CHOLECYSTOKININ-INDUCED INHIBITION OF PRESYMPATHETIC VASOMOTOR NEURONS: DEPENDENCE ON SUB-DIAPHRAGMATIC VAGAL AFFERENTS AND CENTRAL NMDA RECEPTORS IN THE RAT

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*Short running title: RVLM presympathetic neurons and cholecystokinin*

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ABSTRACT

Systemic administration of cholecystokinin (CCK) inhibits a sub-population of RVLM presympathetic vasomotor neurons. This study was designed to determine whether this effect involved sub-diaphragmatic 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, paralysed male Sprague-Dawley rats. The responses of the neurons to CCK (2 and 4 µg/kg, i.v.), phenylephrine (PE; 5 µg/kg, i.v.) and phenylbiguanide (PBG; 5 µg/kg, i.v.) were tested before and after application of the local anesthetic lidocaine (2% w/v gel; 1 ml) to the sub-diaphragmatic vagi at the level of the oesophagus. In 7 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 (MK-801; 1 mg/kg, i.v.), a non-competitive antagonist at N-methyl-D-aspartate (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 sub-diaphragmatic vagal afferents. Furthermore, the data suggest that the CCK activates a central pathway involving NMDA receptors to produce inhibition of RVLM presympathetic neuronal discharge.

Key words: vagal afferent, sympathetic, rostral ventrolateral medulla.
INTRODUCTION

The gastrointestinal peptide cholecystokinin (CCK) is a powerful stimulant of gastrointestinal vagal afferent nerve fibres (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 sub-population of RVLM presympathetic neurons (17, 18). RVLM presympathetic vasomotor neurons are considered to play a major role in 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. Secondly, the selectivity of CCK for a sub-population of RVLM presympathetic neurons may suggest that CCK-sensitive neurons preferentially receive vagal afferent input from the gastrointestinal tract (21).

The neurocircuitry which mediates arterial baroreflex-mediated inhibition of RVLM presympathetic vasomotor neurons and sympathetic vasomotor outflow is described by a trisynaptic model which includes 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 sub-diaphragmatic 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 using male Sprague-Dawley rats (250-380 g) and 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 tracheostomised after induction of anesthesia produced by placement into a chamber saturated with halothane vapour (Fluothane\textsuperscript{TM}, Zeneca, Macclesfield, UK). After cannulation of the trachea, all animals were ventilated artificially with 100% O\textsubscript{2} (1 ml/100 g body weight, 40-60 breaths min\textsuperscript{-1}) 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-pincher 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 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 paralysing agent pancuronium bromide (1-2 mg/kg, i.v) 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 anaesthesia. 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. Note that the pancuronium has detectable vagolytic actions and so reduces the bradycardic actions of phenylbiguanide and phenylephrine.

**Extracellular single unit recording**

Rats were placed into a stereotaxic apparatus and the dorsal cerebellar surface was exposed by removal of a portion of the interparietal bone. A bipolar electrode was 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 the caudal, 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 (T2-T3) enabling antidromic activation of spinally-projecting, barosensitive neurons within 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 metres) 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 O.D.) containing 0.5M sodium acetate and 2% Pontamine sky blue were used to record extracellularly from neurons in the RVLM. The signals were amplified (x1000), filtered (400-4,000 Hz) and monitored using an oscilloscope and an audio amplifier. The effects of CCK (2 and 4 µg/kg, i.v.) or PBG (5 µg/kg, i.v.) on arterial blood pressure and the discharge rate of
RVLM barosensitive, spinally-projecting neurons were recorded and stored onto video tape 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, i.v.). Neuronal discharge rates were measured at rest prior to 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 prior to drug administration.

The doses of CCK and PBG were sub-maximal 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 sub-population of the total population of spinally-projecting, barosensitive cells in the RVLM (17). These are usually neurons with spinal axonal 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, i.v.) or CCK (2 and 4 µg/kg, i.v.) were administered in random fashion.

Blockade of vagal afferent transmission

The following approach was used to interrupt sub-diaphragmatic vagal afferent traffic: a polyethylene cannula was sutured onto a sub-diaphragmatic section of the oesophagus. Care was taken to avoid damage to the anterior and posterior vagal trunks which lie along the oesophagus (15). Lidocaine (1 ml; 2% w/v gel; Orion Laboratories, Welshpool, Western Australia) was applied to the sub-diaphragmatic
vagi through the implanted cannula. The responsiveness of each RVLM presympathetic vasomotor neuron to CCK (2 and 4 µg/kg, i.v.), phenylephrine (5 µg/kg, i.v.), PBG (5 µg/kg, i.v.) and elevation of arterial blood pressure (PE, 5 µg/kg, i.v.) 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, i.v.), phenylephrine (5 µg/kg, i.v.), PBG (5 µg/kg, i.v.) and elevation of arterial blood pressure (PE, 5 µg/kg, i.v.) were tested before and 5 min after blockade of central NMDA receptors using dizocilpine ((+)-MK-801 hydrogen maleate; 1 mg/kg, i.v.; Research Biochemicals International, Natick, MA, USA).

