⁺ currents illustrated by the neurons in Fig. 1, A and B the Na⁺ current illustrated in Fig. 1C had an intermediate activation voltage (−25 mV), and it was sensitive to TTX near the threshold voltage. However, it also had a component of Na⁺ current resistant to TTX at a strong depolarization. Thus by our scoring system, the Na⁺ currents illustrated in Fig. 1 scored 1.0, 0.0, and 0.5, A–C, respectively.

Characterization of I_h. A-type neurons exhibit a large and fast-activating I_h, whereas the I_h in C-type neurons is small and slow activating (16). As with Na⁺ current, some neurons expressed an easily classified I_h, because it was large and fast (Fig. 2A, current traces show I_h for the same neuron illustrated in Fig. 1A), or small and slow (Fig. 2B, current traces show I_h for the same neuron illustrated in Fig. 1B). However, I_h expressed by some neurons had intermediate characteristics (Fig. 2C, current traces show I_h for the same neuron as illustrated in Fig. 1C). Although the I_h illustrated in Fig. 2C current traces activated slowly, this neuron expressed an intermediate amount of I_h. As with the Na⁺ currents, we therefore used a scoring system for I_h. Half of the points assigned for I_h were based on activation kinetics (time to one-half peak current) and half on amplitude, with small scores assigned to characteristics reported to be C-type specific. If time to one-half peak current was <100 ms (test pulse to −110 mV), it scored 0.5 points, if >500 ms, it scored 0.0 points, and values between these extremes scored intermediate points (0.1 points for values between 100 and 200 ms, 0.2 points for values between 200 and 300 ms, etc.). If amplitude was >500 pA, it scored 0.5 points, if <100 pA it scored 0.0 points, and values between the extremes scored intermediate points (0.1 points for 100 to 200 pA, etc.). Thus the currents illustrated in Fig. 2 scored 0.9, 0.0, and 0.2, from A to C, respectively.

Capsaicin sensitivity. Capsaicin activates VR1 (12), which is a nonselective cation channel thought to be primarily expressed on C-type afferent neurons (16, 27). Presence of VR1 was determined by exposing neurons to capsaicin while delivering voltage ramps (Fig. 3). Responses to capsaicin were scored on the basis of increases in holding current at −60 mV. Changes in holding current <−20 pA scored 1.0, changes >−1.0 nA scored 0.0, and intermediate values scored intermediate values (0.1 for every 100 pA increase of holding current). Almost all neurons either did not respond at all to capsaicin (Fig. 3, left traces, shows data from the same neuron illustrated in Figs. 1A and 2A), or responded completely (Fig. 3, right traces, shows data from the same neuron illustrated in Figs. 1B and 2B). Capsaicin responses were reversible (Fig. 3, right current traces). The mixed-type neuron illustrated in Figs. 1C and 2C did not respond to capsaicin (data not shown).

Characteristics of A- vs. C-type neurons. Of 108 neurons included in the data set, 33 (31%) were classified as A-type, 55 (51%) as C-type, and 20 (19%) as mixed (Table 1). Some mixed-type neurons were found to have intermediate characteristics for both their Na⁺ current and I_h, but in some cases a neuron might have a C-type I_h with an A-type Na⁺ current (or vice versa). In two cases, the neurons had almost complete A-type current characteristics yet still responded to capsaicin. These two neurons were classified as mixed. As expected, the

FIG. 3. Effects of capsaicin (10 nM) on whole cell patch-clamp recordings in cultured nodose neurons. Traces above the current recordings illustrate voltage protocol. Left traces: complete insensitivity to capsaicin characteristic of an A-type neuron (same neuron as that illustrated in Figs. 1 and 2, top). Right traces: large and reversible increase in holding current induced by capsaicin characteristic of a C-type neuron (same neuron as that illustrated in Figs. 1B and 2B).
average values for the kinetics of activation and amplitude of \( I_h \) in A- and C-type neurons were significantly different (Table 1). Although resting membrane potential was not different between the groups, C-type neurons tended to have a higher input resistances and larger membrane capacitances (Table 1). However, there was significant overlap between A- and C-type neurons in these characteristics such that neither of these properties could be used to classify neurons.

