Participation of Caudal Ventrolateral Medulla in the Regulation of Gallbladder Motility in Rabbits

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Abstract

To investigate whether the caudal ventrolateral medulla (CVLM) participates in the regulation of gallbladder motility, we studied the effects of microinjection of L-glutamate and other agents into the CVLM on gallbladder pressure (GP) in anesthetized rabbits. A frog bladder connected with a force transducer was inserted into the gallbladder to record the change of GP. Microinjection of L-glutamate into the CVLM decreased GP, While micnoinjection of γ-amino-butyric acid (GABA) increased GP. Microinjection of ketamine, a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, into CVLM increased GP, while microinjection of 6-cyano-7-nitroquinoxaline-2,3-(1H,4H)-dione (CNQX), a competitive (±)-a-amino-3-hydroxy-5- methylisoxazole-4-propionic acid (AMPA)/kainate receptor antagonist, had no significant effect on GP. The effects of L-glutamate was abolished by ketamine, but not by CNQX. Intravenous injection of phentolamine or transection of the spinal cord eliminated the effects of L-glutamate on GP. These results indicate that [1] CVLM participated in the regulation of gallbladder motility; [2] endogenous L-glutamate in CVLM is involved in the regulation mediated by NMDA receptors, the output of which is sent through sympathetic nerve and α-adrenergic receptors.

Key Words: CVLM, gallbladder pressure, NMDA, AMPA, sympathetic nerve

Introduction

As we have reported earlier, both sympathetic and parasympathetic nervous centers participate in the regulation of the motility of extrahepatic biliary system. The nucleus raphe obscurus (NRO) – dorsal motor nucleus of the vagus nerve (DMV) – vagus nerve pathway mainly modulate the phasic contraction of gallbladder (9, 14, 16, 24), while the medial area of hypothalamus (26), paraventricular (PVH) (28), reticular formation in the pontine legmentum (27) and NRO (24) regulate the gallbladder tonic contraction *via* the peripheral sympathetic nerve. The caudal ventrolateral medulla (CVLM) is an important autonomic nervous center that mainly controls the activity of sympathetic fiber. Through the connection with PVH (22), the rostral ventrolateral medulla (RVLM) (1), the nucleus accumbers (15), and the intermediolateral column of the spinal cord (22), CVLM exerted a wide range of regulation on visceral functions. CVLM participate in the regulation of cardiovascular activity and the hypertension-induced hypoalgesia (13). Both electrical and chemical stimulation of the CVLM decrease total lung resistance by withdrawing cholinergic input to airway smooth muscle (21). Stimulation of the CVLM produces inhibition of both frequency and amplitude of gastric antral motility (25). The CVLM was responsible for the inhibition of gastric antral motor activity elicited by acupuncture of "renzhong" (25).

However, the effects of the CVLM on gallbladder motility remain unknown. Glutamate, the excitatory amino acid, and gamma-aminobutyric acid (GABA), the inhibitory amino acid, are widely distributed in central nervous system. They mediate most of the excitatory and inhibitory signal transmission between

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neurons. Many CVLM neurons contained the GABA synthesizing enzyme and glutamic acid decarboxylase (17). Microinjection of N-methyl-D-aspartate (NMDA) or (±)-α-amino-3 -hydroxy-5-methylisoxazole-4-propionic acid (AMPA), the specific glutamate receptors agonists, decreased total resistance of the lung (21). Injection of L-glutamate into the CVLM decreased arterial pressure and increased superior mesenteric conductance, while bilateral injection of GABA increased arterial pressure and decreased superior mesenteric conduction (2). The cardiovascular response evoked by a static muscle contraction increased the release of glutamate and decreased the release of GABA in CVLM (5). Therefore, in this study, glutamate and GABA were used to excite and inhibit the activity of neurons in CVLM, and the effects of CVLM on the regulation of the gallbladder motility were investigated.

