Systemic Cholecystokinin Differentially Affects Baro-activated GABAergic Neurons in Rat Caudal Ventrolateral Medulla

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Cholecystokinin (CCK) is a gastrointestinal peptide released by the intestinal mucosa after ingestion of a meal and plays important roles in digestion and satiety. Digestion is aided by multiple actions of CCK, including changes in gastric motility and secretions (Beglinger 1994) and increased blood flow in the gut (Granger et al. 1980; Heinemann et al. 1996). Systemic CCK appears to enhance the brain’s response to gastric distention by activation of vagal afferent nerves to the brain stem (van de Wall et al. 2005). When given intravenously or stimulated by intraduodenal infusion of fatty acids, CCK can inhibit splanchnic sympathetic nerve activity (sSNA; Sartor and Verberne 2002, 2003; Sartor et al. 2006), which may lessen mesenteric vasoconstriction to promote postprandial gastrointestinal hyperemia. These sympathoinhibitory effects of CCK appear to be specific to the mesenteric circulation because CCK has little effect on blood flow to limbs, skin, or muscle (Chou et al. 1977; Sartor and Verberne 2002).

The neural pathway for CCK-induced inhibition of sSNA requires activation of vagal afferent nerves to the nucleus tractus solitarius (NTS; Sartor and Verberne 2002). In contrast to the uniform inhibition of sympathetic vasomotor nerves evoked by other vagally initiated reflexes, such as the arterial baroreflex and Bezold–Jarisch reflex, the sympathetic responses to CCK vary by target. Whereas sSNA is inhibited by circulating CCK, lumbar SNA innervating the hindlimb is activated (Sartor and Verberne 2002). Elevation in endogenous or exogenous circulating CCK is associated with inhibition of some presympathetic neurons in the rostral ventrolateral medulla (RVLM) (Sartor and Verberne 2002, 2003; Sartor et al. 2006) that are likely to provide the tonic drive to sympathetic vasomotor tone. Interestingly, CCK inhibits some presympathetic RVLM neurons, but not others (Sartor and Verberne 2002, 2003). These data led to the notion that the presympathetic RVLM neurons produce the observed inhibition of sSNA evoked by CCK and that the differential responses of presympathetic RVLM neurons may indicate the sympathetic target of the RVLM neuron (Sartor and Verberne 2002, 2003).

The central mechanisms underlying the CCK-induced alterations in the activity of presympathetic RVLM neurons and SNA are not known. Other sympathoinhibitory reflexes initiated by stimulation of vagal afferents, such as the arterial baroreceptor reflex and the Bezold–Jarisch reflex, require stimulation of inhibitory interneurons in the caudal ventrolateral medulla (CVLM) to activate GABAergic receptors on presympathetic RVLM neurons (Agarwal et al. 1990; Gordon 1987; Sun and Guyenet 1985; Verberne and Guyenet 1992). Indeed, the CVLM contains GABAergic neurons that project toward the RVLM that are activated by increased arterial pressure (AP; baro-activated) and stimulation of the Bezold–Jarisch reflex (Chan and Sawchenko 1998; Minson et al. 1997; Schreihofer and Guyenet 2002, 2003). The present study sought to examine the hypotheses that systemic injection of CCK leads to the activation of GABAergic CVLM neurons and that the CVLM is essential for the production of CCK-induced inhibition of sSNA. The differential responses of baro-activated GABAergic CVLM neurons to CCK may contribute to the diverse responses of presympathetic RVLM neurons and sympathetic outflows observed with systemic CCK.

INTRODUCTION

Cholecystokinin (CCK) is a gastrointestinal peptide released by the intestinal mucosa after ingestion of a meal and plays important roles in digestion and satiety. Digestion is aided by multiple actions of CCK, including changes in gastric motility and secretions (Beglinger 1994) and increased blood flow in the gut (Granger et al. 1980; Heinemann et al. 1996). Systemic CCK appears to enhance the brain’s response to gastric distention by activation of vagal afferent nerves to the brain stem (van de Wall et al. 2005). When given intravenously or stimulated by intraduodenal infusion of fatty acids, CCK can inhibit splanchnic sympathetic nerve activity (sSNA; Sartor and Verberne 2002, 2003; Sartor et al. 2006), which may lessen mesenteric vasoconstriction to promote postprandial gastrointestinal hyperemia. These sympathoinhibitory effects of CCK appear to be specific to the mesenteric circulation because CCK has little effect on blood flow to limbs, skin, or muscle (Chou et al. 1977; Sartor and Verberne 2002).