**Responses to CCK-8.** Overall, 32 of 108 neurons (30%) responded to CCK-8 with a reversible depolarization of the membrane, which in some neurons induced action potential activity (Fig. 4). For A-type neurons, 8 of 33 responded (24%; for examples, Fig. 4, A and B), for C-type neurons, 21 of 55 responded (38%; for examples, Fig. 4, C and D), and for mixed-type neurons, 3 of 20 responded (15%, examples not shown). One mixed-type neuron responded with a hyperpolarizing response (data not shown). Because the numbers of CCK-responsive mixed-type neurons in our sample were very small, they are not considered further. The difference in the percent of A-type vs. C-type neurons that responded to CCK-8 was not significant by \( \chi^2 \) analysis (\( \chi^2 = 1.238, P < 0.25 \)). For CCK-responsive A-type neurons, 2 of 8 (25%) exhibited action potential spiking, whereas for CCK-responsive, C-type neurons 9 of 21 (43%) exhibited action potential spiking. This difference was not statistically significant (\( \chi^2 = 0.209, P < 0.75 \)). In some neurons, hyperpolarizing current injections revealed that the CCK-induced depolarization was associated with a decrease in input resistance, indicating that the depolarization was due to an increase in a depolarizing conductance in the membrane (Fig. 4, A and C); however, in many neurons, changes in input resistance were too small to resolve (data not shown).

The final issue we addressed was whether there was any electrophysiological feature of CCK-responsive neurons that distinguished them from CCK-nonresponsive neurons. The characteristics of both A-type and C-type neurons, separated into CCK-responsive and nonresponsive groups, are summarized in Table 2. We found no difference in any characteristic that could predict CCK-responsiveness vs. nonresponsiveness in A-type neurons. However, CCK-responsive C-type neurons had significantly lower input resistances and more hyperpolarized resting membrane potentials compared with CCK-nonresponsive C-type neurons.

**DISCUSSION**

Our central finding from the experiments described here was that subpopulations of both A- and C-type vagal afferent neurons depolarize in response to CCK. It was expected from previous reports that only C-type neurons would respond to CCK, because CCK-induced inhibition of feeding (24), inhibition of gastric emptying (24, 41), alterations in intestinal motility (47), and stimulation of pancreatic exocrine secretion (31, 33) appear to depend on capsaicin-sensitive vagal afferent neurons. Although most of the primary afferent neurons destroyed by capsaicin are C-type neurons, capsaicin also destroys some A\( \delta \) neurons (27). We did find a few neurons that would have been classified as A type on the basis of their ion currents but that responded to capsaicin. These neurons were classified in the

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**Table 1. Summary of the electrophysiological characteristics of dissociated A-, C-, and mixed-type nodose neurons. Neurons were assigned to type as described in text**

<table>
<thead>
<tr>
<th></th>
<th>A type</th>
<th>C type</th>
<th>Mixed Type</th>
</tr>
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<tbody>
<tr>
<td>Number of neurons (% of total)</td>
<td>33 (31%)</td>
<td>55 (51%)</td>
<td>20 (19%)</td>
</tr>
<tr>
<td>Amplitude of ( I_h ), pA</td>
<td>767 ± 70*</td>
<td>103 ± 11*</td>
<td>215 ± 39</td>
</tr>
<tr>
<td>Time to ( 1/2 ) peak of ( I_h ), ms</td>
<td>167 ± 16*</td>
<td>462 ± 26*</td>
<td>356 ± 39</td>
</tr>
<tr>
<td>Cell input resistance, MΩ</td>
<td>307 ± 38*</td>
<td>649 ± 53*</td>
<td>442 ± 60</td>
</tr>
<tr>
<td>Resting membrane potential, mV</td>
<td>-53 ± 1</td>
<td>-56 ± 1</td>
<td>-56 ± 2</td>
</tr>
<tr>
<td>Cell capacitance, pF</td>
<td>37 ± 2*</td>
<td>47 ± 3*</td>
<td>47 ± 5</td>
</tr>
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</table>

Values are means ± SE. *Significant difference between A- vs. C-type (2-tailed t-test; \( P < 0.025 \)). Time to \( 1/2 \) peak of hyperpolarization-activated current (\( I_h \)) and amplitude values obtained at voltage pulses to -110 mV.

