Cholecystokinin selectively affects presympathetic vasomotor neurons and sympathetic vasomotor outflow

DANIELA M. SARTOR AND ANTHONY J. M. VERBERNE
Clinical Pharmacology and Therapeutics Unit, Department of Medicine, Austin and Repatriation Medical Centre, University of Melbourne, Heidelberg, Victoria 3084, Australia

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Sartor, Daniela M., and Anthony J. M. Verberne. Cholecystokinin selectively affects presympathetic vasomotor neurons and sympathetic vasomotor outflow. Am J Physiol Regulatory Integrative Comp Physiol 282: R1174–R1184, 2002; 10.1152/ajpregu.00500.2001.—Cholecystokinin (CCK) is a potential mediator of gastrointestinal vasodilatation during digestion. To determine whether CCK influences sympathetic vasomotor function, we examined the effect of systemic CCK administration on mean arterial blood pressure (MAP), heart rate (HR), lumbar sympathetic nerve discharge (LSND), splanchic sympathetic nerve discharge (SSND), and the discharge of presympathetic neurons of the rostral ventrolateral medulla (RVLM) in α-chloralose-anesthetized rats. CCK (1–8 μg/kg iv) reduced MAP, HR, and SSND and transiently increased LSND. Vagotomy abolished the effects of CCK on MAP and SSND as did the CCK-A receptor antagonist devazepide (0.5 mg/kg iv). The bradycardic effect of CCK was unaltered by vagotomy but abolished by devazepide. CCK increased superior mesenteric arterial conductance but did not alter iliac conductance. CCK inhibited a subpopulation (~49%) of RVLM presympathetic neurons whereas ~28% of neurons tested were activated by CCK. The effects of CCK on RVLM neuronal discharge were blocked by devazepide. RVLM neurons inhibited by exogenous CCK acting via CCK-A receptors on vagal afferents may control sympathetic vasomotor outflow to the gastrointestinal tract vasculature.

blood pressure; splanchic sympathetic nerve; lumbar sympathetic nerve; rat

CHOLECYSTOKININ (CCK) is a gastrointestinal hormone with actions including gastrointestinal vasodilatation (18), reduced gut motility, gastric acid secretion, and pancreatic secretion (3, 28). In the central nervous system, it is one of the most abundant neuropeptides, probably playing a major role in satiety as well as anxiety-related behavior (6, 38). Of the two types of CCK receptors, the A-type (CCK-A receptor) is predominantly found in the periphery, although it is also present in discrete brain regions and has a higher affinity for the sulfated octapeptide form of CCK. In the brain, the B-type receptor (CCK-B receptor) predominates and has a high affinity for both the sulfated and nonsulfated forms of CCK (13).

Peripherally, CCK has been implicated in postprandial intestinal hyperemia because systemic administration of the peptide produces intestinal vasodilatation (18). In contrast, its effects on the vascular resistance of forelimb, skin, or muscle have been found to be negligible (10), suggesting that the actions of CCK may target specific vascular beds.

CCK receptors are located on vagal afferents (11, 35) and in the nucleus of solitary tract (NTS) (7, 35), a major site of termination of vagal afferents. Although peptides generally do not permeate the blood-brain barrier, CCK may potentially act on neurons of the area postrema, a circumventricular organ with neuronal cell bodies lying outside the blood brain barrier but with projections to several areas within the brain (6). There are also regions within the NTS and the dorsal nucleus of vagus nerve that have altered blood-brain barrier properties potentially facilitating the access of peptides such as CCK (6). Thus CCK may act either directly on the central nervous system to influence sympathetic vasomotor function and/or peripherally via vagal afferent fibers, conveying inhibitory sensory signals to the brain and subsequently to presympathetic vasomotor neurons.

Many studies have characterized the effects of CCK on the discharge of vagal afferent nerve fibers arising from mechano- or chemoreceptors of the gastrointestinal system (5, 23). However, little attention has been paid to the effect of CCK on sympathetic vasomotor outflow or on barosensitive, spinally projecting neurons of the rostral ventrolateral medulla (RVLM) (20).