Materials and Methods

Experiments were preformed on sixty-three healthy adult rabbits of both sexes (2.0~2.5 kg). After fasting for 18~24 h, the rabbits were anesthetized by 20% urethane (1 g/kg, i.v.). They were paralyzed with gallamine trithiodine (2 mg/kg, i.v.) and artificially ventilated. The gallbladder was exposed through a midline abdominal incision. A frog bladder connected with a force transducer was inserted into the gallbladder through a small incision at the fundus to record the gallbladder motility. The right femoral artery was catheterized to monitor blood pressure (BP). The gallbladder pressure (GP) and BP were recorded on a four-channel polygraph recorder (RM-6000, Nihon Kohden, Tokyo, Japan) at a paper speed of 10 mm/min. The animals were then placed prone in a stereotaxic instrument (SN-38712, Narishige, USA). The occipital bone was removed, and the dorsal surface of the medulla was exposed. The microinjection region was in CVLM (0.5-2.5 mm caudal to the obex, 2.5-3.5 mm lateral to the midline, and 2.5-3.5 mm ventral to the medulla surface) (10, 11). A micropipette (30 μm internal diameter, 300 μm external diameter) filled with drug solution was used to inject agents into the CVLM. Individual drugs, in volumes of 100 nl, were microinjected into CVLM. The time taken for one injection was 1 minute. Microinjections were performed in either the right or left CVLM. Bilateral vagus nerves of several rabbits were cut at cervix level. The spinal cords of some rabbits were transected at T3-T4. Anal temperature and BP of all animals were monitored during the experiment and the temperature was kept at 37.5-38.5 °C.

After the experiment, L-glutamate of high concentration and large dose (2 mol/l, 2 μl volume) was microinjected into the same position in CVLM, to destroy the local neurons and cause a lesion *in situ* (Fig. 1). Then the rabbits were killed by air emboli. The medulla was removed and immersed in a solution of 10% formalin for 4 days. The bulbar region were embedded in paraffin wax, serially sectioned at a thickness of 6 μm, and stained with H.E. to facilitate the identification of the lesion site.

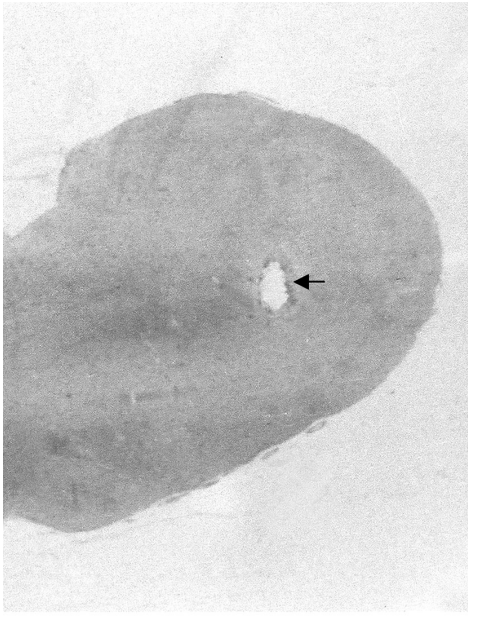

Fig. 1. Coronal section of brain stem at the level of 1.0 mm caudal to obex (right half of the section). The arrow indicates the lesion in the CVLM caused by L-glutamate of high concentration (2 mol/l, 2 μl).

The following drugs were used for microinjection: L-glutamate and ketamine were purchased from Shandong Provincial Biochemical Reagent Center (China); NMDA, AMPA, CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and GABA were purchased from Sigma (USA). All drugs used were freshly prepared and dissolved in distilled water at desired concentration.

Changes of GP were got by subtracting the value of GP 2 min after agents microinjection from that 1 min before the administration. Data were presented as mean ± SD. Student's *t* test was used to compare the data between the experimental and control groups. The criterion for statistical significance was $P < 0.05$.

