CCK-induced inhibition of presympathetic vasomotor neurons: dependence on subdiaphragmatic vagal afferents and central NMDA receptors in the rat

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Verberne, Anthony J. M., and Daniela M. Sartor. CCK-induced inhibition of presympathetic vasomotor neurons: dependence on subdiaphragmatic vagal afferents and central NMDA receptors. Am J Physiol Regul Integr Comp Physiol 287: R809–R816, 2004. First published May 20, 2004; 10.1152/ajpregu.00258.2004.—Systemic administration of cholecystokinin (CCK) inhibits a subpopulation of rostral ventrolateral medulla (RVLM) presympathetic vasomotor neurons. This study was designed to determine whether this effect involved subdiaphragmatic vagal afferents and/or central N-methyl-D-aspartic acid (NMDA) receptors. Recordings were made from CCK-sensitive RVLM presympathetic vasomotor neurons in halothane-anesthetized, paralyzed male Sprague-Dawley rats. The responses of the neurons to CCK (2 and 4 μg/kg iv), phenylephrine (PE; 5 μg/kg iv), and phenylbiguanide (PBG; 5 μg/kg iv) were tested before and after application of the local anesthetic lidocaine (2% wt/vol gel; 1 ml) to the subdiaphragmatic vagi at the level of the esophagus. In seven separate experiments, lidocaine markedly reduced the inhibitory effects of CCK on RVLM presympathetic neuronal discharge rate. In other experiments, the effect of systemic administration of dizocilpine (1 mg/kg iv), a noncompetitive antagonist at NMDA receptor ion channels, on the RVLM presympathetic neuronal responses to CCK, PBG, and PE was tested. In all cases (n = 6 neurons in 6 individual rats), dizocilpine inhibited the effects of CCK, PBG, and PE on RVLM presympathetic neuronal discharge. These results suggest that the effects of systemic CCK on the discharge of RVLM presympathetic neurons is mediated via an action on receptors located on subdiaphragmatic vagal afferents. Furthermore, the data suggest that CCK activates a central pathway involving NMDA receptors to produce inhibition of RVLM presympathetic neuronal discharge.

rostral ventrolateral medulla

THE GASTROINTESTINAL PEPTIDE cholecystokinin (CCK) is a powerful stimulant of gastrointestinal vagal afferent nerve fibers (1, 12, 24, 25) and has selective effects on sympathetic vasomotor outflow that are dependent on intact vagal afferents (17). In accord with its effects on sympathetic vasomotor outflow, CCK selectively inhibits the discharge of a subpopulation of rostral ventrolateral medulla (RVLM) presympathetic neurons (17, 18). RVLM presympathetic vasomotor neurons are considered to play a major role in the generation of sympathetic vasomotor outflow, control of sympathetic cardiovascular reflexes, and arterial blood pressure (3, 8). While it seems firmly established that the cardiovascular effects of CCK are dependent on vagal afferent mechanisms, it would seem prudent to seek support for this concept at the single neuronal level. Second, the selectivity of CCK for a subpopulation of RVLM presympathetic neurons may suggest that CCK-sensitive neurons preferentially receive vagal afferent input from the gastrointestinal tract (21).

The neurocircuitry that mediates arterial baroreflex-mediated inhibition of RVLM presympathetic vasomotor neurons and sympathetic vasomotor outflow is described by a trisynaptic model, which includes N-methyl-D-aspartic acid (NMDA) receptor-mediated activation of propriomedullary GABAergic neurons in the caudal ventrolateral medulla (6, 8). Such a scheme may also be applicable in the case of CCK-induced inhibition of RVLM presympathetic vasomotor neurons (21). Thus in addition to examining the role of subdiaphragmatic vagal afferents in the actions of systemic CCK on sympathetic vasomotor outflow, the importance of central NMDA receptors has been assessed by examining the effect of administration of a centrally active NMDA receptor antagonist, dizocilpine (MK-801), on CCK-induced inhibition of RVLM presympathetic neurons.