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M E T H O D S

A subset of individually recorded, baro-activated CVLM neurons was filled with biotinamide using a previously described juxtacellular labeling method to confirm their GABAergic phenotype (Schreihofer and Guyenet 1997, 2002, 2003; Schreihofer et al. 1999, 2000).

Physiological data analyses
The Neurolog system (www.digitimer.com) was used to measure AP, mean AP, and HR. The SNA was amplified and filtered (10-Hz to 3-kHz band-pass with a 60-Hz notch filter; Differential AC amplifier 1700, A-M Systems). The raw SNA signal was full-wave rectified and averaged into 1-s bins. The baseline SNA (100%) was defined as the activity immediately preceding each physiological test and the minimum SNA (0%) was determined after injection of a high dose of phenylephrine or clonidine at the end of the experiment (10 μg/kg iv, Sigma; Mandel and Schreihofer 2006; Schreihofer and Guyenet 2000). Changes in SNA were expressed as a percentage change from baseline.

Recordings of individual CVLM neurons were made with an intracellular amplifier in bridge mode (Neuroprobe 1600, A-M Systems) to allow monitoring of the recording during injection of current through the recording electrode. The neuronal activity was amplified, filtered (300 Hz to 5 kHz), and counted in 1-s bins. Fluctuations in neuronal firing rate after injection of CCK that were <0.5 spikes/s in either direction were considered to be unrelated to the drug treatment because this variability is observed in spontaneous activity. Neurons that showed a change in activity with injection of CCK that was near this threshold were examined twice to confirm the response was the result of CCK.

All analog physiological variables were converted to digital signals (Micro 1401, Cambridge Electronic Design; www.ced.co.uk) and viewed on-line (Spike2 software, Cambridge). For CVLM recording experiments baseline values were measured for 30 s before injection of CCK. The CCK-induced changes for AP, SNA, and unit activity were measured for 2 min, and the initial responses reported reflect the lowest or highest (for activated CVLM neurons) value during the 15-s period after the onset of responses to CCK. The CCK-induced changes in HR, which are likely produced by direct actions on the heart (Gaw et al. 1995; Zhao et al. 2002), were used as a reliable onset time for examining the other physiological responses to CCK (see examples in Fig. 5).

Histology of labeled CVLM neurons
At the end of each experiment with juxtacellularly labeled CVLM neurons, the rat was perfused transcardially with phosphate-buffered saline (250 ml, pH 7.4) and then 4% paraformaldehyde (500 ml, pH 7.4). The brain was removed and stored in fixative for 48 h at 4°C. The brain stem was cut into 30-micron coronal sections using a Vibratome and stored in a cryoprotectant solution at −20°C (Schreihofer and Guyenet 1997). Individual biotinamide-filled, baro-activated CVLM neurons were processed to confirm their location within the CVLM and expression of GAD67 mRNA as previously reported (Mandel and Schreihofer 2006; Schreihofer and Guyenet 2002, 2003). The biotinamide-filled neurons were revealed by incubating the tissue with streptavidin Alexa 488 (Molecular Probes). Expression of GAD67 mRNA was detected using antisense digoxigenin-labeled cRNA probes (Schreihofer and Guyenet 2003; Schreihofer et al. 1999) generated from a full-length cDNA encoding GAD67 (2.7 kb, generously supplied by Dr. A. J. Tobin, University of California, Los Angeles, CA; Erlander et al. 1991). The digoxigenin-labeled ribo-probe was revealed by incubation with a sheep polyclonal anti-digoxigenin antibody conjugated to alkaline phosphatase followed by incubation with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate, 4-toluidine salt, which produced a blue-brown reaction product in the somas of neurons that express GAD67 mRNA (Mandel and Schreihofer 2006; Schreihofer and Guyenet 2003; Schrei-
hofer et al. 1999). Epifluorescence was used to visualize biotinamide-filled CVLM neurons, and brightfield illumination was used to visualize the GAD67 mRNA hybridization reaction product (Schreihofer and Guyenet 2003). Examples of biotinamide-filled CVLM neurons were captured using a digital camera (MagnaFire, Optronics; www.olympusamerica.com). The resulting files were imported into Adobe Photoshop, where they were converted to gray scale with the levels and sharpness adjusted to optimize visualization of the filled neurons.