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**Fig. 4. Cholecystokinin (CCK)-8-induced depolarizations in nodose neurons. Standard whole cell patch-clamp techniques were used to record membrane potential as described in MATERIALS AND METHODS. CCK-8 (100 nM) was applied for the duration of the labeled bar. Downward spikes indicated by an asterisk in A and C are responses to hyperpolarizing current injections. Action potentials illustrated in B and D have been clipped above -30 mV. Typically, action potentials would depolarize to potentials positive to +30 mV (not shown). Results illustrated in A and B were from neurons classified as A-type; results illustrated in C and D were from neurons classified as C-type.**
mixed category and thus are not part of the A-type neuronal population we characterized. Only capsaicin-insensitive neurons were placed in our A-type category. Nonetheless, CCK clearly activated a significant number of these neurons. Therefore, some vagally mediated actions of CCK may be mediated by capsaicin-insensitive neurons.

Electrophysiological characterization of nodose neurons. Several specific attributes of ionic conductances have been associated with A- vs. C-type neurons in the nodose ganglia (16, 21, 62). However, we found these attributes did not cleanly segregate in many neurons. Even when fiber conduction velocity is used to classify neurons, there are some neurons that have velocities intermediate between that expected for C- and A-type fibers (62). Because judgments needed to be made in classifying neurons, we devised a scoring system to make the judgments objective. The scoring system enabled us to eliminate neurons that had ambiguous characteristics and ensured that neurons classified as A- or C-type had distinctive ionic characteristics.

The electrophysiological characteristics we found for A- and C-type neurons are in good agreement with values previously reported by other investigators. Most investigators report that nodose neurons have a resting membrane potential between −50 and −60 mV whether measured by intracellular electrode in intact ganglia (21, 62) or by patch-clamp techniques in dissociated cells (16). The observation that C-type cells have a larger membrane capacitance is in agreement with Gallego and Eyzaguirre (21) and Stansfeld and Wallis (62) but at odds with Doan and Kunze (16). Similar to us, Stansfeld and Wallis (62) found that C-type cells have a higher resting membrane resistance. However, Gallego and Eyzaguirre (21) did not find a significant difference in resting membrane resistance. Doan and Kunze (16) did not specifically address resting membrane resistance in A- vs. C-type cells, but given that they found evidence that \(I_h\) was active in setting the resting membrane potential and A-type neurons have much larger \(I_h\) it would be expected that C-type neurons would have a higher membrane resistance. Magnitudes of \(I_h\) found by Doan and Kunze (16) are about twice as large as those we observed for reasons that are not clear. Finally, we found that 31% of our neurons were categorized as A type (capsaicin insensitive), although a few mixed-type neurons also were capsaicin insensitive. This is in reasonable agreement with the findings of Carobi (11), who found that neonatal capsaicin reduced neuronal numbers in nodose ganglia to ~30% of controls.

The functional significance of distinctive ionic characteristics among vagal afferents or the potential adaptive value of different conduction velocities for different vagal afferent fibers is not known. Possibilities include that different fiber types carry different specific sensory information and/or innervate unique structures.

For example, Berthoud et al. (3) found that capsaicin treatment almost completely denervated the small intestine, whereas a significant portion of gastric innervation (50−70%) appeared to survive capsaicin treatment. Thus intestinal innervation appears to be primarily C-type neurons, whereas the majority of gastric innervation may be A-type. We are unaware of any other studies that address the composition of neuronal type in other vagally innervated structures. It is interesting to note that the presence of both A- and C-type vagal afferent neurons are preserved across small mammal species, including herbivore (62), carnivore (21), and omnivore (Ref. 16, present study), each with significant differences in diet, suggesting that they must be of physiological significance.

Electrophysiological actions of CCK on dissociated nodose neurons. Previous reports (6, 13, 19, 34, 42) indicate that CCK activates vagal afferent fibers both in vivo and in ex vivo nerve/organ preparations. Consistent with these reports, we found that cultured nodose neurons also depolarize in response to CCK. The response rate we observed (30%) is in good agreement with previous studies that indicate 33% of neurons in the nodose ganglia express mRNA coding for CCK-A receptors (9) and 38% of cultured nodose ganglia neurons respond to CCK with an increase in intracellular Ca\(^{2+}\) (58). Hepatic vagal C fibers respond to intravenous CCK-8 with a rapid (20–30 s) increase in firing, which then returns to near-baseline activity in ~1 min (13). This dynamic is similar to our observations in isolated nodose neurons (Fig. 4), which suggests the transient nature of the responses we observed is not an artifact of the isolated neuron preparation or the whole cell-patch configuration.

