THE SYMPATHOINHIBITORY EFFECTS OF SYSTEMIC
CHOLECYSTOKININ ARE DEPENDENT ON NEURONS IN THE CAUDAL
VENTROLATERAL MEDULLA IN THE RAT

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ABSTRACT

The gastrointestinal hormone cholecystokinin (CCK) inhibits a subset of presympathetic neurons in the rostroventrolateral medulla (RVLM) that may be responsible for driving the sympathetic vasomotor outflow to the gastrointestinal circulation. We tested the hypothesis that the central neurocircuitry of this novel sympathoinhibitory reflex involves a relay in the caudal ventrolateral medullary (CVLM) depressor area. Blood pressure and greater splanchnic sympathetic nerve discharge (SSND) or lumbar sympathetic nerve discharge (LSND) were monitored in anesthetised, paralysed male Sprague-Dawley rats. The effects of phenylephrine (PE, 10 µg/kg i.v.; baroreflex activation), phenylbiguanide (PBG, 10 µg/kg i.v.; von Bezold-Jarisch reflex) and CCK (4 or 8 µg/kg, i.v.) on SSND or LSND, were tested before and after bilateral injection of 50-100 nl of the GABA$_A$ agonist muscimol (1.75 mM; n=6, SSND; n=7, LSND) or the excitatory amino acid antagonist kynurenate (55 mM; n=7, SSND) into the CVLM. PE and PBG elicited splanchnic and lumbar sympathoinhibitory responses that were abolished by bilateral muscimol or kynurenate injection into the CVLM. Similarly, the inhibitory effect of CCK on SSND was abolished following neuronal inhibition within the CVLM. In contrast, CCK-evoked lumbar sympathoexcitation was accentuated following bilateral CVLM inhibition. In control experiments (n=7), these agents were injected outside the CVLM and had no effect on splanchnic sympathoinhibitory responses to PE, PBG and CCK. In conclusion, neurons in the CVLM are necessary for the splanchnic but not lumbar sympathetic vasomotor reflex response to CCK. This strengthens the view that subpopulations of RVLM neurons supply sympathetic vasomotor outflow to specific vascular territories.
KEYWORDS

Splanchnic sympathetic nerve, lumbar sympathetic nerve, cholecystokinin, reflex, rat
INTRODUCTION

It is widely accepted that presympathetic neurons in the rostral ventrolateral medulla (RVLM) play a pivotal role in cardiovascular regulation (3, 4, 17) and that reflex regulation of blood pressure is dependent on an inhibitory link in the caudal ventrolateral medulla (CVLM) (18, 24, 25).

Many have speculated that RVLM neurons may be “hardwired” to provide tonic sympathetic drive to specific types of vascular beds, although solid evidence for this proposition has been elusive (4). In support of this argument, we have recently demonstrated that a subset of neurons in the rostral ventrolateral medulla (RVLM) are sensitive to intravenous administration of the gastrointestinal hormone cholecystokinin (CCK) (15, 16, 21) and have proposed that this population of neurons may specifically supply the sympathetic vasomotor drive to the gastrointestinal circulation.

CCK exerts its inhibitory effects on a subset of barosensitive and spinally-projecting RVLM neurons that are predominantly fast-firing and have fast-conducting spinal axons, via an action at CCK₁ receptors located on subdiaphragmatic vagal afferent fibers (16). CCK-induced inhibition of RVLM presympathetic neurons is also dependent on activation of central NMDA receptors (21). Administration of secretagogues into the duodenum produces a selective inhibition of CCK-sensitive RVLM neurons as a consequence of the physiological release and likely paracrine action, of gastrointestinal hormones and peptides. (14). Furthermore, systemic CCK administration differentially affects sympathetic vasomotor outflow; splanchnic sympathetic nerve discharge (SSND) is inhibited by CCK while lumbar sympathetic nerve discharge (LSND) is augmented (15). Similarly, systemic CCK administration significantly increases mesenteric conductance but has little or no effect on iliac conductance (15). These results strengthen the argument for a “viscerotopic” role of
RVLM presympathetic neurons and suggest that CCK-sensitive RVLM neurons may play an important role in gastrointestinal circulatory control.

The baroreflex and the von Bezold-Jarisch reflex are believed to utilise a parallel if not identical trisynaptic medullary pathway that incorporates an inhibitory relay in the rostral tip of the CVLM (2, 9, 20). It is plausible that the CCK-induced gastrointestinal circulatory reflex also utilises a similar trisynaptic pathway. Neurons in the NTS that respond to subdiaphragmatic vagal afferent stimulation form a separate pool of neurons that are distinguishable from NTS neurons responding to either baroreflex or chemoreceptor stimulation (12). NTS neurons that are responsive to sub-diaphragmatic vagal afferent stimulation may be identical to those responsible for the CCK-induced reflex inhibition of RVLM presympathetic vasomotor neurons following stimulation of CCK₁ receptors on subdiaphragmatic vagal afferents. Furthermore, these neurons may in turn, relay an excitatory signal to a particular subset of CVLM neurons which may be specific in their inhibition of CCK-sensitive RVLM neurons.