**CCK and inhibition of the CVLM**

Microinjections of substances were delivered to the brain stem using single-barrel glass pipettes pulled and cut to a tip of 40–50 µm. Glutamate, muscimol, or buffered saline (pH 7.4) were pressure-injected in a volume of 100 nl (Schreihofer and Sved 1992; Schreihofer et al. 2005) over a period of 30 s. The CVLM sites were functionally located by observing decreases in AP (>20 mmHg) after microinjection of glutamate (1 nmol). Once functional sites were located, the pipette was withdrawn, rinsed with water, and filled with muscimol (100 pmol/100 nl). This γ-aminobutyric acid type A (GABA<sub>A</sub>) receptor agonist was microinjected into CVLM to globally inhibit the cell bodies within the region. In control rats buffered saline was microinjected into the CVLM. Bilateral injections were made one side at a time with about 1 min between injections.

With the muscimol- or saline-filled pipette in place in one CVLM, baseline readings of mean AP and HR were recorded for 30 s. Then CCK was injected (8–10 µg/kg, iv), and the initial changes in AP, HR, and sSNA were examined (30 s after onset of HR response). Five minutes later muscimol or buffered saline was microinjected into the CVLM on each side. The changes in AP, HR, and sSNA were recorded 1–2 min after the second microinjection, when peaks in these variables had reached a plateau. Then the injection of CCK was repeated. After responses to CCK, the measurements, of a sympathetic response to PBG (20 µg/kg, iv) was used to confirm that the bilateral microinjections of muscimol had functionally inhibited the CVLM. These responses were always present in rats after bilateral microinjection of saline into the CVLM. At the end of the experiment the pipette was withdrawn and rats were deeply anesthetized with isoflurane (5%). Each rat was transcardially perfused with 4%, pH 7.4) as previously described (Mandel and Schreihofer 2006; Schreihofer and Guyenet 2003). Eight of the CVLM neurons that were activated by CCK (7/35), although the inhibition of sSNA was comparable to that observed with CVLM neurons activated by CCK (Fig. 1A). In addition, three baro-activated CVLM neurons were briskly activated immediately preceding the sympathoinhibitory response, as previously reported (Schreihofer and Guyenet 2003). PBG produced a 91.7 ± 3.8% inhibition of SNA associated with a 14.7 ± 5.4-fold increase in the activity of the CVLM neurons.

**Inhibition of the CVLM by bilateral microinjection of muscimol produces large increases in sSNA. To confirm that enhanced sympathetic drive does not alter the observed sympathetic responses to CCK after inhibition of the CVLM, CCK-induced changes in sSNA were examined before and 5 min after injection of hydralazine (10 mg/kg, iv), which decreased AP to raise sSNA.**

**Statistics**

In the CVLM neuronal recording experiments changes in CVLM neuronal activity, sSNA, mean AP, and HR in response to CCK were each analyzed by paired t-test after separating the CVLM neurons by the type of response to CCK (excited, inhibited, or unaffected). In the microinjection experiments the responses to CCK and PBG before and after microinjection of saline or muscimol into the CVLM were each compared by two-way ANOVA with repeated measures followed by Tukey–Kramer post hoc tests. In all cases values were considered to be significantly different when P < 0.05.