Results

Effects of L-glutamate and GABA Microinjected into CVLM

In 25 rabbits, microinjection of L-glutamate

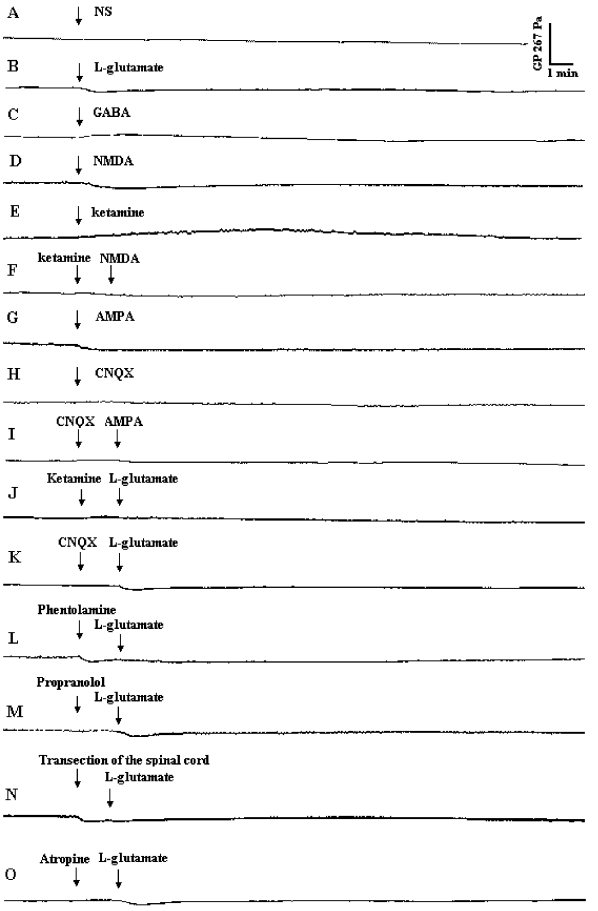

Fig. 2. The effects of several reagents microinjected into the CVLM on gallbladder pressure (GP). A, Normal Saline (NS); B, L-glutamate (106 mmol/l, 100 nl); C, γ-amino-n-butyric acid (GABA, 1 mol/l, 100 nl); D, N-methyl-D-aspartate (NMDA, 0.5 mmol/l, 100 nl); E, ketamine (90 mmol/l, 100 nl); F, NMDA (0.5 mmol/l, 100 nl) after ketamine (90 mmol/l, 100 nl); G, (±)-α-amino-3-hydroxy-5-methylisoxazole- 4-propionic acid (AMPA, 0.1 mmol/l, 100 nl); H, 6-cyano-7-nitroquinoxaline -2,3-(1H,4H)-dione (CNQX, 2 mmol/l, 100 nl); I, AMPA (0.1 mmol/l, 100 nl) after CNQX (2 mmol/l, 100 nl); J, L-glutamine (106 mmol/l, 100 nl) after ketamine (90 mmol/l, 100 nl); K, L-glutamine (106 mmol/l, 100 nl) after CNQX (2 mmol/l, 100 nl); L, L-glutamine (106 mmol/l, 100 nl) after injection of phentolamine (1.5 mg/kg); M, L-glutamine (106 mmol/l, 100 nl) after injection of propranolol (1.5 mg/kg); N, L-glutamine (106 mmol/l, 100 nl) after transection of the spinal cord; O, L-glutamine (106 mmol/l, 100 nl) after injection of atropine (0.2 mg/kg).

(106 mmol/l, 100 nl), the neuroexcitatory amino acid, into the CVLM decreased GP (Fig. 2B, 3 and Fig. 4). This effect began at approximately 5 s after the microinjection, and resulted in maximal changes (-40 ± 10 Pa, P<0.001) after 1 min and returned to the baseline within 6 min after the microinjection. In 6 rabbits, 5 different concentrations of L-glutamate (8, 17, 43, 85, 106, 170 mmol/l) were microinjected into CVLM. There was a dose-response relationship between L-glutamate and GP decrease (Fig. 3).

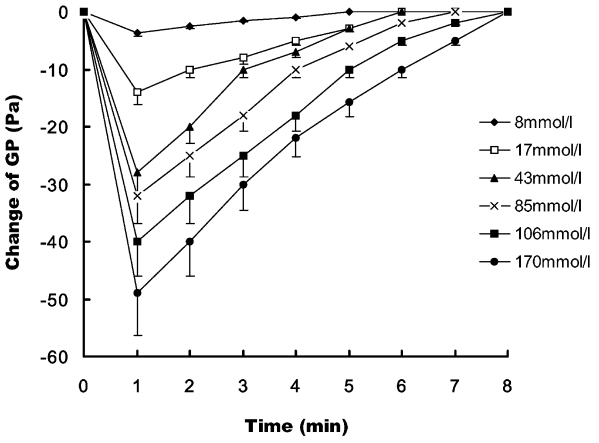

Fig. 3. Dose-dependent effects of L-glutamate (8, 17, 43, 85, 106, 170 mmol/l) microinjected into the CVLM on the changes of gallbladder pressure (GP).