METHODS

All experiments were performed with the use of male Sprague-Dawley rats (250–380 g). The protocols were 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, where 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 with the use of a servo-controlled heating pad. The left carotid artery and left jugular vein were cannulated to measure arterial blood pressure and heart rate and for intravenous drug administration, respectively.

After the completion of all surgery and ascertaining an appropriate level of anesthesia, as judged by application of the tests described above, the paralyzing agent pancuronium bromide (1–2 mg/kg iv) was administered. After neuromuscular blockade was established, the stability of the arterial blood pressure and heart rate record and the absence of a pressor response to firm hindlimb toe pinch were used as indications of adequate anesthesia. 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

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RVLM. The signals were amplified and recorded extracellularly from neurons in the ventral medulla. The magnitude of the field potential was used to identify the celiac, medial, and ventral contours of the facial motor nucleus, as previously described (2, 22). A bipolar electrode was also placed into the dorsolateral funiculus of the thoracic spinal cord nucleus, as previously described (2, 22). A bipolar electrode was also placed on the mandibular branch of the right facial nerve, which, when stimulated (0.1 ms pulses, 0.5 Hz, 0.3–1.0 mA), produced an antidromic field potential within the facial motor nucleus of the ventral medulla. The magnitude of the field potential was used to identify barosensitive neurons in the RVLM. Invariant antidromic latency and the collision test 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 meters) by the antidromic latency (in seconds). Only barosensitive cells, which were collision-test positive and were inhibited by systemic administration of CCK, were included in the study. Glass microelectrodes (2 mm outer diameter) containing 0.5 M sodium acetate and 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 with the use of an oscilloscope and an audio amplifier. The effects of CCK (2 and 4 μg/kg iv) or phenylbiguanide (PBG; 5 μg/kg iv) on arterial blood pressure and the discharge rate of RVLM barosensitive, spinally projecting neurons were recorded and stored onto videotape together with the blood pressure responses. Barosensitivity of the spinally projecting neurons was judged by their response to elevation of arterial blood pressure produced by systemic administration of the vasoconstrictor agent phenylephrine (PE; 5 μg/kg iv). Neuronal discharge rates were measured at rest before manipulation of arterial blood pressure levels or injection of any drugs. A change in discharge rate was calculated by counting the total number of spikes over the period of the response and expressing this as a percentage of the total number of spikes observed over a period of the same duration before drug administration. The doses of CCK and PBG were submaximal and were chosen on the basis of previous reports (17, 20, 22).

Only one neuron was studied in each experiment. CCK-sensitive RVLM presympathetic neurons are a subpopulation of the total population of spinally projecting, barosensitive cells in the RVLM (17). These are usually neurons with spinal afferent conduction velocities in the lightly myelinated range. After confirmation of the identity of a CCK-sensitive RVLM presympathetic vasomotor neuron, doses of PBG (5 μg/kg iv) or CCK (2 and 4 μg/kg iv) were administered in random fashion.

Blockade of vagal afferent transmission. The following approach was used to interrupt subdiaphragmatic vagal afferent traffic: a polyethylene cannula was sutured onto a subdiaphragmatic section of the esophagus. Care was taken to avoid damage to the anterior and posterior vagal trunks, which lie along the esophagus (15). Lidocaine (1 ml; 2% wt/vol gel; Orion Laboratories, Welshpool, Western Australia) was applied to the subdiaphragmatic vagi through the implanted cannula. The responsiveness of each RVLM presympathetic vasomotor neuron to CCK (2 and 4 μg/kg iv), PE (5 μg/kg iv), PBG (5 μg/kg iv), and elevation of arterial blood pressure (PE, 5 μg/kg iv) was tested before and 10–15 min after application of lidocaine. In three separate experiments, the cervical vagi were exposed bilaterally and the effects of CCK were tested before and after bilateral application of lidocaine.