**RESULTS**

**Effects of CCK on SNA and AP**

In the 92 rats used in the following experiments, the injection of CCK significantly decreased sSNA and AP. Within 15 s the maximal decrease in sSNA was −31.8 ± 2.8%, although the response was variable (range = +37 to −77%). In nine of these cases CCK did not evoke the expected inhibition. In addition, the CCK-mediated sympathoinhibition was not a smooth, steady-state response, but vacillated during the reduced period of sSNA (see Figs. 1A and 2B), perhaps as the result of opposing effects of CCK on sSNA or baroreflex-mediated responses to the fluctuating AP. Repeated injections within an animal produced a consistent sympathetic response, suggesting the variability in sympathetic responses between rats may arise from slight differences in the state of the animal.

Injection of CCK also produced a mild depressor response within 15 s (−9.8 ± 1.5 mmHg) in most rats. In a few cases AP did not decrease (see example in Fig. 4B) despite a significant decrease in sSNA, perhaps as a result of the opposing effects of CCK on different sympathetic nerves (Sartor and Verberne 2002). The initial depressor response was followed by a second depressor response (within 30 s; Fig. 2C) that did not correspond to inhibition of sSNA, but coincided with a transient rise in sSNA (Fig. 2B) that was likely to be baroreflex mediated (see Fig. 1, A and B).

**Effects of CCK on baro-activated, pulse-modulated CVLM neurons**

In 29 rats 35 neurons recorded within the CVLM were briskly activated by increased AP (Fig. 1A and B; in six rats one neuron was recorded on each side of the medulla). In addition, each of these neurons displayed activity that was strongly modulated by the AP pulse, especially when AP was raised by constriction of the aortic snare (Schreihofer and Guyenet 2003). Most of the baro-activated CVLM neurons were examined with PBG (26/35) and all of these neurons were briskly activated immediately preceding the sympathoinhibitory response, as previously reported (Schreihofer and Guyenet 2003). PBG produced a 91.7 ± 3.8% inhibition of SNA associated with a 14.7 ± 5.4-fold increase in the activity of the CVLM neurons.

Most of these baro-activated CVLM neurons were moderately activated by CCK (25/35; 3.4 ± 1.1-fold increase; Table 1) and the elevated activity was sustained over the period of sympathoinhibition (Figs. 1A and 2). The activity of a subset of the baro-activated neurons was unaffected by injection of CCK (7/35), although the inhibition of sSNA was comparable to that observed with CVLM neurons activated by CCK (Fig. 1B, Table 1). In addition, three baro-activated CVLM neurons were inhibited by CCK and one of these neurons was completely silenced during the inhibition of sSNA (Fig. 2A, Table 1).

Ten of these baro-activated CVLM neurons were juxtaganglionic filled with biotinamide to confirm a GABA/Aergic phenotype (Mandel and Schreihofer 2006; Schreihofer and Guyenet 2003). Eight of the CVLM neurons that were activated by
CCK and two of the neurons that were unaffected by CCK expressed GAD67 mRNA (Fig. 3).

Effects of CCK on baro-inhibited CVLM neurons

In 34 rats we recorded 44 CVLM neurons that were silenced when AP was raised by constriction of the aortic snare (baro-inhibited, Fig. 4). In 10 rats one cell was recorded on each side of the medulla. Most baro-inhibited neurons examined were inhibited by PBG immediately before the onset of the inhibition of sSNA (35 of the 39 cells tested). The three other cells showed a burst of excitation with PBG followed by inhibition in two of them.

In the majority of baro-inhibited CVLM neurons (34/44) injection of CCK led to a decrease in firing rate (Fig. 4A, Table 2) that was sustained for several minutes, and three of these neurons were silenced by CCK (Fig. 4A). A subset of baro-inhibited CVLM neurons was unaffected by injection of CCK (10/44; Fig. 4B, Table 2), despite a significant decrease in sSNA (Fig. 4B, Table 2).

Effects of CCK on sSNA, AP, and HR after inhibition of the CVLM

To determine whether the CVLM is an essential component of the central pathway for CCK-induced decreases in SNA and AP, we measured responses to CCK before and after bilateral inhibition of neuronal cell bodies in the CVLM by microinjection of the GABA_A receptor agonist muscimol. To confirm the CVLM was functionally inactivated, we examined reflexive changes in SNA and HR produced by activation of the Bezold–Jarisch reflex with PBG (Schreihofer et al. 2005; Verberne et al. 1992). To demonstrate that responses to CCK and PBG could be reliably observed twice in the time frame of the experiment, we also examined responses before and after bilateral microinjection of buffered saline (pH 7.4) into the CVLM.