One previous study (18) has examined the effects of CCK on dissociated neurons from nodose ganglia. In this study, intracellular electrodes were used, and they found a resting membrane potential (~56 mV) similar to what we found; however, the input resistances they measured (~22 MΩ) were less than one-tenth those we

### Table 2. Summary of the characteristics of A-type and C-type neurons that were responsive or nonresponsive to 100 nM CCK-8

<table>
<thead>
<tr>
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<th>CCK responsive</th>
<th>Nonresponsive</th>
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<tr>
<td><strong>A-type neurons</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of neurons</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>Amplitude of CCK response, mV</td>
<td>11.0 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Amplitude of (I_h), pA</td>
<td>600 ± 60</td>
<td>821 ± 89</td>
</tr>
<tr>
<td>Time to ½ peak (I_h), ms</td>
<td>132 ± 15</td>
<td>178 ± 20</td>
</tr>
<tr>
<td>Cell input resistance, MΩ</td>
<td>346 ± 41</td>
<td>295 ± 48</td>
</tr>
<tr>
<td>Resting membrane potential, mV</td>
<td>−55 ± 2</td>
<td>−53 ± 2</td>
</tr>
<tr>
<td>Cell capacitance, pF</td>
<td>36 ± 3</td>
<td>37 ± 2</td>
</tr>
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</table>

| **C-type neurons**       |                |               |
| Number of neurons        | 21             | 34            |
| Amplitude of CCK response, mV | 15.1 ± 1.7    |               |
| Amplitude of \(I_h\), pA  | 114 ± 20       | 96 ± 14       |
| Time to ½ peak \(I_h\), ms | 452 ± 37       | 469 ± 36      |
| Cell input resistance, MΩ| 428 ± 55       | 785 ± 126     |
| Resting membrane potential, mV | −62 ± 2     | −53 ± 2      |
| Cell capacitance, pF     | 43 ± 4         | 49 ± 4        |

Values are means ± SE. *Significant difference between CCK-responsive and nonresponsive neurons (2-tailed t-test, \(P<0.05\)). Time to ½ peak \(I_h\) and amplitude values obtained at voltage pulses to −110 mV.
measured, suggesting that the intracellular electrode was inducing significant damage to the integrity of the neuronal membrane. These investigators found that 66% of the neurons responded to CCK with a fast depolarizing response and ~10% responded with a slow depolarizing response. The responses we observed are similar to the slow depolarizing responses they observed. The explanation for the 66% response rate for the fast response is not apparent given that only 33% of nodose neurons express CCK-A receptors and just 9% express CCK-B receptors (9). The neuronal types responding to CCK were not assessed in this previous study.

The majority of electrophysiological studies have found that CCK depolarizes neurons in the peripheral nervous system (35, 38, 51), spinal cord (48), and brain (8, 10, 14, 57, 71, 72). However, hyperpolarizing responses have also been observed in some neurons (8, 38, 73), and one study found that CCK augments the hyperpolarizing effects of dopamine in the ventral mesencephalon (10). Depolarizing effects of CCK have been associated with increases (35, 38, 48, 51, 57), decreases (8, 35, 51, 71), or no change (35, 51) in input resistance, even within the same preparation (35, 51). Although we occasionally could resolve a decrease in input resistance associated with CCK-induced depolarization in cultured nodose neurons, more often no change in input resistance was detectable. It appears that identification of the conductance modified by CCK in nodose neurons will require more sensitive techniques than membrane potential recordings.

We found that CCK-responsive C-type neurons had more hyperpolarized resting membrane potentials and lower resting membrane resistances than non-CCK-responsive C-type neurons, suggesting the presence of a greater amount of background K⁺ conductance in CCK-responsive neurons. The significance of this observation is not yet apparent. **Capsaicin sensitivity of vagal afferent neurons.** Capsaicin is a neurotoxin that destroys some populations of sensory neurons by activating VR1s, which are non-selective cation channels (12). Degeneration of capsaicin-sensitive fibers and cell bodies results from increased excitation and eventual calcium overload (4). Although when VR1 was first cloned (12), it was reported that mRNA for VR1 was not present in nodose ganglia; other studies have suggested the presence of VR1 in nodose ganglia. For example, [3H]resiniferatoxin, a VR1 agonist, exhibits specific binding to some nodose ganglion neurons (63). Also, careful neuroanatomical studies indicate that subpopulations of vagal afferent neurons degenerate after capsaicin treatment (3, 28, 29, 45). More recently, in situ hybridization demonstrated the presence of VR1 mRNA in nodose ganglia (25). In the rat, systemic treatment with capsaicin destroys most vagal afferent intraganglionic laminar endings (IGLEs) in the myenteric plexus of the small intestine, the cecum, and colon (3, 29). However, >70% of the vagal afferent IGLEs of the stomach and esophagus do not degenerate after capsaicin, and more than half of vagal afferent intramuscular arrays of the stomach are resistant to capsaicin (3). Consistent with these findings, vagal responses to gastric distension are not abolished by prior capsaicin treatment (44). These results suggest that we might expect a majority of capsaicin-sensitive abdominal afferents to innervate nongastric target organs.