The present study was designed to characterize the effects of intravenously administered CCK on mean arterial blood pressure (MAP), heart rate (HR), lumbar sympathetic nerve discharge (LSND), splanchic sympathetic nerve discharge (SSND), and regional vascular conductance. In addition, we examined the effect of CCK on the discharge of presympathetic neurons of the RVLM to determine whether CCK could discriminate subgroups of these neurons. Finally, we examined the importance of vagal afferents and the specific CCK receptor subtype responsible for the effects of CCK on sympathetic vasomotor function.
METHODS

All experiments were performed using male Sprague-Dawley rats (250–380 g). This study was approved by the Ethical Review Committee of the Austin and Repatriation Medical Centre (Heidelberg, Victoria, Australia) and complied with the principles outlined in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

General procedures. Rats were tracheostomized after induction of anesthesia produced by placement into a chamber saturated with halothane vapor (Fluothane, Zeneca, Macclesfield, UK). After cannulation of the trachea, all animals were ventilated artificially with 100% O2 (1 ml/100 g body wt, 40–60 breaths/min) containing 1.3–1.5% halothane. The deep surgical level of anesthesia produced by halothane was maintained throughout the entire surgical procedure during which the absence of firm paw-pinch and corneal probing responses were used to verify the depth of anesthesia. Core temperature was maintained at 36–38°C using a servo-controlled heating pad. The left carotid artery and left jugular vein were cannulated to measure arterial blood pressure and HR and for intravenous drug administration, respectively.

After surgery was completed, the inspired halothane concentration was gradually reduced to zero and α-chloralose (70 mg/kg, iv) was administered slowly over a 20- to 30-min period. Once an appropriate level of anesthesia was achieved, as ascertained by application of the tests described above, the paralyzing agent pancuronium bromide (1 mg/kg, iv) was administered. After neuromuscular blockade was established, the stability of the arterial blood pressure and HR record and the absence of a pressor response to firm hindlimb toe pinch were used as indications of adequate anesthesia. Supplements of α-chloralose (20–25 mg/kg, iv) were administered as needed according to the above criteria or on an hourly basis. Adequacy of anesthesia was also confirmed before administration of pancuronium supplements (0.3–0.5 mg/kg). Pancuronium was supplemented hourly or as indicated by a muscle twitch response to spinal stimulation.

Sympathetic nerve recording. LSND and SSND were recorded in separate groups of rats. The lumbar sympathetic nerve trunk between L3 and L5 was exposed via a midline abdominal incision after isolation and section of the iliolumbar blood vessels. Splanchnic nerve discharge was recorded from the greater splanchnic nerve after exposure via a retroperitoneal incision. Doses of CCK (1–8 μg/kg) were administered via the jugular vein as a bolus injection, and the effects on LSND or SSND were recorded using an alternating current-coupled amplifier and filter (30–3,000 Hz) and stored on videotape (PCM recorder, Vetters Instruments, Rebersburg, PA) together with the arterial blood pressure signal. Changes in HR were recorded using a polygraph for subsequent analysis. After CCK administration, rats either underwent bilateral cervical vagotomy or received the CCK-A receptor antagonist devazepide (30) (0.5 mg/kg, iv) or vehicle. Once MAP, HR, and sympathetic nerve discharge were stabilized after α-chloralose administration, doses of CCK (1–8 μg/kg) were administered in random order and each dose was given at least twice. At the conclusion of each experiment in which sympathetic nerve discharge was recorded, the α2-adrenoceptor agonist clonidine (200 μg/kg, iv) was administered. At this dose, clonidine produced a pronounced increase in arterial blood pressure and subsequent reflexly mediated sympathoinhibition as well as a powerful central sympathetic inhibitory effect. The residual nerve discharge signal remaining after clonidine administration was regarded as noise that, on subsequent computer analysis, was systematically subtracted from the full-wave rectified signal and so established the zero level of nerve discharge. Sympathetic nerve discharge was analyzed offline, full-wave rectified, and averaged over 1-s intervals using a computer-based data acquisition system as described previously (21, 44). The level of resting sympathetic nerve discharge recorded at the beginning of the experiment was used as the level of 100% nerve discharge and was derived by averaging the signal over a 30-s period. Sympathetic nerve discharge was therefore quantified as arbitrary units of activity. Responses to CCK were measured at the nadir of the initial depressor phase of the response by averaging the nerve discharge over the duration of the maximal period of the response. In all experiments, other than those in which single unit recording was performed, the animals were killed by overdose with halothane (4% in the inspired air).