Eighteen rats were prepared for microinjections of muscimol into the CVLM. Baseline AP was 112 ± 4 mmHg and HR was 337 ± 7 bpm. Before inhibition of CVLM, injection of CCK significantly decreased SNA, AP, and HR (Figs. 5A and 6, A–C). Similarly, injection of PBG significantly decreased SNA, HR, and AP (Fig. 6, A–C). Bilateral microinjection of muscimol into the CVLM increased AP (74 ± 4 mmHg), HR (51 ± 7 bpm), and SNA (to 276 ± 27%). Five minutes after inhibition of the CVLM, injection of CCK now evoked robust increases in SNA (Figs. 5B and 6A). The injections of CCK appeared to effectively enter the systemic circulation in all cases because HR was still decreased, likely by direct effects on the heart (Figs. 5B and 6C; Gaw et al. 1995; Zhao et al. 2002). The decreases in SNA, AP, and HR observed with injection of PBG were reversed to significant increases in all three variables after inhibition of the CVLM (Figs. 5C and 6, A–C).

Eleven rats were prepared for bilateral microinjection of buffered saline into the CVLM as a control group. In these rats baseline AP was 106 ± 3 mmHg and HR was 315 ± 9 bpm. The CVLM sites were determined functionally by microinjection of glutamate, which decreased AP by 25 ± 4 mmHg. The responses to CCK and PBG before and after microinjections of saline into the CVLM were comparable (Figs. 5, D vs. E and 6, D–F).

Effects of CCK after hydralazine-induced increases in sSNA

Inhibition of the CVLM eliminated the sympathoinhibitory response to CCK and unmasked a robust sympathoexcitatory response (Fig. 5, A vs. B). Activation of CCK receptors at the

FIG. 1. Typical responses of baro-activated caudal ventrolateral medulla (CVLM) neurons to systemically injected cholecystokinin (CCK). A: increasing arterial pressure (AP) by constriction of an abdominal aortic snare (at first arrow) produced a burst of activity in the recorded CVLM neuron, followed by a silent period as the release of the snare produced a transient decrease in AP. Injection of CCK (at second arrow) activated the CVLM neuron and decreased splanchnic sympathetic nerve activity (sSNA) and AP. B: increasing AP (at first arrow, snare) excited the CVLM neuron, followed by a brief period of silence as the release of the snare decreased AP. Injection of CCK (at second arrow) did not alter the activity of the CVLM neuron but decreased sSNA, AP, and heart rate (HR). This neuron was classified as insensitive to systemically administered CCK. Both of these baro-activated CVLM neurons showed prominent pulse modulation of activity and activation by injection of phenyl biguanide (PBG).

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sympathetic ganglia appears to enhance sympathetic neurotransmission (e.g., Mo and Dun 1986). Thus the effects of CCK on sympathetic ganglia and postganglionic SNA may be influenced by the level of SNA, which is substantially increased after inhibition of the CVLM. To determine whether the CCK-induced sympathoexcitatory response could be a result of the heightened state of SNA produced by inhibition of the CVLM, we examined CCK responses after a hydralazine-induced increase in SNA. Hydralazine decreased AP by 36/11006 8 mmHg and increased SNA to 200/11006 15% (n = 6; see Fig. 7). Under these conditions, CCK continued to inhibit SNA (−28 ± 3%), suggesting the sympathoexcitatory response to CCK after inhibition of the CVLM was not a result of the elevated SNA.

## DISCUSSION

Circulating CCK stimulates vagal afferent nerves to evoke inhibition of sSNA, which may contribute to postprandial hyperemia. Analogous to other vagal reflexes that regulate sympathetic vasomotor tone, such as the arterial baroreflex and Bezold–Jarisch reflex, CCK appears to inhibit sSNA by decreasing the activity of presympathetic neurons in the RVLM (Sartor and Verberne 2002, 2003). In the present study systemic injection of CCK led to the activation of the majority of recorded baro-activated neurons in the CVLM, and inhibition of the CVLM abolished CCK-induced inhibition of sSNA. These observations suggest that baro-activated GABAergic CVLM neurons produce CCK-induced inhibition of presympathetic RVLM neurons and sSNA, as previously reported for other vagally mediated sympathoinhibitory reflexes. In addition, we observed that some baro-activated neurons of the CVLM are inhibited or unaffected by systemic CCK. These data suggest the CVLM may also contribute to the diverse responses of presympathetic RVLM neurons and sympathetic nerves to systemic CCK (Sartor and Verberne 2002).