It was the purpose of this study to determine whether the sympathetic vasomotor gastrointestinal reflex mediating the splanchnic sympathoinhibitory effects of CCK utilises an inhibitory relay in the CVLM or alternately, a relay in the midline medulla which has previously been shown to modulate sympathetic vasomotor outflow (22). It was also of interest to determine whether the sympathoexcitatory effects of CCK on LSND were independent of the CVLM. In order to test these hypotheses, we examined the effects of interruption of neurotransmission in the CVLM using two approaches: (a) microinjection of the GABAₐ agonist muscimol for direct inhibition of CVLM neurons and (b), microinjection of the non-specific glutamate receptor antagonist kynurenate for blockade of excitatory synaptic transmission in the CVLM.
METHODS

Animals. All experiments were performed using male Sprague-Dawley rats (250-400 g, n=28) purchased from the Animal Resource Center (Perth, Western Australia). This study was approved by the Ethical Review Committee of Austin Health (Heidelberg, Victoria, Australia) and complied with principles outlined in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and those outlined in the Use of Animals for Scientific Purposes and the American Physiological Society’s Guiding Principles in the Care and Use of Animals.

Materials. CCK-octapeptide (CCK-8, sulfated form) was purchased from the American Peptide Company, Sunnyvale, CA, U.S.A. Phenylbiguanide (PBG), phenylephrine (PE), muscimol and kynurenate were purchased from Sigma-Aldrich Chemical, Milwaukee, WI. Fluorescent microbeads (FluoSpheres; 0.04\,\mu\text{m}, orange fluorescent; 540/560 nm) were purchased from Molecular Probes, Eugene, Oregon, USA. Kynurenate and muscimol were dissolved in artificial cerebrospinal fluid (CSF) (19).

Animal preparation. All animals were anesthetised with halothane (Fluothane, Zeneca, Macclesfield, U.K.), tracheostomised and ventilated with 100% O\textsubscript{2} containing 1.3-1.5% halothane (50-60 breaths/min; 1 ml/100g). Adequacy of anesthetic depth was verified by absence of a response to firm toe-pinch or corneal probing. Following procedures described previously (15, 16), the right carotid artery and jugular veins of animals were cannulated for the measurement of arterial blood pressure and for intravenous drug administration, respectively. Following completion of all surgery pancuronium (1-2 mg/kg, i.v.) was administered to induce neuromuscular blockade. A servo-controlled heating pad was used to maintain core temperature at 36-38\degree C.

Recording of sympathetic nerve discharge. LSND and SSND were recorded in separate groups of animals. A midline abdominal incision was used to isolate and section the
ilio-lumbar blood vessels for exposure of the left lumbar sympathetic nerve trunk (between L3-L5). SSND was recorded from the left greater splanchnic nerve that was accessed via a retroperitoneal incision and isolated at the segment distal to the suprarenal ganglion. Isolated nerves were placed onto the bared tips of two Teflon-coated silver wires (bare diameter 250 µm; A-M Systems, Everett, WA, U.S.A.), embedded in silicone sealant (Kwik-Cast, Coherent Life Sciences, Hilton, South Australia) and externalised through the sutured wound.

**RVLM neuronal recording and microinjection of kynurenate and muscimol.**

Glass microelectrodes (2 mm OD) filled with 2% Pontamine sky blue in 0.5M sodium acetate (impedance 5-10 MΩ) were used for extracellular recording of RVLM neurons. Action potentials were amplified (x1000) with an intracellular amplifier in bridge mode (Intra 767, World Precision Instruments, Sarasota, FL, U.S.A.), filtered (400 Hz to 4 kHz bandpass; Fintronics, Orange, CT, U.S.A.), counted via a window discriminator and monitored using an oscilloscope and audio amplifier. The procedures used for location, identification and extracellular single unit recording of RVLM neurons have been described previously (15, 16).

Following the location of a barosensitive RVLM neuron, the recording electrode was replaced with a microinjector containing muscimol (1.75mM) or kynurenate (55mM): FluoSpheres (9:1). Microinjectors were made from glass micropipettes (20-30µm OD) attached to a 1 µl Hamilton syringe. For injection into CVLM, the microinjector was moved 1.6 mm caudal and 0.4 mm dorsal, relative to the location of the identified RVLM neuron. For control experiments, microinjections of muscimol (1.75mM; 50nl): FluoSpheres (9:1) were made into the midline medulla at the level of RVLM, into the midline medulla at the level of CVLM (1.6mm caudal and 0.4mm...
dorsal to RVLM site) or bilaterally at a more rostral location (1.4 mm caudal and 0.4 mm dorsal to RVLM).