Blockade of central NMDA receptors. Responses of RVLM presympathetic vasomotor neurons to CCK (2 and 4 μg/kg iv), PE (5 μg/kg iv), PBG (5 μg/kg iv), and elevation of arterial blood pressure (PE, 5 μg/kg iv) were tested before and 5 min after blockade of central NMDA receptors using dizocilpine [(+)-MK-801 hydrogen maleate; 1 mg/kg iv; Research Biochemicals International; Natick, MA].

Previous experiments conducted in our laboratory have demonstrated that the effects of PBG and CCK are reproducible over time (16).

Histological analysis of recording sites. Recording sites within the RVLM were marked by iontophoretic deposition of Pontamine sky blue.

Fig. 1. The effects of cholecystokinin (CCK; 4 μg/kg iv) on the discharge of a rostral ventrolateral medulla (RVLM) presympathetic vasomotor neuron before and after application of lidocaine (local anesthetic gel, 1 ml; 2% wt/vol) to the subdiaphragmatic vagi adjacent to the esophagus. A: discharge of the neuron is slowed by systemic injection of CCK injected at the marker (●). B: after application of lidocaine, the effect of CCK on the discharge of the RVLM neuron is reduced. AP, arterial blood pressure; FR, firing rate; HR, heart rate.
blue from the recording electrode. At the conclusion of each unit recording experiment the animals were deeply anesthetized with pentobarbitone 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 and the brains were collected for histological verification of recording sites. The brains were sectioned with the use of a cryostat and mounted onto gelatin-subbed slides and were stained for Nissl substance with Cresyl violet. Recording sites were identified under the light microscope and were mapped onto standard maps of the rat brain with reference to a rat brain atlas (14). All RVLM presympathetic vasomotor neurons described in this study were located within 500 μm of the caudal pole of the facial motor nucleus as described previously (16–18, 22).

**Data analysis and statistics.** Extracellular action potentials, arterial blood pressure, heart rate, and stimulation pulses were recorded onto
RVLM PRESYMPATHETIC NEURONS AND CHOLECYSTOKININ

and 4 nal discharge (Figs. 6 and 9). The inhibitory effects of CCK (2 μg/kg iv) on RVLM presympathetic neuronal discharge (Fig. 7). Dizocilpine administration markedly reduced the inhibitory effects of CCK (4 μg/kg iv) on RVLM presympathetic neuronal discharge from −60 ± 11% and −84 ± 5% to −9 ± 5% and −20 ± 5%, respectively (P < 0.05 for both comparisons; Fig. 9).

The hypertensive and bradycardic effects of CCK were not affected significantly by vagal anesthesia (Fig. 4; P > 0.05). Similarly, the effects of neither PE nor PBG on arterial blood pressure and heart rate were altered significantly by vagal anesthesia (Fig. 4; see also Fig. 2). The reduction in RVLM neuronal discharge rate in response to PE was reduced significantly by lidocaine application to the vagus from −99 ± 1% to −64 ± 12% (Figs. 3 and 4; P < 0.05). However, the peak MAP responses to PE were also reduced significantly by lidocaine treatment (P < 0.05; pre-lidocaine 124 ± 7 mmHg; post-lidocaine 105 ± 5 mmHg, n = 7).

The neuronal inhibitory response to PBG was not significantly altered by vagal anesthesia (−98 ± 2% compared with −78 ± 7%; P < 0.05; Figs. 2 and 4).

The mean axonal conduction velocity of the RVLM presympathetic neurons in the vagal anesthesia group was 4.1 ± 0.5 m/s (n = 7 neurons in separate experiments).

In three separate experiments, lidocaine was applied bilaterally to the vagi at the cervical level. In all three cases, bilateral lidocaine application to the cervical vagi blocked the responses to systemic CCK administration (Fig. 5).