### TABLE 1. Initial effects of systemic cholecystokinin (CCK) on the activity of baro-activated neurons in the CVLM and splanchnic sympathetic nerve activity (sSNA)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Baseline</th>
<th>After CCK</th>
<th>Change in sSNA</th>
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<tbody>
<tr>
<td>CCK-activated</td>
<td>25</td>
<td>2.5 ± 0.5</td>
<td>6.4 ± 0.8</td>
<td>−27.2 ± 5.2*</td>
</tr>
<tr>
<td>CCK-insensitive</td>
<td>7</td>
<td>3.2 ± 1.7</td>
<td>3.2 ± 1.8</td>
<td>−18.4 ± 8.4*</td>
</tr>
<tr>
<td>CCK-inhibited</td>
<td>3</td>
<td>3.0 ± 0.4</td>
<td>0.8 ± 0.6</td>
<td>−38.7 ± 16.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. CVLM neuronal activity is expressed in spikes/second; change in sSNA is expressed as percent change from baseline. *P < 0.05 compared to baseline. Baseline mean AP in this group of rats was 115 ± 2 mmHg.
A critical assumption of the present study is that individually recorded baro-activated CVLM neurons innervate the presympathetic RVLM neurons to promote decreases in SNA and AP. Although adjacent cholinergic vagal motor neurons in the CVLM display some physiological properties that are indistinguishable from GABAergic CVLM neurons, they do not express GAD67 mRNA (Schreihofer and Guyenet 2003). For this reason, in the present study a GABAergic phenotype was conclusively established for a subset of CVLM neurons that responded to CCK. In addition, we cannot demonstrate that individually recorded CVLM neurons make synaptic contacts with presympathetic RVLM neurons. Available evidence suggests the CVLM contains GABAergic neurons that tonically inhibit presympathetic RVLM neurons to decrease SNA and mediate sympathetic cardiovascular reflexes (Gordon 1987; Schreihofer et al. 2005; Verberne and Guyenet 1992; Willette et al. 1984). In agreement, the activity of many baro-activated CVLM neurons is inversely related to the activity of presympathetic RVLM neurons and SNA under resting conditions and with the production of sympathetic reflexes that require the CVLM and RVLM (Schreihofer and Guyenet 2003). CVLM neurons with these physiological properties have been shown to project toward the RVLM and express GAD67 mRNA (Agarwal and Calaresu 1991; Jeske et al. 1993; Schreihofer and Guyenet 2003). Thus although the ability of the baro-activated CVLM neurons to regulate the presympathetic RVLM neuronal activity is speculative, these neurons are excellent candidates for the critical inhibitory interneurons that mediate sympathoinhibitory reflexes in the brain stem.

In the present study systemic CCK also led to altered firing rates of baro-inhibited neurons in the CVLM. In contrast to the baro-activated CVLM neurons, previous studies showed these neurons are not GABAergic, but are catecholaminergic neurons of the caudal portion of the C1 cell group (Mandel and Schreihofer 2006; Schreihofer and Guyenet 2003; Stornetta et al. 1999; Verberne et al. 1999). Several observations suggest these are not the neurons that inhibit presympathetic RVLM neurons to decrease SNA and AP. These neurons do not appear to innervate the RVLM, but instead project to forebrain regions associated with autonomic function (Verberne et al. 1999).