The arterial blood pressure and sympathetic nerve responses to the von Bezold-Jarisch reflex (PBG; 10 µg/kg i.v.), the baroreflex (PE; 10 µg/kg i.v.) and the CCK reflex (CCK; 4 or 8 µg/kg, i.v.) were tested just prior to and directly subsequent to microinjection of muscimol (50-100nl) or kynurenate (50-100nl) into the CVLM. The doses of CCK, PBG and PE used in this study were submaximal, as determined from previous studies (15, 20, 23).

Data analysis and statistics. Arterial blood pressure, heart rate, sympathetic nerve activity and extracellular unit potentials were recorded using a data acquisition system (Cambridge Electronic Design, Cambridge, UK) and Spike2 software. Sympathetic nerve discharge was analysed off-line, and was full-wave rectified and averaged over 1 s intervals. The α2-adrenoceptor agonist clonidine (200 µg/kg, i.v.) was administered at the conclusion of each experiment in which sympathetic nerve discharge was recorded. At this dose, clonidine produces a pronounced increase in arterial blood pressure accompanied by reflexly-mediated sympathoinhibition and a powerful central sympathoinhibitory effect (5). The residual signal remaining after clonidine administration was regarded as noise and was systematically subtracted from the full-wave rectified signal to establish the zero level of nerve discharge in subsequent computer analyses. The level of resting sympathetic nerve discharge recorded at the beginning of the experiment was used as the 100% level of nerve discharge and was derived by averaging the signal over a 30 s period. Sympathetic nerve discharge was quantified as arbitrary “units” of activity, and calculated using the following formula: (signal – zero level)/(100% level-zero level)*100.
Systemic administration of CCK often produced a biphasic arterial blood pressure response that comprised an initial depressor response followed by a longer-lasting secondary pressor response. SND responses to CCK were measured at the nadir of the depressor phase of the response by averaging the nerve discharge over this period. Following microinjection of muscimol or kynurenate into the CVLM, the initial depressor response to CCK was often abolished, but the effects on SND were measured within the same time period as for pre-treatment. The effects of PE on SND were measured at the peak of the arterial pressor response and for PBG at the nadir of the depressor response.

Data are expressed as means ± SE. The paired t-test was used to calculate the level of significance between means, and normality of the data was tested using the method of Kolmogorov and Smirnov (GraphPad Instat version 3.05 for Windows 95, GraphPad Software, San Diego, California, USA).

**Histological localization of stimulation and recording site.** At the end of each experiment, rats were deeply anesthetised before transcardial perfusion with phosphate-buffered saline (PBS; 0.05M, pH 7.4) followed by 4% formaldehyde (Riedel-de Haen, Seelze, Germany) in PBS. The brain was removed and stored in 4% formaldehyde/ PBS until required for sectioning.

Sections (40 µm) were cut at –25°C on a cryostat (Cryocut 1800, Grale Scientific; Ringwood, Victoria, Australia) and collected onto gelatin slides and subsequently cover-slipped using Vectashield mounting medium for fluorescence (Vector Laboratories, Inc, Burlingame, CA). Sections were scanned under a fluorescence microscope (Olympus BX60, Tokyo, Japan) with a wide band green filter (bandpass 510-550 nm) for identification of the fluorescent bead injection sites. The sections displaying the ‘center’ of the injection site were photographed using an MCID
imaging system (Imaging Research, Inc, Ontario, Canada) and a 24-bit 3CCD colour video camera (DXC-9100P; Sony, Tokyo, Japan; resolution 782 x 582 pixels). Coverslips were removed from slides containing sections with the fluorescent microinjection sites and were processed for Nissl staining using Cresyl Violet acetate (Sigma-Aldrich, Castle Hill, NSW, Australia). Sections were subsequently re-coverslipped with D.P.X (Sigma-Aldrich, Castle Hill, NSW, Australia) prior to re-examination under light microscopy, and re-photographed for histological correlation. Injection sites were mapped with reference to a standard rat brain atlas (13).