Extracellular single unit recording. Extracellular single unit recording was performed in experiments separate from those in which sympathetic nerve activity was recorded. In addition to the general procedures outlined above, an inflatable occlusive cuff was placed around the abdominal aorta just below the level of the diaphragm. This was used to produce a precisely controlled elevation of arterial blood pressure to stimulate the baroreceptors. Rats were then placed into a stereotaxic apparatus, and the dorsal cerebellar surface was exposed by removal of a portion of the interpeduncular bone. A bipolar electrode was placed on the mandibular branch of the right facial nerve that, when stimulated (0.1-ms pulses, 0.5 Hz, 0.3–1.0 mA), produced an antidromic field potential within the facial nucleus of the ventral medulla. The magnitude of the field potential was used to identify the caudal, medial, and ventral contours of the facial nucleus as previously described (8, 46). A bipolar electrode was also placed into the dorsolateral funiculus of the thoracic spinal cord (T2-T3), enabling antidromic activation of spinally projecting, barosensitive neurons within the RVLM. The collision test, invariant antidromic latency, and high frequency following (twin pulses, 3-ms pulse interval) were used to establish the antidromic nature of spikes produced by spinal stimulation (0.5 Hz, 0.5-ms duration, 0.3–2.5 mA intensity). Conduction velocities of spinal axons were calculated by dividing the straight-line distance between the recording electrode and the spinal stimulating electrode (in m) by the antidromic latency (in s). Glass microelectrodes (2 mm OD) containing 0.5 M sodium acetate-2% Pontamine sky blue were used to record extracellularly from neurons in the RVLM. The signals were amplified (×1,000), filtered (400–4,000 Hz), and monitored using an oscilloscope and an audio amplifier. The effects of CCK (1–8 μg/kg, iv) on arterial blood pressure and the discharge rate of RVLM barosensitive, spinally projecting neurons were recorded and stored on videotape together with blood pressure responses. Neuronal discharge rates were measured at rest before manipulation of arterial blood pressure levels or injection of any drugs. A change in discharge rate in response to CCK was measured by comparing the resting level of discharge prior to CCK administration with the discharge rate directly corresponding to the nadir of the depressor response. The doses of CCK were chosen on the basis of previous reports (26, 31, 41) and also by performing preliminary experiments that determined the threshold for the effects of CCK on arterial blood pressure, RVLM unit discharge, and sympathetic nerve discharge.

Regional vascular conductance measurement. In experiments separate from those in which sympathetic nerve discharge was recorded, miniaturized Doppler flow probes (Iowa Doppler Products, Iowa City, IA) were placed onto the super-
rior mesenteric artery and an iliac artery for measurement of superior mesenteric and hindlimb vascular conductance. Conductance (given in kHz·mmHg⁻¹·1,000) was calculated by dividing flow (measured in kHz Doppler shift, which is directly proportional to blood velocity) by MAP.

Histological analysis of recording sites. At the conclusion of each unit recording experiment, the animals were deeply anesthetized with pentobarbital sodium (Nembutal, Rhone Merieux Australia, Pinkenba, Queensland, Australia; 60 mg/kg, ip) before transcardiac perfusion with 4% formaldehyde-Tris-buffered saline (0.05 M, pH 7.6) solution. The brains were then collected for histological verification of recording sites. Recording sites within the RVL were marked by iontophoretic deposition of Pontamine sky blue from the recording electrode. The brains were sectioned using a cryostat, mounted onto gelatin-subbed slides, and stained for Nissl substance with cresyl violet. Recording sites were identified under the light microscope and mapped onto standard maps of the rat brain with reference to a rat brain atlas (37) (see Fig. 11).

Data analysis and statistics. Arterial blood pressure, sympathetic nerve discharge, single-unit activity, and stimulation pulses were recorded onto videotape using a pulse code modulation data acquisition recorder (Vetter Instruments). These signals were analyzed subsequently using customized computer-assisted data acquisition software. Data are expressed as means ± SE. Differences between means were compared by one-way ANOVA followed by a Tukey-Kramer test. P < 0.05 was considered to be significant.

Drugs. α-Chloralose (30 mg/ml; Sigma-Aldrich, Castle Hill, Australia) was dissolved in 2% sodium tetraborate. Phenylbiguanide (PBG; Aldrich Chemical, Milwaukee, WI) and CCK octapeptide (sulfated form; American Peptide, Sunnyvale, CA) were dissolved in normal saline (0.9% wt/vol NaCl). Devazepide (L-364,718; Merck Research Laboratories, Rahway, NJ) was dissolved in DMSO and polyethyleneglycol 400 (9:1) followed by 1:1 dilution with normal saline.