Central NMDA receptor blockade. Administration of the centrally active noncompetitive NMDA receptor antagonist dizocilpine had a biphasic effect on arterial blood pressure. Shortly after dizocilpine administration, MAP increased from 93 ± 3 to 108 ± 2 mmHg and this was followed by a prolonged decrease to 56 ± 3 mmHg.

NMDA receptor blockade markedly reduced the inhibitory effects of CCK (4 μg/kg iv) on RVLM presympathetic neuronal discharge (Figs. 6 and 9). The inhibitory effects of CCK (2 and 4 μg/kg iv) on RVLM presympathetic neuronal discharge were reduced by dizocilpine administration from −76 ± 11% and −78 ± 13% to −5 ± 3% and −12 ± 6%, respectively (P < 0.05 for both comparisons; Fig. 9). Dizocilpine administration had no significant effect on the MAP or heart rate responses to PBG, PE, and CCK (Fig. 9).

The reduction in neuronal discharge rate induced by PBG was reduced from −97 ± 3% to −27 ± 10% (Figs. 7 and 9; P < 0.05; n = 6 neurons) after dizocilpine administration. Similarly, the baroreflex-mediated inhibition of neuronal discharge rate induced by PE was reduced from −97 ± 3% to −38 ± 9% (P < 0.05; n = 6 neurons) after dizocilpine administration (Figs. 8 and 9). However, the peak MAP responses to PE were reduced significantly by dizocilpine treatment (P < 0.05; pre-dizocilpine 128 ± 8 mmHg; post-dizocilpine 119 ± 9 mmHg, n = 6).

The mean axonal conduction velocity of the RVLM presympathetic neurons in the dizocilpine treatment group was 3.3 ± 0.7 m/s (n = 6 neurons in separate experiments).

DISCUSSION

This study has demonstrated that systemically administered CCK produces inhibition of a subpopulation of RVLM presympathetic neurons via a mechanism that is dependent on intact subdiaphragmatic vagal afferents and central NMDA receptors.

Subdiaphragmatic vagal local anesthesia. Subdiaphragmatic vagal anesthesia reduced resting mean arterial pressure (MAP) from 85 ± 3 mmHg (n = 7) to 65 ± 4 mmHg (P < 0.05). Blockade of subdiaphragmatic vagal afferent transmission markedly reduced the inhibitory effects of CCK (4 μg/kg iv) on RVLM presympathetic neuronal discharge (Fig. 1). Vagal lidocaine application significantly reduced the inhibitory effects of CCK (2 and 4 μg/kg iv) on RVLM presympathetic neuronal discharge from −60 ± 11% and −84 ± 5% to −9 ± 5% and −20 ± 5%, respectively (P < 0.05 for both comparisons; Fig. 4).

The hypotensive and bradycardic effects of CCK were not affected significantly by vagal anesthesia (Fig. 4; P > 0.05). Similarly, the effects of neither PE nor PBG on arterial blood pressure and heart rate were altered significantly by vagal anesthesia (Fig. 4; see also Fig. 2). The reduction in RVLM neuronal discharge rate in response to PE was reduced significantly by lidocaine application to the vagus from −99 ± 1% to −64 ± 12% (Figs. 3 and 4; P < 0.05). However, the peak MAP responses to PE were also reduced significantly by lidocaine treatment (P < 0.05; pre-lidocaine 124 ± 7 mmHg; post-lidocaine 105 ± 5 mmHg, n = 7).

The neuronal inhibitory response to PBG was not significantly altered by vagal anesthesia (−98 ± 2% compared with −78 ± 7%; P < 0.05; Figs. 2 and 4).