RESULTS

Splanchnic SND responses to PE, PBG and CCK before and after bilateral muscimol injection into the CVLM. In six rats, the effects of PE, PBG and CCK on SSND were assessed before and after bilateral muscimol microinjections (50 nl equivalent to 87.5 pmol /side) into CVLM. As a result of bilateral muscimol microinjections, resting arterial pressure rose from 84 ± 4 mmHg to 117 ± 5 mmHg ($P < 0.01$) and resting SSND rose from 92 ± 3 units to 146 ± 14 units ($P < 0.01$). Prior to bilateral muscimol microinjection, PE (10 µg/kg i.v.), PBG (10 µg/kg i.v.) and CCK (4 or 8 µg/kg, i.v.) elicited robust splanchnic sympathoinhibitory responses (Figs. 1A, 1C) that were subsequently reduced (for PE) or reversed (for PBG and CCK) following muscimol into CVLM (Figs. 1B, 1C). The hypertensive response to PE was significantly reduced from 36 ± 5 mmHg to 16 ± 2 mmHg ($P < 0.05$) following bilateral muscimol microinjection into CVLM, due to the resultant increase in baseline AP. Nevertheless, maximal AP levels reached following PE administration before and after bilateral muscimol microinjections were comparable ($P > 0.05$). Following bilateral muscimol microinjection, the hypotensive effect of PBG was abolished or occasionally reversed (-
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42 ± 8 mmHg pre-muscimol, 0 ± 3 mmHg post-muscimol; \( P < 0.01 \), and that of CCK was reversed (-8 ± 2 mmHg pre-muscimol; 3 ± 2 mmHg post-muscimol; \( P < 0.05 \)). The CVLM injection sites for these experiments are depicted in Figure 1D.

**Splanchnic SND responses to PE, PBG and CCK before and after bilateral kynurenate injection into the CVLM.** In seven rats, the effects of PE, PBG and CCK on SSND were assessed before and after bilateral kynurenate microinjections (50 - 100 nl equivalent to 2.75 - 5.5 nmol /side) into CVLM. As a result of bilateral kynurenate microinjections, resting arterial pressure rose from 81 ± 5 mmHg to 97 ± 5 mmHg \( (P < 0.01) \) and resting SSND rose from 78 ± 8 units to 162 ± 27 units \( (P < 0.05) \). Prior to bilateral kynurenate microinjection, PE (10 \( \mu \)g/kg i.v.), PBG (10 \( \mu \)g/kg i.v.) and CCK (4 \( \mu \)g/kg, i.v.) produced robust splanchnic sympathoinhibitory responses (Figs. 2A, 2C) that were subsequently significantly reversed following kynurenate into CVLM (Figs 2B, 2C). The hypertensive response to PE was similar before and after bilateral kynurenate microinjection into CVLM (36 ± 5 mmHg pre-kynurenate; 24 ± 4 mmHg post-kynurenate; \( P > 0.05 \)) and maximal AP levels reached following PE administration before and after bilateral kynurenate microinjections were also comparable \( (P > 0.05) \). Following bilateral kynurenate microinjection, the hypotensive effect of PBG was abolished or occasionally reversed (-32 ± 5 mmHg pre-kynurenate, 0 ± 3 post-kynurenate; \( P < 0.01 \), and that of CCK reversed (-8 ± 2 mmHg pre-kynurenate; 3 ± 2 mmHg post-kynurenate; \( P < 0.05 \)). The CVLM injection sites for these experiments are depicted in Figure 2D. In two experiments (excluded from the group), the injection sites were more rostral, lateral (right hand side) and medial (left hand side), and had no effect on either baseline levels or on PE, PBG and CCK responses.
Lumbar SND responses to PE, PBG and CCK before and after bilateral muscimol injection into the CVLM. The effects of PE, PBG and CCK on SSND were assessed before and after bilateral muscimol microinjections (100 nl, equivalent to 175 pmol/side) into CVLM in 7 rats. As a result of bilateral muscimol microinjections, resting arterial pressure rose from $81 \pm 4$ mmHg to $120 \pm 6$ mmHg ($P < 0.01$) and resting SSND rose from $105 \pm 13$ units to $179 \pm 25$ units ($P < 0.01$). Prior to bilateral muscimol microinjection, PE (10 µg/kg i.v.) and PBG (10 µg/kg i.v.) produced robust lumbar sympathoinhibitory responses (Figs 3A, 3C) that were subsequently reversed following muscimol into CVLM (Figs. 3B, Fig 3C). CCK (4 µg/kg, i.v.) on the other hand, elicited a sympathoexcitatory response (Figs 3A, 3C) that was potentiated following bilateral muscimol microinjection (Figs. 3B, Fig 3C). The hypertensive response to PE was not significantly altered following bilateral muscimol microinjection into CVLM ($35 \pm 2$ mmHg pre-muscimol, $22 \pm 4$ mmHg post-muscimol; $P > 0.05$). Maximal AP levels elicited by PE administration after bilateral muscimol microinjections were significantly greater than those before microinjection ($P > 0.05$). However, PE administration post-muscimol resulted in a sympathoexcitatory rather than lumbar sympathoinhibitory response. Following bilateral muscimol microinjection, the hypotensive effect of PBG was reversed ($-43 \pm 5$ mmHg pre muscimol, $5 \pm 3$ post muscimol; $P < 0.001$), as was that of CCK ($-10 \pm 2$ mmHg pre muscimol; $7 \pm 2$ mmHg post muscimol; $P < 0.001$). The CVLM injection sites for these experiments are depicted in Figure 3D. In one experiment (excluded from the group), the injection sites were more rostral and medial (left hand side), and had no effect on either baseline levels or on PE, PBG and CCK responses.