RESULTS

Effects of CCK on MAP, HR, and LSND. The effects of CCK (1 and 8 μg/kg) on MAP, HR, and LSND are shown in Figs. 1 and 2. The measured response coincided with the periods indicated by the shaded areas. CCK consistently produced a dose-dependent depressor response that was consistently accompanied by a dose-dependent bradycardia (see Fig. 2). The depressor response was often biphasic: an initial brief depressor response was followed by a more prolonged depressor phase. In 26% of the cases, the depressor response produced by CCK was followed by a more sustained secondary increase in MAP. The secondary pressor response did not occur in all animals and was not studied further, in contrast to the initial depressor and bradycardic effects of CCK, which were observed in all experiments. CCK (1–8 μg/kg) produced significant increases in LSND (P < 0.05, n = 9) that coincided with the nadir of the initial depressor response and were not clearly dose dependent.

Effect of CCK on SSND: effects of bilateral cervical vagotomy and CCK-A receptor blockade. CCK produced a significant decrease in SSND that was dose dependent, immediate, and sustained over a period of several minutes before returning to baseline levels (Figs. 3 and 4, prevagotomy). On occasions when CCK produced a depressor response followed by a more pronounced secondary pressor response, the inhibitory effect on the splanchnic nerve discharge was unchanged, suggesting that blood pressure changes were not primarily responsible for the decrease in activity.

Bilateral cervical vagotomy abolished the depressor responses to CCK, which thereafter resulted in small increases in MAP and SSND that were not clearly dose dependent and not significantly different from baseline. The effect of bilateral vagotomy on the MAP responses to CCK administration was only significant for the two higher doses of CCK tested (4 and 8 μg/kg). Bilateral cervical vagotomy abolished the inhibitory effect of CCK on SSND for all doses tested (Fig. 4; n = 8; P < 0.01 for 1 μg/kg dose; P < 0.001 for all other doses; Tukey-Kramer multiple comparisons test). The bradycardic response to CCK was unaffected by bilateral vagotomy (Fig. 4; P > 0.05).

Systemic administration of the selective CCK-A receptor antagonist devazepide (0.5 mg/kg, iv) produced a significant increase in MAP (95 ± 4 and 108 ± 7 mmHg, before and after devazepide, respectively; P > 0.05) and a significant decrease in HR (445 ± 6 and 420 ± 9 beats/min, before and after devazepide, respectively; P < 0.05) but did not significantly alter SSND (101 ± 11 and 93 ± 8 U before and after devazepide, respectively; P > 0.05).

Devazepide significantly reduced the effects of CCK on MAP, HR, and SSND (Figs. 5 and 6; P < 0.05, respectively; Tukey-Kramer multiple comparisons test).
test; \( n = 9 \) experiments). During devazepide administration, a significant elevation of arterial blood pressure (\( P < 0.05 \)) but not sympathetic nerve discharge was observed (Fig. 5). Devazepide administration abolished the effects of CCK injection on MAP, HR, and SSND (Fig. 6). Recovery from the effects of CCK-A receptor blockade produced by systemic administration of devazepide was not observed over the course of the remainder of the experiment (for up to 2 h). Administration of vehicle had no statistically significant effects on the SSND, MAP, or HR responses to CCK (\( n = 4; P > 0.05 \)).

**Effect of CCK on regional blood flow.** In six experiments, CCK produced significant increases in superior mesenteric conductance ranging from 1.3 ± 0.1 to 1.7 ± 0.1 kHz·mmHg\(^{-1} \cdot 1,000\) accompanied by dose-dependent depressor responses ranging from \(-4 \pm 1\) to \(-10 \pm 2\) mmHg. The increases in superior mesenteric conductance were not clearly dose related. CCK produced only small changes in iliac conductance, ranging from \(0.0 \pm 0.1\) to \(0.3 \pm 0.3\) kHz·mmHg\(^{-1} \cdot 1,000\) (Fig. 7).