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 from the recording electrode. At the conclusion of each unit recording experiment the animals were deeply anesthetised with pentobarbitone sodium (Nembutal, Rhone Merieux Australia, Pinkenba, Queensland, Australia; 60 mg/kg, i.p.) prior to 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. Brains were sectioned using a cryostat and mounted onto gelatin-subbed slides and were stained for Nissl substance using 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 video tape using a PCM data acquisition system (Vetter Instruments, Rebersburg, PA, USA). Signals were analysed off-line using a Cambridge Electronic Design data acquisition system (CED, Cambridge, UK) and Spike2 software. Data are expressed as means ± s.e. mean. Changes in neuronal discharge rate were calculated by expressing the peak change in discharge rate (in spikes/s) occurring in response to each agent tested as a percentage of the basal discharge rate immediately prior to administration of the test agent. Differences between means were compared by repeated measures ANOVA followed by a Bonferroni modified t-test using GraphPad InStat version 3.05 (GraphPad Software, San Diego, CA, U.S.A.). $P<0.05$ was considered as the level of significance.

Drugs
Phenylbiguanide (PBG; Aldrich Chemical Co., Milwaukee, WIS, USA), and cholecystokinin octapeptide (sulphated form; American Peptide Co., Sunnyvale, CA, USA) were dissolved in normal saline (0.9% w/v NaCl). PBG and CCK were injected in a volume of 0.1 ml/kg.
RESULTS

Sub-diaphragmatic vagal local anaesthesia

Sub-diaphragmatic vagal anesthesia reduced resting MAP from 85±3 mmHg (n=7) to 65±4 mmHg (P<0.05). Blockade of sub-diaphragmatic vagal afferent transmission markedly reduced the inhibitory effects of CCK (4 µg/kg, i.v.) on RVLM presympathetic neuronal discharge (Fig. 1). Vagal lidocaine application significantly reduced the inhibitory effects of CCK (2 and 4 µg/kg, i.v.) 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). 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 & 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 & 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 non-competitive NMDA receptor antagonist dizocilpine had a biphasic effect on arterial blood pressure. Shortly after dizocilpine administration, MAP increased from 93±3 mmHg 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, i.v.) on RVLM presympathetic neuronal discharge (Figs. 6 & 9). The inhibitory effects of CCK (2 and 4 µg/kg, i.v.) 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 & 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 & 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 cholecystokinin produces inhibition of a sub-population of RVLM presympathetic neurons via a mechanism which is dependent on intact sub-diaphragmatic vagal afferents and central NMDA receptors.

Interruption of vagal afferent traffic arising in sub-diaphragmatic 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, since sub-diaphragmatic 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 since lidocaine application to the sub-diaphragmatic vagi blocked the response to systemic CCK but not PBG. This finding also suggests that the lidocaine did not spread sufficiently to anesthetise cardiopulmonary vagal afferents. We have previously observed that the inhibitory responses of RVLM presympathetic neurons to systemic administration of PBG and CCK are very stable (16). This suggests that the effects of sub-diaphragmatic vagal anesthesia or NMDA 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 sub-diaphragmatic vagal afferents. To our knowledge, this study is the first demonstration of the inhibitory influence of the sub-diaphragmatic vagal afferents on circulatory function. A potential underlying mechanism for this action of CCK may involve (i) CCK-induced activation of gastrointestinal vagal afferents (9), (ii)
activation of NTS neurons leading to (iii) activation of an intramedullary inhibitory pathway which leads to (iv) 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 (1995) found that sub-diaphragmatic vagal stimulation predominantly activates RVLM presympathetic vasomotor neurons in the anesthetized rabbit (5). 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 sub-group of sub-diaphragmatic vagal afferents which have an inhibitory influence on sympathetic vasomotor function. Perhaps selective stimulation of specific sub-diaphragmatic 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 sub-diaphragmatically may be expected to also block transmission in splanchnic afferents, these are unlikely to be of any importance in the inhibitory actions of CCK since in a prior study we demonstrated that the sympathoinhibitory effects of CCK are blocked by section of the cervical vagi (17). 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 which 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 pancuronium.

Another major finding in the present study, was that CCK-induced inhibition of RVLM presympathetic neuronal discharge was reduced by central NMDA receptor blockade with dizocilpine. Although the precise location(s) of the site of action of the NMDA receptor antagonist is presently unknown, it is likely that NMDA receptors located in the NTS and/or caudal ventrolateral medulla play a role. NMDA receptor blockade in the CVLM blocks aortic depressor nerve stimulation-evoked depressor responses (6, 11). NMDA receptors probably are not critically involved in mediating baroreflex-associated neurotransmission in the NTS (7, 10, 23). Precise localisation of the NMDA receptors involved in CCK-induced sympathoinhibition is of major importance since it will determine whether this reflex is similar to that of the trisynaptic baroreflex model.