Splanchnic SND responses to PE, PBG and CCK before and after muscimol injection outside of the CVLM. In three rats, the effects of PE, PBG and CCK on SSND were...
assessed before and after bilateral muscimol microinjections (50 nl equivalent to 87.5 pmol, per side) into a location rostral to CVLM (1.0-1.4 mm caudal to identified RVLM neuron). In 2 other experiments, single microinjections (50 nl equivalent to 87.5 pmol) were made in subsequent order into the midline medulla at the level of RVLM then CVLM (see methods for exact locations). As a result of muscimol microinjections into all of these locations outside of the CVLM (grouped together n=7), resting arterial pressure did not change (91 ± 3 mmHg pre-muscimol; 97 ± 7 mmHg post-muscimol; P > 0.05) nor did resting SSND (98 ± 3 units pre-muscimol; 102 ± 8 units post-muscimol; P > 0.05). The sympathoinhibitory responses to PE (10 µg/kg i.v.), PBG (10 µg/kg i.v.) and CCK (4 or 8 µg/kg, i.v.) were unaffected following muscimol microinjections outside of CVLM (Figs. 4A, 4B, 4C), reflected by the stability of the hypertensive response to PE and the hypotensive responses to PBG and CCK, pre- and post-muscimol microinjection (P > 0.05 for all). The injection sites for these experiments are depicted in Figure 4D.

DISCUSSION

The present study demonstrates for the first time that the sympathoinhibitory reflex initiated by systemic administration of CCK acts via a pathway that is similar to that of the von Bezold-Jarisch reflex and the baroreflex. Importantly, it demonstrates the differential effects of CCK on splanchnic versus lumbar sympathetic vasomotor outflows, and highlights significant differences in the central circuitry involved in regulating these outflows.

As with the von Bezold-Jarisch reflex and the baroreflex (6, 8, 20), we have shown that the CCK-evoked gastrointestinal reflex can be disrupted by blockade of excitatory amino acid transmission with bilateral kynurenate microinjection into the CVLM. Similarly, all three reflexes can be blocked or attenuated by direct inhibition of
these neurons with bilateral microinjection of the GABA\textsubscript{A} receptor agonist muscimol, into the CVLM. On the other hand, injection of muscimol outside the confines of the CVLM had no significant effect on these sympathoinhibitory reflexes. This confirms the anatomical specificity of the effect of muscimol.

In accordance with previous studies (20), our experiments demonstrated that activation of the von Bezold-Jarisch reflex elicits a hypotensive response that is accompanied by a robust lumbar and splanchnic sympathoinhibitory response. Following inhibition of CVLM neurons, the depressor response to PBG was either abolished or converted to a slight pressor response, and the sympathoinhibitory response was transformed into a sympathoexcitatory response. Similarly, blockade of excitatory amino acid transmission by injection of kynurenate into CVLM abolished the depressor response to systemic PBG administration and reversed the sympathoinhibitory response to a sympathoexcitatory response. It has previously been postulated that the sympathoexcitatory response to PBG may result from the simultaneous activation of excitatory sympathetic cardiac afferents whose effects become unmasked on removal of the more powerful inhibitory component, or alternately from activation of peripheral chemoreceptors (20).

Activation of the baroreflex by systemic administration of PE produced a pressor response that was accompanied by profound lumbar and splanchnic sympathoinhibitory responses. Following bilateral microinjection of muscimol or kynurenate into CVLM, the sympathoinhibitory effects of PE were either significantly reduced or converted into sympathoexcitatory responses. The reduction in the pressor effects of PE following muscimol microinjection (lumbar and splanchnic nerves) can be attributed to the rise in pressure as a consequence of CVLM blockade, but is unlikely to have contributed to the resultant sympathoexcitatory effects or near abolition of the sympathoinhibitory effects of PE. Similar to the scenario discussed above for PBG, PE
Caudal ventrolateral medulla and CCK-induced sympathoinhibition may also activate cardiac sympathetic afferents, the effects of which may only be unmasked on removal of the more powerful inhibitory component (10).

Activation of the gastrointestinal vasomotor reflex by peripheral CCK administration resulted in an initial depressor response that was accompanied by splanchnic sympathoinhibition and lumbar sympathoexcitation. The depressor response elicited by CCK was often followed by a secondary, more prolonged pressor response that was not studied further. Following bilateral muscimol or kynurenate injection into CVLM, the depressor response to CCK was either abolished or transformed into a slight pressor response. Both of these treatments transformed the splanchnic sympathoinhibitory responses elicited by CCK into sympathoexcitatory responses. On the other hand, the lumbar sympathoexcitatory response elicited by CCK was augmented following bilateral muscimol injection into CVLM.