Intramucosal nerve endings in the small intestine are a likely target of CCK action because of their proximity to I cells that release CCK. In the study by Berthoud et al. (3), capsaicin-induced damage to vagal afferent intramucosal nerve endings was not accessed because of the small size of these endings. On the other hand, mucosal application of capsaicin, which is thought to be specific for destroying mucosal vagal afferent nerve endings without affecting vagal nerve endings in muscular layers (33), eliminated the stimulation of pancreatic secretion by physiological stimuli and exogenous CCK (33) and transiently attenuated reduction of food intake by intestinal infusion of oleic acid (64), a satiation stimulus that is reversed by CCK-A receptor antagonists. Taken together, these data are consistent with the possibility that many of the capsaicin-sensitive C-type neurons that respond to CCK innervate the small intestine. Additional experiments using retrogradely labeled vagal afferents are necessary to test this hypothesis.

**Significance of CCK activation of capsaicin-insensitive neurons.** To date, most vagally mediated actions of CCK have been associated with activation of capsaicin-sensitive vagal afferent neurons. Our results indicated that compared with capsaicin-insensitive A-type vagal afferent neurons, capsaicin-sensitive C-type neurons seemed more likely to respond to CCK, their responses to CCK tended to be larger, and triggering of action potentials by CCK occurred more frequently in these neurons. However, at our sample size, these differences did not reach statistical significance. The nature of CCK-triggered reflexes mediated by A-type vagal afferent neurons is not known. The one vagally mediated physiological response reported to be insensitive to capsaicin is the stimulation of pancreatic secretion (24), although others (31, 33) have reported that CCK stimulated pancreatic secretion is abolished by capsaicin pretreatment. One difference between these two studies is that Guan et al. (24) used 10-fold less capsaicin to induce neuronal degeneration than Li and colleagues (31, 33). The study of Guan et al. (24) demonstrated that their treatment was sufficient to prevent inhibition of food intake and gastric emptying by CCK. These results suggest there may be a range of sensitivities of vagal afferent neurons to destruction by capsaicin treatment. Conceivably, CCK-induced pancreatic secretion may rely on neurons that are more resistant to capsaicin than those mediating inhibition of food intake and gastric emptying.

It is possible that CCK modulates the action of other sensory stimuli mediated by A-type vagal afferent fibers. As discussed above, some vagal afferent neurons that respond to gastric distension are not destroyed by capsaicin (3, 44). Schwartz et al. (55, 56) have reported that CCK modulates the response of vagal afferent
neurons to gastric distension. Our observations would be consistent with a potential modulatory role for CCK on capsaicin-insensitive gastric mechanoreceptive neurons. However, additional experiments will be necessary to determine whether the A-type neurons activated by CCK that we observed are indeed associated with capsaicin-insensitive gastric mechanoreceptive innervation. Finally, the presence of CCK-sensitive vagal afferent fibers resistant to capsaicin may explain the persistence of the residual satiating action of relatively high doses of exogenous CCK in capsaicin-treated rats (43, 61).