**Effect of CCK on discharge of RVLM presympathetic vasomotor neurons.** Ventrolateral medullary bulbo-splanchnic barosensitive neurons were recorded in 15 separate experiments and tested for responsiveness to systemic administration of CCK. These neurons were (1) located within 500 \( \mu \)m of the caudal pole of the facial nucleus, (2) spontaneously active, and (3) silenced by elevation of arterial blood pressure to \(>130–150\) mmHg and by intravenous administration of PBG (10 \( \mu \)g/kg, iv) (Fig. 8B). For all neurons tested, spinal cord stimulation produced a constant latency spike that could be collided with the spontaneous neuronal spikes and therefore satisfied the conditions of the collision test (Fig. 8A). CCK administration caused either dose-dependent inhibition of the discharge of these cells (19/39 cells tested; 49%), an increase in the discharge rate (11/39 cells tested; 28%), or no change (9/39 cells tested; 23%). The degree of inhibition of neuronal discharge produced by CCK was dose dependent and ranged from \(-20\%\) reduction in discharge rate for the lowest CCK dose tested (1 \( \mu \)g/kg) to complete inhibition of discharge produced by the highest doses tested (4 or 8 \( \mu \)g/kg). The inhibitory effects of CCK on neuronal discharge preceded the effects of CCK on arterial blood pressure.

The activation of neuronal discharge by CCK ranged from \(-50\%\) to 400\% increase above resting discharge. Figure 9 depicts the responses of a neuron activated by CCK. The activation ranged from \(-200\%\) increase in discharge rate with the lower dose of CCK (1 \( \mu \)g/kg) to

![Graph showing effects of CCK on arterial blood pressure and sympathetic nerve responses](image)

**Fig. 2.** Effects of systemic administration of CCK on arterial blood pressure, LSND, and heart rate (HR). CCK (1–8 \( \mu \)g/kg) reduced MAP and HR and increased LSND. \( \Delta \)MAP, \( \Delta \)HR, and \( \Delta \)LSND are changes relative to preinjection levels. Values are means ± SE; \( n = 9 \) experiments. *\( P < 0.05 \).

**Fig. 3.** Arterial blood pressure and splanchnic sympathetic nerve responses to systemic administration of CCK: effect of bilateral cervical vagotomy. The effects of CCK (1 and 8 \( \mu \)g/kg) on MAP and splanchnic sympathetic nerve discharge (SSND) before and after bilateral cervical vagotomy are shown. The responses were taken as the average over the periods indicated by the shaded bars. Vagotomy reduced the sympathoinhibitory responses to CCK and abolished the depressor responses. CCK was administered at the time indicated by ●.
Neurons were classified as nonresponsive to CCK if it was judged that the inhibition was secondary to an increase in arterial blood pressure. This was clearly evident compared with neurons that were inhibited by CCK prior to any increase in MAP.

Neurons that were inhibited by CCK administration had a significantly higher basal discharge rate (16 ± 2 spikes/s; range: 0.5–33 spikes/s; n = 19 cells) and axonal conduction velocity (3.5 ± 0.4 m/s; range: 0.5–5.9 m/s) than those activated by CCK administration (discharge rate: 8 ± 2 spikes/s; range: 1–18 spikes/s; P < 0.05; and conduction velocity: 1.1 ± 0.3 m/s; range: 0.5–2.8 m/s; n = 11 cells; P < 0.05 for both comparisons). Neurons unaffected by CCK administration had a discharge rate of 5 ± 1 spikes/s (range: 0.5–11 spikes/s; n = 9 cells) and a conduction velocity of 0.9 ± 0.3 m/s (range: 0.4–3.1 m/s), which were significantly lower than those observed for the cells inhibited by CCK (P < 0.05 for both comparisons).

Effects of CCK-A receptor blockade on response of RVLM presympathetic neurons to CCK. We investigated the effects of devazepide on the response of RVLM presympathetic neurons to CCK. In five of six cases, CCK inhibited the discharge rate of the cell by 20–100%, depending on the dose of CCK as described above. Figure 10A shows the responses of an RVLM medullospinal barosensitive neuron to CCK (1 and 4 μg/kg). In this example, CCK inhibited the discharge rate of the cell by 20–70% depending on the dose of CCK. These responses were subsequently completely abolished by administration of the CCK-A receptor antagonist devazepide (Fig. 10B). After devazepide administration, only a small pressor response was observed upon readministration of CCK. Similarly, for one cell that was activated by CCK, devazepide completely abolished the CCK-induced effect (data not shown). Devazepide administration abolished the responses to CCK but did not alter the inhibition of neuronal discharge produced by PBG or baroreceptor activation (aortic occlusion) (Fig. 10B). During administration of devazepide, a significant elevation of arterial blood pressure (P < 0.05) but not unit discharge was observed.