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Fig. 4. Summary of the effects of application of lidocaine (local anesthetic gel, 1 ml, 2% wt/vol) onto the subdiaphragmatic vagi adjacent to the esophagus on the responses to systemic administration of CCK (2 μg/kg iv), PE (5 μg/kg iv), and PBG (5 μg/kg iv). The changes in mean arterial blood pressure (ΔMAP), HR, and RVLM presympathetic neuronal discharge rate (ΔFiring rate) produced by each agent before (open bars) and after (solid bars) lidocaine treatment are shown. Data are presented as means ± SE and represent data obtained in 7 separate experiments. *P < 0.05.
Interruption of vagal afferent traffic arising in subdiaphragmatic branches of the vagus was achieved by topical application of the local anesthetic lidocaine. It was expected that this treatment would abolish the neuronal responses to CCK but not PBG, because subdiaphragmatic vagal afferents are responsive to systemically administered CCK and PBG activates 5-HT₃ receptors located on cardiopulmonary vagal afferents (19, 21). This prediction proved correct.

Fig. 5. The effects of CCK (4 μg/kg iv) on the discharge of an RVLM presympathetic vasomotor neuron before and after bilateral application of lidocaine (local anesthetic gel, 0.5 ml, 2% wt/vol) to the cervical vagi. A: discharge of the cell is markedly reduced after CCK injection (●). B: after lidocaine treatment the effects of CCK are blocked.

Fig. 6. The effects of CCK (4 μg/kg iv) on the discharge of an RVLM presympathetic vasomotor neuron before and after systemic administration of 1 mg/kg iv dizocilpine (N-methyl-D-aspartate receptor ion channel antagonist). A: discharge of the neuron is slowed by systemic injection of CCK injected at the marker (●). B: after administration of dizocilpine, the effect of CCK on the discharge of the RVLM neuron is blocked.
because lidocaine application to the subdiaphragmatic vagi blocked the response to systemic CCK but not PBG. This finding also suggests that the lidocaine did not spread sufficiently to anesthetize cardiopulmonary vagal afferents. We (16) have previously observed that the inhibitory responses of RVLM presympathetic neurons to systemic administration of PBG and CCK are very stable. This suggests that the effects of subdiaphragmatic vagal anesthesia or systemic injection of PBG or CCK at the marker (●) were blocked by administration of dizocilpine, an N-methyl-D-aspartate receptor ion channel antagonist (1 mg/kg iv). A: discharge of the neuron is slowed by systemic injection of PBG at the marker (●). B: after administration of dizocilpine, the effect of PBG on the discharge of the RVLM neuron is blocked.

Fig. 7. The effects of PBG (5 μg/kg iv) on the discharge of an RVLM presympathetic vasomotor neuron before and after systemic administration of dizocilpine (N-methyl-D-aspartate receptor ion channel antagonist; 1 mg/kg iv). A: discharge of the neuron is slowed by systemic injection of PBG injected at the marker (●). B: after administration of dizocilpine, the effect of PBG on the discharge of the RVLM neuron is blocked.

Fig. 8. The effects of PE (5 μg/kg iv) on the discharge of an RVLM presympathetic vasomotor neuron before and after systemic administration of dizocilpine (N-methyl-D-aspartate receptor ion channel antagonist; 1 mg/kg iv). A: discharge of the neuron is slowed by systemic injection of PE injected at the marker (●). B: after administration of dizocilpine, the effect of PE on the discharge of the RVLM neuron is markedly reduced. Note that some variation in spike size occurred during the response to PE. Accurate counting of the spike rate was ensured by adjustment of the window discriminator.
NMDDA receptor blockade were not attributable to development of tachyphylaxis.

Despite systemic administration, the inhibitory effects of CCK on RVLM presympathetic neuronal discharge were mediated by an action at CCK receptors on subdiaphragmatic vagal afferents. To our knowledge, this study is the first demonstration of the inhibitory influence of the subdiaphragmatic vagal afferents on circulatory function. A potential underlying mechanism for this action of CCK may involve 1) CCK-induced activation of gastrointestinal vagal afferents (9), 2) activation of the nucleus of tractus solitarius (NTS) neurons leading to 3) activation of an intermedullary inhibitory pathway, which leads to 4) inhibition of RVLM presympathetic neurons. In support of this contention, systemic administration of CCK induces Fos expression in the NTS via vagal afferents (4).