Advantages and disadvantages of the cultured vagal afferent preparation. The study of vagal afferent neurons in culture has several advantages over in vivo preparations. First, by definition, in vivo preparations include end organs innervated by the vagal afferent fibers. In most cases, nonneural and intrinsic neural cells within the end organs express CCK receptors (1, 40, 49, 50, 52, 53, 60, 65, 66). Therefore, recordings of vagal afferent responses to CCK in vivo may well represent responses of the end organ and not direct responses of vagal afferents themselves. For example Blackshaw and Grundy (6) found that CCK-induced responses of some intestinal afferents were secondary mechanoreponses to CCK-induced activation of intestinal smooth muscle. The use of cultured neurons obviates the problem of indirect responses, because no end-organ tissue is present; hence all responses are direct effects of CCK on the vagal afferents themselves. Second, cultured nodose neurons afford the best preparation to functionally characterize the biochemical and biophysical nature of responses to CCK in actual visceral afferent neurons. The opportunity to do such characterizations in fibers recorded in vivo is very limited, not only because of the presence of other tissues, but also because the small size of vagal afferent fibers makes isolating and identifying single fibers for biochemical or biophysical analysis impractical.

Naturally, the benefits of cultured afferent cell bodies are accompanied by some limitations. First, it is possible that loss of the neuronal processes during isolation of the nodose neurons could result in alteration of their functional phenotypes, such that the cells recorded do not represent the qualities of CCK receptors as they are expressed in the terminals of intact neurons. Second, it is probable that, in vivo, CCK sensitivity of vagal afferents is localized primarily to the terminals of vagal afferent neurons. Therefore, the physiological implications of responses recorded from cultured afferent cell bodies must be acknowledged as speculative. With regard to the notion that the phenotypic properties of isolated vagal afferents may not be representative of neurons of the intact ganglion, it is important to note that the proportion of cultured neurons found to be sensitive to CCK is very near the proportion of nodose ganglion neurons reported to express mRNA for the CCK-A receptor (9). Also, the proportion of neurons found to be CCK-sensitive agrees well with the proportion of vagal fibers that respond to CCK during extracellular recording in vivo (26). Finally, previous studies have established that vagal afferent neurons from the nodose ganglia retain their electrophysiological characteristics in short-term culture (30, 39). Because all of our experiments were conducted within 48 h of isolating the vagal afferent cell bodies, we feel that the neurons we recorded most likely are the same phenotypically CCK-sensitive neurons examined by other methods in vivo.

Limits of the cultured neuron preparation places on interpretation of our results requires added circum- spectation. The fact that many, if not most, vagal afferent CCK binding sites seem destined for peripheral terminals and axons strongly suggests that these sites are adapted to monitor local CCK levels in the extracellular space. Because endogenous CCK is secreted into the extracellular space in close proximity to vagal afferent terminals (2), it is likely that the concentration at the terminals is well above the plasma CCK concentration. Although the actual local CCK concentration is not known, we do know that inhibition of gastric emptying (36) and reduction of food intake by CCK (67) are mediated by low affinity sites of the CCK-A receptor. This site would seem to be well adapted to detecting nanomolar concentrations of CCK close to the site of release, i.e., “paracrine” CCK. Our previous finding (58) that most, if not all, responses by cultured vagal afferents seem to be mediated by the low affinity CCK-A receptor site is consistent with the interpretation that at least some vagal responses to CCK may be paracrine in nature. Furthermore, although the cell body may not be the “normal” location from which CCK responses are evoked in vivo, several autoradiographic studies (22, 37, 69) indicate that cell bodies of neurons within the nodose ganglia do express CCK receptors. Therefore, if one accepts that the receptive phenotype of the vagal afferent neurons is maintained in culture, it seems reasonable that responses of these neurons would be reflective of responses that occur at the neuron terminals in vivo.

In conclusion, CCK depolarized subpopulations of both C-type and A-type vagal afferent neurons in culture. The percentage of nodose neurons that responded to CCK was in fairly close agreement with the proportion of nodose neurons that express CCK receptor mRNA and that respond to CCK with an increase in cytosolic Ca2+. The observation that CCK induces a decrease in input resistance in some vagal afferents is consistent with CCK-mediated activation of a depolarizing conductance. However, a decrease in input resistance was not consistently observed in all vagal afferent neurons; thus the nature of the specific conductance modified by CCK and the cellular mechanism by which CCK modifies this conductance remain to be determined. The fact that CCK-sensitive vagal afferent neurons can be segregated into at least two distinct phenotypes suggests that it may be possible to associate distinct projections and CCK-mediated responses with each phenotype. Finally, the consistency of our results with previous in vivo and ex vivo models suggest that further examinations of cultured nodose neurons will enable us to detail the cellular mechanisms by
which CCK-sensitive vagal afferent neurons participate in control of feeding and gastrointestinal function.

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DISCLOSURES

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