Localization of RVLM recording site. The location of a typical recording site from the single unit recording experiments is shown in Fig. 11. This was based on the histological identification of the Pontamine sky blue deposits made within the RVLM. All RVLM units were recorded within 300 μm of this site.

DISCUSSION

CCK modifies motor activity of the gastrointestinal tract and modulates satiety through a mechanism dependent on vagal afferent nerve fibers (6). In addition to having these regulatory actions, CCK also acts on gastrointestinal blood flow (18, 39). Digestion is accompanied by intestinal vasodilatation (postprandial hyperemia) (42), which is dependent on an intact vagus but not mediated by parasympathetic vasodilator activity (36). The mechanisms associated with postpran-
dial hyperemia appear to be complex (16) and may include release of local factors such as nitric oxide, prostaglandins, and polypeptides such as secretin, neurotensin, and CCK. A potential mechanism that may play a permissive role in postprandial hyperemia is withdrawal of sympathetic vasomotor drive to the gastrointestinal vasculature. CCK is a potential mediator of such a mechanism since it is released on consumption of a meal in response to the presence of certain nutrients (33).

In this study, the doses of CCK that produced effects on arterial blood pressure, RVLM unit discharge, and sympathetic vasomotor discharge exceeded those that have been used to elicit changes in vagal afferent nerve discharge (5). However, these have usually been administered by close arterial injection and this route of administration was not considered feasible in our experiments because of the concurrent measurement of sympathetic nerve discharge or the use of the aortic snare to raise arterial blood pressure. The doses of CCK used in the present study would also lead to plasma concentrations that would exceed those observed after food consumption. Then again, it is most probable that CCK acts in a paracrine fashion, i.e., it is released from enteroendocrine cells and accesses vagal afferent terminals in the mucosal lamina propria (4, 6). In view of this, a comparison of CCK blood levels produced on exogenous administration of the peptide to those observed after meal consumption is irrelevant because the latter reflect only “spill-over” into the circulation.

**Differential effects of CCK on sympathetic vasomotor outflow.** CCK did not influence sympathetic vasomotor outflow uniformly since it produced a dose-dependent activation of LSND and inhibition of SSND. The phase of activation was also followed by inhibition if arterial blood pressure increased after the initial CCK-induced depressor response. This suggests that the late inhibition was mediated by activation of the baroreflex.

In contrast, CCK uniformly inhibited SSND and activation of SSND was never observed. CCK-induced
splanchnic sympathetic inhibition was mediated by a vagal reflex since bilateral vagotomy inhibited the splanchnic sympathoinhibitory effect. The splanchnic sympathoinhibitory response produced by CCK administration was mediated by activation of CCK-A receptors since it was abolished by devazepide (30).

It appears that anesthesia qualitatively alters the responses to CCK. Bachelard et al. (2) reported pressor responses to CCK administration accompanied by generalized vasoconstriction in awake rats (2). These responses to CCK in awake rats were not reduced in rats pretreated with capsaicin, suggesting the responses were not mediated by vagal afferents. Other studies (32) have reported depressor responses that were occasionally accompanied by pressor responses as observed in the present study. However, in these previous studies (2, 32) it was not established whether the effects were mediated by the sympathetic nervous system or by direct actions of CCK on the vasculature.

**Cardiac actions of CCK.** CCK produced an immediate bradycardic effect that was abolished by devazepide but not by vagotomy. This suggests that the bradycardic effect was mediated by CCK-A receptors but not through activation of vagal afferent or efferent discharge. Bradycardic actions of CCK have been described in the pithed rat (17) and also in the isolated perfused rat heart (32). CCK-induced bradycardia was insensitive to blockade of α-adrenoceptors, β-adrenoceptors, or muscarinic receptors and was unaffected by ganglion blockade or adrenalectomy (17). Similarly, CCK-8 does not produce bradycardia in a rat strain that lacks CCK-A receptors (25). Taken together, these data exclude the possibility that CCK produces bradycardia by reflex inhibition of cardiac sympathetic drive.

![Fig. 7. Effects of CCK on blood pressure and superior mesenteric and iliac conductance.](image)

**Fig. 7.** Effects of CCK on blood pressure and superior mesenteric and iliac conductance. CCK (1–8 μg/kg) reduced MAP (open bars) and increased superior mesenteric artery conductance (gray bars). Iliac conductance (filled bars) was not altered significantly by CCK administration. Values are means ± SE; n = 6 experiments. *P < 0.05.