Our previous studies have demonstrated that the sympathoinhibitory effects of CCK are due to activation of CCK$_1$ receptors on subdiaphragmatic vagal afferents (14-16, 21). We hypothesized that RVLM neurons that are inhibited by CCK may be a subgroup responsible for supplying the sympathetic vasomotor outflow to the gastrointestinal vasculature, and those that are activated by CCK may belong to a different subgroup that drive the sympathetic vasomotor outflow to skeletal muscle vasculature (14, 15). We have constructed a model that attempts to explain the results of the present study and the function of different subtypes of CCK-sensitive RVLM neurons. Figure 5 predicts the outcome of varying proportions of (simultaneous) excitatory/inhibitory signals to CCK-inhibited and CCK-activated RVLM neurons. Thus, putative gastrointestinal RVLM neurons that are inhibited by CCK receive a predominantly inhibitory input, whereas those that are activated receive an input that is predominantly excitatory. In support of this hypothesis, there is evidence for a direct excitatory projection from NTS to RVLM as reported for the sympathetic chemoreflex,
which operates independently of the CVLM (7). Alternatively, direct excitatory inputs from supramedullary structures such as the PVN (1), may play a role.

A possible scenario is that CCK activates CCK\textsubscript{1} receptors on subdiaphragmatic vagal afferents, thereby transmitting excitatory signals to neurons in the NTS. A specific subset of NTS neurons (12) may in turn, send both direct excitatory and inhibitory (via a relay in the CVLM) signals to RVLM presypathetic vasomotor neurons. In the case of CCK-inhibited RVLM neurons, a more powerful inhibitory drive from CVLM may outweigh the direct excitatory drive, thereby producing an overall inhibitory effect and a decrease in sympathetic vasomotor outflow to the gastrointestinal vasculature. As a consequence of withdrawal of inhibitory input from CVLM (with either bilateral kynurenate or muscimol microinjections into CVLM), the weaker excitatory input may be unmasked, thereby resulting in CCK-induced sympathoexcitation as seen in our splanchnic nerve experiments (see Figs 1, 2 & 5a).

In the case of CCK-activated neurons, the excitatory drive may outweigh the inhibitory drive to produce an overall sympathoexcitatory response, as observed for the lumbar nerve experiments (see Figs 3 & 5b). As a result of bilateral injection of muscimol into CVLM and withdrawal of inhibitory signals to RVLM, the remaining excitatory component would be expected to entirely dominate, reflecting the augmentation in sympathoexcitation observed in our study. The different effects of CCK on lumbar versus splanchnic nerve discharge are of interest, given that these sympathetic outflows behave uniformly to stimuli such as the von Bezold-Jarisch and baroreflex.

Stimulation of the midline medullary raphe area has been shown to inhibit RVLM presypathetic vasomotor neurons (22). Lesions of this midline area have little effect on baroreflex sympathoinhibition, suggesting that the midline raphe is unlikely to be involved in baroreflex function (11). As with the baroreflex and the von Bezold-
Jarisch reflex, muscimol injection into the caudal midline raphe- both at the level of RVLM and at the level of CVLM, had no effect on the CCK-elicited gastrointestinal reflex. Similarly, bilateral microinjection of the GABA_A agonist into an area just rostral to the CVLM had no significant effect on any of these reflexes. It is unlikely that injection of artificial CSF in itself contributed to the effects of muscimol or kynurenate since previous studies have shown that microinjections of artificial CSF into the CVLM have no significant effects on resting MAP and HR or the von Bezold-Jarisch reflex (19). In common to all three reflexes is their dependence on an inhibitory relay that incorporates the CVLM.

In conclusion, this study has demonstrated that CCK can evoke differential responses in the sympathetic vasomotor supply to the gastrointestinal and skeletomuscular vascular beds, strengthening the argument for a “viscerotopic” organisation of RVLM presympathetic vasomotor neurons. While CCK elicits splanchnic sympathoinhibition that may lead to gastrointestinal vasodilation, it also produces lumbar sympathoexcitation that may lead to hindlimb vasoconstriction. These responses are probably due to the recruitment of different subpopulations of RVLM presympathetic vasomotor neurons: those that are inhibited by CCK and those that are activated by CCK (14-16). A major finding of this study is that the central circuitry involved in the sympathoinhibitory effects of CCK is similar to that proposed for the von Bezold-Jarisch and the baroreflex in that it incorporates an inhibitory relay in the CVLM. On the other hand, the sympathoexcitatory effects of CCK are independent of a relay in the CVLM, but may be dependent on a direct projection from the NTS or supramedullary structures. We have hypothesized that CCK-inhibited and CCK-activated RVLM presympathetic vasomotor neurons may receive both direct excitatory inputs from NTS or supramedullary structures and indirect inhibitory inputs from CVLM. However, it is the relative strength of these inputs that determines the net
inhibitory/excitatory effect on the two neuronal subpopulations and therefore on the sympathetic vasomotor outflows they drive.