### TABLE 2. Initial effects of systemic CCK on the activity of baro-inhibited neurons in the CVLM and sSNA

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Baseline</th>
<th>After CCK</th>
<th>Change in sSNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK-inhibited</td>
<td>34</td>
<td>3.5 ± 0.4</td>
<td>1.1 ± 0.2*</td>
<td>−29.1 ± 3.8*</td>
</tr>
<tr>
<td>CCK-insensitive</td>
<td>10</td>
<td>1.8 ± 0.6</td>
<td>1.5 ± 0.6</td>
<td>−24.5 ± 3.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE. CVLM neuronal activity is expressed in spikes/second; change in sSNA is expressed as percent change from baseline. *P < 0.05 compared to baseline. Baseline mean AP in this group of rats was 109 ± 2 mmHg.
The differential responses of the baro-activated CVLM neurons do not appear to be due to the efficacy of CCK in evoking inhibition of sSNA because an equivalent inhibition of sSNA was achieved in rats where CVLM neurons were excited, inhibited, or unaffected (see Table 1). However, the physiological consequences of the differential responses of the CVLM neurons to CCK are not certain. Do the baro-activated CVLM neurons that respond differentially to CCK all innervate the same sympathetic targets, or do they provide selective influence to particular SNAs? If baro-activated CVLM neurons displaying these three types of responses to CCK all influence sSNA, this may explain why the maximal CCK-induced sympathoinhibition is only roughly 30% compared with the near abolition of sSNA by PBG, which vigorously activates all baro-activated CVLM neurons. Alternatively, several observations support the notion that individual baro-activated CVLM neurons may selectively influence the activity of different sympathetic nerves. Whereas PBG activates all baro-activated CVLM neurons and universally inhibits barosensitive SNAs, CCK produces differential responses in baro-activated CVLM neurons and in sSNA versus lumbar SNA (Sartor and Verberne 2002; Verberne and Guyenet 1992). In addition, in the present study the activity of the baro-activated, CCK-activated CVLM neurons mirrored the fluctuations in sSNA throughout the 2-min period of recording, but baro-activated CVLM neurons that were unaffected or inhibited by CCK did not follow the patterns of SNA over this same time course (Fig. 2). Although more evidence is clearly needed to establish differential control of various sympathetic outflows by the CVLM, these data support the notion that individual CVLM neurons are differentially regulated by a physiological stimulus that produces diverse sympathetic responses.

In contrast to the established roles of vagal afferent nerves and ventrolateral medulla for CCK-induced inhibition of sSNA, the mechanism underlying CCK-induced activation of SNA is not known. Examinations of sympathetic responses to CCK were previously performed using recordings of postganglionic sympathetic fibers. It is possible that some of the sympathoexcitatory responses to CCK are mediated by CCK receptors in the autonomic ganglia themselves. For instance, activation of mesenteric and pancreatic ganglionic neurons with CCK facilitates nicotinic neurotransmission (Ma and Szurszewski 1996; Mo and Dun 1986; Schumann and Kreulen 1986), which could enhance SNA by a peripheral mechanism. Here, CCK-mediated activation of ganglionic nerves may be more pronounced when sympathetic ganglionic neurotransmission is elevated. In the present study we sought to determine whether the elevation in sSNA produced by the inhibition of CVLM could be responsible for the subsequent activation of sSNA by CCK. However, in the face of hypotension-induced sympathoactivation, CCK still produced an inhibition of sSNA (Fig. 7), suggesting CCK-mediated sympathoactivation cannot be explained solely as a response that occurs during times of elevated SNA. Clearly, the sympathetic responses to CCK are complex and appear to involve simultaneous sympathoexcitatory and sympathoinhibitory inputs to sSNA. Whether the vagus nerves or any central pathways are involved in the