![Fig. 8. Inhibitory effect of CCK on the discharge of a rostral ventrolateral medulla (RVLM) medullospinal barosensitive neuron.](image)

**Fig. 8.** Inhibitory effect of CCK on the discharge of a rostral ventrolateral medulla (RVLM) medullospinal barosensitive neuron. A: collision test. A constant latency antidromic spike (A) is elicited by spinal cord stimulation (arrow; traces 1, 2, and 4 from top). The delay between the spontaneous spike (S) and the spinal stimulus (7.2 ms) exceeds the critical interval for collision. Reduction of the delay between the spontaneous spike and the spinal stimulus to the critical interval (6.8 ms) results in collision of the antidromic spike (trace 3 from top). The additional spikes appearing with each spike are of inconstant latency and are due to orthodromic activation of the neuron by spinal stimulation. B: inhibition of neuronal discharge by brief aortic occlusion (AOc) and subsequent elevation of MAP. CCK (1 and 4 μg/kg) and phenylbiguanide (PBG) were administered at the times indicated by • and produced reversible inhibition of the discharge of the neuron.
and suggest instead that CCK has a direct effect on the heart, perhaps by influencing the pacemaker cells. In support of this conclusion, it has been demonstrated (25) that CCK-A receptor mRNA is restricted to the atria of the heart.

**Differential effects of CCK on RVLM presympathetic neurons.** A very limited number of studies (19, 24, 32) have examined the influence of peripheral CCK on cardiovascular and sympathetic vasomotor function but the present study is the first report of the effects of CCK on the discharge of RVLM presympathetic neurons. The properties of these neurons have been described previously in numerous studies (8, 43, 45) and may be summarized as follows: 1) they have a spinally projecting axon with conduction velocities in the range of 0.4–8 m/s; 2) they are completely silenced by elevation of arterial blood pressure and have a pronounced pulse synchronous discharge; 3) they are found most frequently within 0–500 μm caudal to the caudal pole of the facial nucleus; and 4) the vast majority are silenced by activation of vagal cardiopulmonary afferents (46). The effect of CCK on RVLM presympathetic neurons was not uniform across the population of cells studied. Although RVLM medullospinal neurons responded uniformly to activation of cardiopulmonary vagal afferents (5-HT3 receptor activation with PBG), this was not the case for their response to CCK since some cells were profoundly inhibited, some were activated, and others were unaffected by CCK. An additional difference between the effects of PBG and CCK was the duration of their inhibitory effects: the response to PBG lasted only 2–3 s whereas the response to CCK had a duration of 2–3 min. We (46) have previously described a difference in the response of ventrolateral medulla barosensitive cells with projections to the hypothalamus to PBG. These observations suggest that there may be differences in the types of vagal afferent input these neurons receive (indirectly), particularly those of subdiaphragmatic origin.

Selective effects of peripherally administered CCK on the activity of central neurons have been described previously (26). Leng et al. (26) reported that CCK differentially influenced the discharge of neurons in the supraoptic nucleus of hypothalamus. The neurons activated by CCK were concluded to be oxytocin-producing cells whereas those unaffected by CCK were considered to be vasopressin cells. The differential effects of substances such as CCK and PBG on RVLM presympathetic neurons suggest that vagal afferents are heterogeneous with respect to their effects on sympathetic vasomotor function. Vagal stimulation evokes a mixed response in peripheral sympathetic nerves and RVLM presympathetic neurons (43), indicating that some populations of vagal afferents exert an inhibitory effect on sympathetic vasomotor function whereas others have an excitatory action.

CCK may prove to be a useful tool for distinguishing functional subclasses of RVLM presynaptic vasomotor neurons. An attractive hypothesis, also supported by the effects of CCK on sympathetic outflow, is that RVLM medullospinal neurons inhibited by CCK target sympathetic preganglionic neurons that inner-
activate the abdominal visceral vascular smooth muscle. Neurons that were inhibited by CCK had discharge rates and conduction velocities that were significantly greater than those activated or unaffected by CCK. This, in turn, suggests that CCK inhibits RVLM non-adrenergic (non-C1) neurons since this subpopulation is most strongly associated with the fast-firing, fast-conducting neuronal phenotype (40).