**Perspectives**

Previous studies have shown that cardiopulmonary vagal and baroreceptor reflex pathways are dependent on the integrity of the CVLM. The present study indicates that the circulatory reflex initiated by CCK-induced stimulation of subdiaphragmatic afferents also operates using similar intramedullary circuitry. Furthermore, this study demonstrates that the sympathetic vasomotor outflow to the gastrointestinal tract and to skeletal muscle vasculature are controlled differentially.
REFERENCES


FIGURE LEGEND

Figure 1. A & B: Arterial blood pressure and splanchnic sympathetic nerve discharge (SSND) responses to systemic administration of phenylephrine (PE, 10 µg/kg, i.v.), phenylbiguanide (PBG, 10 µg/kg, i.v.) and cholecystokinin (CCK, 8 µg/kg, i.v.) before (panel A) and after (panel B) bilateral muscimol microinjection into the CVLM. Responses in panels A and B are obtained from the same preparation. Bottom trace represents mean arterial pressure, center trace represents raw SSND and top trace represents rectified and integrated SSND. Sympathoinhibitory responses to PE are abolished and those of PBG and CCK are reversed following bilateral muscimol injection into CVLM. C: Group data demonstrating the effects of PE (10 µg/kg, i.v.), PBG (10 µg/kg, i.v.) and CCK (4-8 µg/kg, i.v.) on SSND (expressed as percentage change in SSND) pre- and post- muscimol injection into CVLM (n=6). All three responses are significantly altered by muscimol injection into CVLM. D: Representative coronal section showing bilateral muscimol injection sites. * P<0.01, ** P<0.001.

Abbreviations: 12, hypoglossal nucleus; LRt, lateral reticular nucleus; py, pyramidal tract; ROb, raphe obscurus; sp5, spinal trigeminal tract.

Figure 2. A & B: Arterial blood pressure and splanchnic sympathetic nerve discharge (SSND) responses to systemic administration of phenylephrine (PE, 10 µg/kg, i.v.), phenylbiguanide (PBG, 10 µg/kg, i.v.) and cholecystokinin (CCK, 4 µg/kg, i.v.) before (panel A) and after (panel B) bilateral kynurenate microinjection into CVLM. Responses in panels A and B are obtained from the same preparation. Bottom trace represents mean arterial pressure, center trace represents raw SSND and top trace represents SSND that has been rectified and integrated. Sympathoinhibitory response to PE is abolished and the sympathoinhibitory responses to PBG and CCK are reversed,
following bilateral kynurenate injection into CVLM. C: Group data demonstrating the effects of PE, PBG and CCK on SSND (expressed as percentage change in SSND) pre- and post- kynurenate injection into CVLM (n=7). All three responses are significantly altered by kynurenate injection into CVLM. D: Representative coronal section showing bilateral kynurenate injection sites. * \( P<0.01 \), ** \( P<0.001 \). Abbreviations: 12, hypoglossal nucleus; LRt, lateral reticular nucleus; py, pyramidal tract; ROb, raphe obscurus; sp5, spinal trigeminal tract.