Gieroba and colleagues (5) found that subdiaphragmatic vagal stimulation predominantly activates RVLM presympathetic vasomotor neurons in the anesthetized rabbit. However, electrical stimulation of the vagus would excite virtually all afferent fibers present in the vagus, and, evidently, some of these produce sympathoexcitation. Presumably, CCK activates a subgroup of subdiaphragmatic vagal afferents, which have an inhibitory influence on sympathetic vasomotor function. Possibly selective stimulation of specific subdiaphragmatic vagal branches (15) may yield sympathoinhibitory responses.

Apart from identifying the specific site of the action of CCK on sympathetic vasomotor outflow, this study highlights an interesting physiological principle: that signals arising from the gastrointestinal tract may be conveyed to the central nervous system along a vagal pathway to influence central sympathetic vasomotor outflow.

Although lidocaine applied subdiaphragmatically may be expected to also block transmission in splanchnic afferents, these are unlikely to be of any importance in the inhibitory actions of CCK because in a prior study (17) we demonstrated that the sympathoinhibitory effects of CCK are blocked by section of the cervical vagi. In support of this conclusion, it was found that bilateral application of lidocaine to the cervical vagi also blocked the responses to CCK.

Lidocaine application also produced a reduction in arterial blood pressure that probably resulted from anesthesia of the nearby sympathetic nerve trunks. This also explains the reduced baroreflex response to elevation of arterial blood pressure using the vasoconstrictor agent PE on the discharge of RVLM presympathetic neurons. Thus while the arterial blood pressure increases produced by PE before and after lidocaine were similar, the actual peak pressure was lower after lidocaine.

The bradycardic effects of CCK were not influenced by either dizocilpine or lidocaine treatment, and these observations reinforce the view that this action of CCK is mediated by activation of CCK1 receptors in the heart (13, 17). Similarly, the bradycardic effects of baroreflex activation or in response to activation of cardiopulmonary afferents were minimal and were not altered by either lidocaine treatment or dizocilpine. It is likely that the bradycardic responses to PE and PBG were blocked by the vagolytic actions of Figure 9. Summary of the effects of systemic administration of dizocilpine (N-methyl-D-aspartate receptor ion channel antagonist; 1 mg/kg iv) on the responses to systemic administration of CCK (2 and 4 μg/kg iv), PE (5 μg/kg iv), and PBG (5 μg/kg iv). The changes in mean arterial blood pressure (ΔMAP), HR, and RVLM presympathetic neuronal discharge rate (ΔFiring rate) produced by each agent before (open bars) and after (solid bars) dizocilpine treatment are shown. Data are presented as means ± SE and represent data obtained in 6 separate experiments. *P < 0.05.
by withdrawing sympathetic vasoconstrictor drive to the gastrointestinal tract.

In summary, this study has demonstrated that the actions of CCK on the discharge of RVLM presympathetic vasomotor neurons is mediated by activation of CCK receptors located on subdiaphragmatic vagal afferents. This indicates that signals arising from the targets of vagal afferent innervation within the abdominal viscera and presumably within the gastrointestinal tract may influence sympathetic vasomotor outflow. In addition, the neurocircuitry that mediates CCK-induced sympathetic inhibition involves central NMDA receptors.

**Perspectives**

The actions of CCK on sympathetic vasomotor function may constitute a new gastrointestinal-cardiovascular reflex. CCK, and perhaps several other agents derived from the gastrointestinal tract, may modulate gastrointestinal blood flow by regulation of gastrointestinal vasomotor outflow via an action at receptors located on subdiaphragmatic vagal afferents. The selective actions of CCK on a subpopulation of RVLM presympathetic vasomotor neurons may also provide a means of identifying similar but parallel intramedullary pathways involved in sympathetic vasomotor control.

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