**CCK and gastrointestinal blood flow.** CCK has been regarded (10) as a potential mediator of gastrointestinal vasodilatation associated with digestion. CCK is released from type I secretory cells of the gastrointestinal mucosa in response to ingestion of a meal (29, 34). Specific types of nutrients are probably detected by chemosensitive afferent endings in the intestinal mucosa, and these signals are relayed, in part, via the abdominal vagus nerve (15). The mechanisms that have been proposed for the effects of CCK on gastric blood flow include activation of vagal afferents, which then activate vagal efferent discharge and release of ACh (22). An additional mechanism, suggested by the results of the present study, may involve withdrawal of sympathetic vasomotor tone to submucosal arterioles, resulting in vasodilatation. This conclusion is consistent with the finding that CCK administration also produced an increase in superior mesenteric conductance but had little influence on iliac conductance. However, the effects of CCK on superior mesenteric conductance were modest and not clearly dose dependent. This is most likely because resting gastrointestinal blood flow is already elevated under general anesthesia (1) and so further increases above an elevated baseline may not be possible.

A number of mechanisms may operate in parallel to produce CCK-mediated vasodilatation, e.g., gastrointestinal vasodilatation is produced by activation of local vasodilator systems as well as through withdrawal of sympathetic vasoconstrictor drive. It re-
mains to be established whether RVLM medullospinal neurons that are inhibited by CCK are those that drive sympathetic vasomotor outflow to the gastrointestinal tract. However, it is clear that CCK differentially influences the discharge of RVLM presympathetic neurons. Previous studies (45, 46) have demonstrated that the vast majority of RVLM presympathetic neurons are inhibited by activation of vagal cardiopulmonary 5-HT3 receptors. These findings suggest that the majority of RVLM neurons receive inhibitory cardiopulmonary receptor input but do not explain the selectivity of the actions of CCK. A possible explanation that accounts for the effects of CCK is that only a subpopulation of RVLM neurons receive inhibitory input from subdiaphragmatic vagal afferents responsive to CCK. Hillsley and Grundy (23) reported that mesenteric vagal afferents responded to activation of either CCK-A receptors or 5-HT3 receptors but not both, suggesting the existence of a subpopulation of CCK-sensitive vagal afferent fibers.

We found that bilateral cervical vagotomy abolished the effects of CCK on SSND, indicating that CCK administration activated receptors on vagal afferent fibers, which then resulted in sympathoinhibition produced by inhibition of a specific subset of presympathetic vasomotor neurons. A large body of evidence (12, 14, 27) supports the view that many of the effects of CCK are mediated through vagal afferent nerve fibers. Peripheral administration of CCK induces expression of the immediate early gene c-fos in a number of brain structures, including the NTS, the lateral parabrachial nucleus, the lateral subdivision of the central nucleus of the amygdala, the hypothalamic paraventricular nucleus, and the bed nucleus of the stria terminalis (9, 12, 14, 27, 48). In many cases (12, 14, 27), the effects of CCK on central Fos expression were prevented by prior vagotomy. Similarly, induction of brain Fos expression was prevented by CCK-A receptor blockade (12, 48).

Interestingly, Fos expression in the area postrema and NTS produced by intraduodenal administration of glucose was markedly reduced by administration of devazepide (47). These observations suggest that nutrient stimulation of the gastrointestinal tract signals the central nervous system through release of CCK that activates CCK-A receptors on vagal afferent fibers terminating in the NTS. The results of the present study suggest that similar mechanisms may operate to modulate sympathetic vasomotor outflow to the gastrointestinal tract.

Perspectives

This study has demonstrated that CCK has differential effects on 1) the discharge of presympathetic vasomotor neurons, 2) sympathetic vasomotor outflow, and 3) regional hemodynamic function. In addition, we provided evidence that these effects of CCK are mediated by vagal afferents through activation of CCK-A receptors. Potentially, these actions of CCK may be associated with the increased gastrointestinal blood flow observed after CCK administration and may be a mechanism underlying postprandial hyperemia. Further study is needed to determine whether RVLM presympathetic neurons inhibited by CCK are located within a specific region of the ventrolateral medulla or are associated with a specific neuronal phenotype. Similarly, it remains to be determined whether these neurons represent a homogeneous class, e.g., presympathetic vasomotor neurons that drive sympathetic preganglionic neurons controlling gastrointestinal vascular smooth muscle. Finally, the results of these experiments suggest that physiological stimuli that produce endogenous CCK release (e.g., food consumption) should also inhibit some RVLM presympathetic neurons.

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REFERENCES

16. Gallavan RH and Chou CC. Possible mechanisms for the initiation and maintenance of postprandial intestinal hyper-