Figure 3. A & B: Arterial blood pressure and lumbar sympathetic nerve discharge (LSND) responses to systemic administration of phenylephrine (PE, 10 \( \mu \)g/kg, i.v.), phenylbiguanide (PBG, 10 \( \mu \)g/kg, i.v.) and cholecystokinin (CCK, 4 \( \mu \)g/kg, i.v.) before (panel A) and after (panel B) bilateral muscimol microinjection into CVLM. Responses in panels A and B are obtained from the same preparation. Bottom trace represents mean arterial pressure, center trace represents raw LSND and top trace represents LSND that has been rectified and integrated. Sympathoinhibitory responses to PE and PBG are reversed following bilateral muscimol injection into CVLM, and the sympathoexcitatory response to CCK is augmented after bilateral muscimol injection. C: Group data demonstrating the effects of PE, PBG and CCK on LSND (expressed as percentage change in LSND) pre- and post- muscimol injection into CVLM (n=7). All three responses are significantly altered by muscimol injection into CVLM. D: Representative coronal section showing bilateral muscimol injection sites for these experiments. * \( P<0.01 \), ** \( P<0.001 \). Abbreviations: 12, hypoglossal nucleus; LRt, lateral reticular nucleus; py, pyramidal tract; ROb, raphe obscurus; sp5, spinal trigeminal tract.
Figure 4. A & B: Arterial blood pressure and splanchnic sympathetic nerve discharge (SSND) responses to systemic administration of phenylephrine (PE, 10 µg/kg, i.v.), phenylbiguanide (PBG, 10 µg/kg, i.v.) and cholecystokinin (CCK, 4 µg/kg, i.v.) before (panel A) and after (panel B) muscimol microinjection into the midline medulla at the level of RVLM. Responses in panels A and B are obtained from the same preparation. Bottom trace represents mean arterial pressure, center trace represents raw SSND and top trace represents SSND that has been rectified and integrated. Sympathoinhibitory responses to PE, PBG and CCK remain intact following bilateral muscimol injection into CVLM. C: Group data for the experiments in which muscimol was injected into the midline medulla at the level of RVLM, into the midline medulla at the level of CVLM and bilaterally at a location 1.4 mm caudal to an identified RVLM neuron, and demonstrates the effects of PE (10 µg/kg, i.v.), PBG (10 µg/kg, i.v.) and CCK (4-8 µg/kg, i.v.) on SSND (expressed as percentage change in SSND) pre- and post-muscimol injection into these areas (n=7). All three responses remain unaltered following muscimol injections outside of CVLM. D: Single muscimol injections into the midline medulla at the level of RVLM (i: -11.8 mm from bregma; n=2); bilateral muscimol injections at a location 1.4 mm caudal and 0.4 mm dorsal to RVLM (ii, n=3), or single muscimol injections into the midline medulla at the level of CVLM, 1.6 mm caudal and 0.4 mm dorsal to RVLM (iii, n=2). Abbreviations: 12, hypoglossal nucleus; LPGi, lateral paragigantocellular nucleus; LRt, lateral reticular nucleus; py, pyramidal tract; ROb, raphe obscurus; sp5, spinal trigeminal tract.

Figure 5. Schematic depicting the balance of excitatory and inhibitory inputs to two subpopulations of RVLM presympathetic vasomotor neurons: those that are inhibited by CCK (5A; represented by circle within RVLM) and those that are activated by CCK
Caudal ventrolateral medulla and CCK-induced sympathoinhibition (5B; represented by square within RVLM). Heavy lines represent more dominant inputs. A: RVLM neurons inhibited by CCK may be specific for supplying the sympathetic vasomotor outflow to the gastrointestinal vasculature. This neuronal subset may receive more substantial inhibitory signals from CVLM (including baroreceptor signals), resulting in an overall reduction in splanchnic sympathetic vasomotor outflow (grey block arrow). On removal of the inhibitory signal from CVLM with kynurenate or muscimol injections into CVLM (X), the excitatory signal is unmasked, reversing the sympathoinhibition to sympathoexcitation (black block arrow). B: RVLM neurons activated by CCK may be specific for supplying the sympathetic vasomotor outflow to skeletal muscle vasculature. These neurons may receive more powerful excitatory signals (from NTS or some other source), resulting in an increase in lumbar sympathetic vasomotor outflow (grey block arrow). On withdrawal of inhibitory signals from CVLM with muscimol microinjections into the area (X), there is an augmentation of the sympathoexcitatory response (black block arrow).
Figure 1

A  Pre-muscimol

B  Post-muscimol

C

D
Figure 2

A  Pre-kynurenate

B  Post-kynurenate

C

D

* P < 0.01
** P < 0.001
Figure 3

A  Pre-muscimol

![Graph showing PE, PBG, and CCK responses before muscimol administration.](image)

B  Post-muscimol

![Graph showing PE, PBG, and CCK responses after muscimol administration.](image)

C

<table>
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<th></th>
<th>PE</th>
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<th>CCK</th>
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* * P < 0.01
** ** P < 0.001

D

![Diagram showing brain structures.](image)
Figure 4

A  Pre-muscimol

B  Post-muscimol

C

D

Figures showing pre- and post-muscimol effects on neural activity and percent change in SSND units.
Figure 5

A  SPLANCHNIC sympathetic vasomotor outflow

- CCK-activated RVLM neurons
- Sympathoinhibition is reversed on withdrawal of inhibitory signal from CVLM (X)

NTS

Activation of CCK\textsubscript{1} receptors on subdiaphragmatic vagal afferents

RVLM

CVLM

Excitatory signal

Inhibitory signal

B  LUMBAR sympathetic vasomotor outflow

- CCK-activated RVLM neurons
- Sympathoexcitation is augmented on withdrawal of inhibitory signal from CVLM (X)

NTS

Activation of CCK\textsubscript{1} receptors on subdiaphragmatic vagal afferents

RVLM

CVLM

Excitatory signal

Inhibitory signal