FIG. 5. Representative responses to CCK before and after microinjection of muscimol or saline into the CVLM. A: injection of CCK produced increases in sSNA, AP, and HR. B: in the same rat shown in A, 5 min after bilateral microinjection of muscimol into the CVLM (note higher scales for sSNA and AP arising from increases produced by muscimol) injection of CCK produced a robust increase in sSNA with decreases in AP and HR. C: in the same rat shown in A and B, 10 min after injection of CCK, injection of PBG evoked increases in sSNA and AP with no effect on HR, indicating functional inhibition of the CVLM. D: in another rat, injection of CCK produced decreases in sSNA, AP, and HR. E: in the same rat shown in D, 5 min after bilateral microinjection of saline into the CVLM, injection of CCK produces the same decreases in sSNA, AP, and HR. F: in the same rat shown in D and E, 10 min after injection of CCK, injection of PBG produces decreases in sSNA, AP, and HR (after a brief rise in sSNA and AP), indicating a functional CVLM.
activation of lumbar SNA or sSNA after inhibition of the CVLM remains to be determined. Several of the CCK-mediated cardiovascular responses observed in the present study are likely to be attributable to peripheral actions of CCK directly at the blood vessels and heart. For example, the second phase of the depressor response to CCK in the present study persisted after inhibition of the CVLM (Fig. 5B) and did not coincide with a reduction in sSNA (Fig. 2). During this period the decrease in AP was instead associated with a transient reduction in the activity of baro-activated, CCK-activated CVLM neurons and a small rise in SNA (Fig. 2). This depressor response may have arisen from a CCK-mediated vasodilation that occurs at the blood vessels (Sanchez-Fernandez et al. 2004). The bradycardia evoked by CCK is also likely to be the result of actions at the target organ because activation of myocardial CCK_A receptors slows HR (Gaw et al. 1995; Zhao et al. 2002). In agreement, inhibition of the CVLM did not alter the bradycardic response to CCK, suggesting that a contribution by this sympathoinhibitory pathway is relatively minor. In addition, it is not likely that the persistent bradycardia arose from the activation of adjacent vagal motor neurons in the CVLM. Given the large size of the microinjections of muscimol into the CVLM in the present study, nearby cardiac vagal motor neurons are also likely to be inhibited. Indeed, after microinjections of muscimol into the CVLM, the vagally mediated bradycardia normally elicited by PBG was abolished (see Fig. 6C). Here the change in SNA reflects the difference between baseline and the peak SNA within 15 s after the onset of the change in HR.

In summary, clearly the autonomic and cardiovascular responses to CCK are complex, with diverse responses observed in splanchnic and lumbar sympathetic nerves and both direct and indirect influences of CCK on cardiovascular targets. Existing data suggest that CCK-induced inhibition of sSNA is mediated by altering the activity of neurons in the CVLM and RVLM. However, mechanisms underlying the excitatory effects of CCK on lumbar SNA are not known. In addition, opposing responses to CCK can also be observed within a sympathetic nerve. When the sympathoinhibitory effect of CCK on sSNA is eliminated by inhibition of the CVLM, a powerful CCK-mediated excitation of sSNA is unmasked. Analogous to the heterogeneous responses to systemic CCK

**FIG. 6.** Effects of inhibition of the CVLM on sympathetic and cardiovascular responses to CCK and PBG. Filled bars = before microinjections into the CVLM; open bars = same rats after microinjections into the CVLM. A–C: rats with microinjections of muscimol into the CVLM. D–F: rats with microinjections of saline into the CVLM. *Significant change with drug treatment (CCK or PBG), P < 0.05. †Significant difference from response observed before microinjections of muscimol into the CVLM, P < 0.05. Changes are the difference between the baseline (30 s before injection) and the peak or trough occurring within 15 s of the onset of change in heart rate. For PBG, the brief burst in SNA occurring in a few rats was not analyzed and the values reflect the longer-lasting sympathoinhibition (see Fig. 5F). After inhibition of the CVLM with muscimol the sympathoexcitatory response was observed in all rats in the absence of sympathoinhibition (see Fig. 5C). Here the change in SNA reflects the difference between baseline and the peak SNA within 15 s after the onset of the change in HR.

**FIG. 7.** Typical example of the effects of hydralazine on SNA and AP responses to injection of CCK. Before hydralazine injection of CCK (at first arrow) decreased sSNA and AP. Injection of hydralazine (at second arrow) decreased AP and increased sSNA. During hydralazine-induced sympathoexcitation injection of CCK (at third arrow) still produced decreases in sSNA and AP.
observed in SNA, barosensitive neurons in both the CVLM and RVLM are differentially affected by CCK. These distinct responses to CCK may provide a roadmap to the innervation of specific sympathetic targets by neurons in the CVLM and RVLM.

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