Approximately 50% of the RVLM presympathetic vasomotor neurons are sensitive to CCK (17, 18). In contrast, virtually all are sensitive to systemic administration of
PBG (22). The selectivity of CCK for a sub-population of RVLM presympathetic neurons may be related to its preferential effects on splanchnic sympathetic outflow (17). On the other hand, PBG inhibits lumbar, splanchnic, renal and adrenal sympathetic outflow (for review see (21)). The functional significance of the effects of CCK may be that it, alone, or in concert with other substances released upon consumption of food, may contribute to gastrointestinal hyperemia (post-prandial hyperemia) 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 sub-diaphragmatic 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 which mediates CCK-induced sympathoinhibition 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 sub-diaphragmatic vagal afferents. The selective actions of CCK on a sub-population of RVLM presympathetic vasomotor neurons may also provide a means of identifying...
similar but parallel intramedullary pathways involved in sympathetic vasomotor control.
ACKNOWLEDGEMENTS

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REFERENCES


Figure Legends

Figure 1. The effects of cholecystokinin (CCK; 4 µg/kg, i.v.) on the discharge of an RVLM presympathetic vasomotor neuron before and after application of lidocaine (local anesthetic gel, 1 ml, 2% w/v) to the sub-diaphragmatic vagi adjacent to the oesophagus. A, The 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. Abbreviations: AP, arterial blood pressure; FR, firing rate; HR, heart rate.

Figure 2. The effects of phenylbiguanide (PBG; 4 µg/kg, i.v.) on the discharge of an RVLM presympathetic vasomotor neuron before and after application of lidocaine (local anesthetic gel, 1 ml, 2% w/v) to the sub-diaphragmatic vagi adjacent to the oesophagus. A, The discharge of the neuron is silenced by systemic injection of CCK injected at the marker (●). B, After application of lidocaine the effect of PBG on the discharge of the RVLM neuron is reduced slightly. Abbreviations: AP, arterial blood pressure; FR, firing rate; HR, heart rate.

Figure 3. The effects of the vasoconstrictor phenylephrine (PE; 5 µg/kg, i.v.) on the discharge of an RVLM presympathetic vasomotor neuron before and after application of lidocaine (local anesthetic gel, 1 ml, 2% w/v) to the sub-diaphragmatic vagi adjacent to the oesophagus. A, The discharge of the neuron is silenced by systemic injection of PE injected at the marker (●). B, After application of lidocaine the effect of PE on the discharge of the RVLM neuron is reduced. Note that the peak rise in pressure in response to PE is less after lidocaine. Abbreviations: AP, arterial blood pressure; FR, firing rate; HR, heart rate.

Figure 4. Summary of the effects of application of lidocaine (local anesthetic gel, 1 ml, 2% w/v) onto the sub-diaphragmatic vagi adjacent to the oesophagus on the responses to systemic administration of
cholecystokinin (CCK; 2 & 4 µg/kg, i.v.), phenylephrine (PE; 5 µg/kg, i.v.) and phenylbiguanide (PBG; 5 µg/kg, i.v.). The changes in mean arterial blood pressure (ΔMAP), heart rate and RVLM presympathetic neuronal discharge rate (ΔFiring rate) produced by each agent before (open columns) and after (filled columns) lidocaine treatment are shown. Data are presented as mean±s.e.m. and represent data obtained in 7 separate experiments. * P<0.05.

Figure 5. The effects of cholecystokinin (CCK; 4 µg/kg, i.v.) on the discharge of an RVLM presympathetic vasomotor neuron before and after bilateral application of lidocaine (local anesthetic gel, 0.5 ml, 2% w/v) to the cervical vagi. A, The discharge of the cell is markedly reduced after CCK injection (●). B, After lidocaine treatment the effects of CCK are blocked. Abbreviations: AP, arterial blood pressure; FR, firing rate.

Figure 6. The effects of cholecystokinin (CCK; 4 µg/kg, i.v.) 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, i.v.). A, The 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. Abbreviations: AP, arterial blood pressure; FR, firing rate; HR, heart rate.

Figure 7. The effects of phenylbiguanide (PBG; 5 µg/kg, i.v.) 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, i.v.). A, The 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. Abbreviations: AP, arterial blood pressure; FR, firing rate; HR, heart rate.
Figure 8. The effects of phenylephrine (PE; 5 µg/kg, i.v.) 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, i.v.). A, The 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 occurred during the response to PE. Accurate counting of the spike rate was ensured by adjustment of the window discriminator. Abbreviations: AP, arterial blood pressure; FR, firing rate; HR, heart rate.

Figure 9. Summary of the effects of systemic administration of dizocilpine (N-methyl-D-aspartate receptor ion channel antagonist; 1 mg/kg, i.v.) on the responses to systemic administration of cholecystokinin (CCK; 2 & 4 µg/kg, i.v.), phenylephrine (PE; 5 µg/kg, i.v.) and phenylbiguanide (PBG; 5 µg/kg, i.v.). The changes in mean arterial blood pressure (ΔMAP), heart rate and RVLM presympathetic neuronal discharge rate (ΔFiring rate) produced by each agent before (open columns) and after (filled columns) dizocilpine treatment are shown. Data are presented as mean±s.e.m. and represent data obtained in 6 separate experiments. * P<0.05.
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