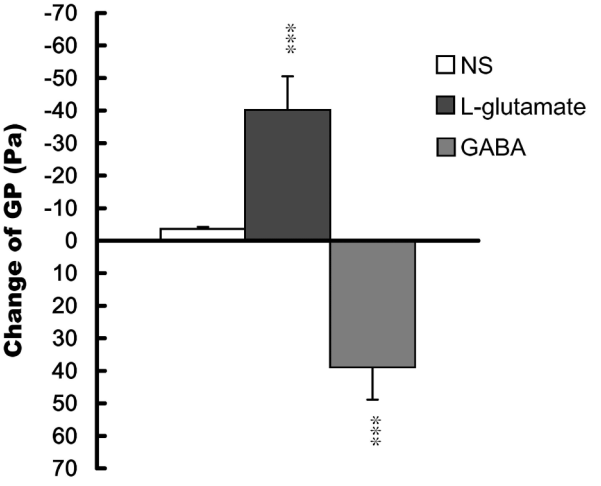

Fig. 4. Average maximal changes in gallbladder pressure (GP) after microinjections of Normal Saline (NS), L-glutamate (106 mmol/l, 100 nl) and γ-amino-butyric acid (GABA, 1 mol/l, 100 nl) into the CVLM. ***P<0.001 vs NS.

In 18 rabbits, microinjection of GABA (1 mol/l, 100 nl) into the CVLM increased GP (+39±10 Pa, P<0.001) (Fig. 2C and Fig. 4). This effect began at approximately 5 s after the microinjection, reached a peak at 2-3 min, and then returned to the baseline within 7 min.

Effects of NMDA Receptors on the Motility of Gallbladder

In 15 rabbits, NMDA (0.5 mmol/l, 100 nl), the specific NMDA receptor agonist, decreased GP (-43 ± 12 Pa, P < 0.001) after it was microinjected into CVLM (Fig. 2D and Fig. 5B).

In 10 rabbits, ketamine (90 mmol/l, 100 nl), a

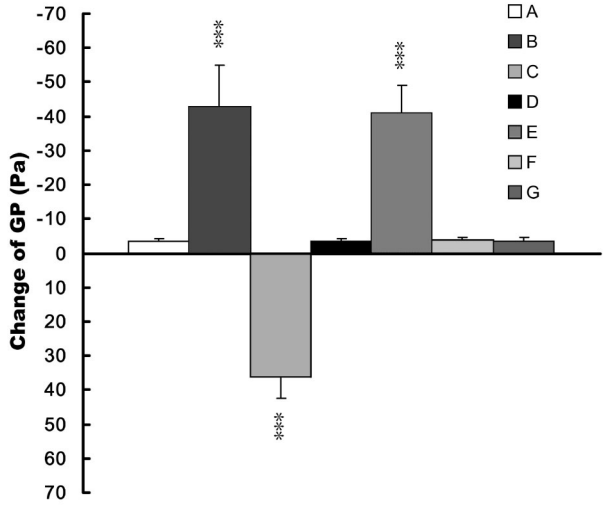

Fig. 5. The effects of microinjection of normal saline (NS) (A); N-methyl-D-aspartate (NMDA, 0.5 mmol/l, 100 nl) (B); ketamine (90 mmol/l, 100 nl) (C); NMDA (0.5 mmol/l, 100 nl) after ketamine(90 mmol/l, 100 nl) (D); α-amino-3 -hydroxy-5-methylisoxazole-4-propionic acid (AMPA, 0.1 mmol/l, 100 nl) (E); 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 2 mmol/l, 100 nl) (F) and AMPA (0.1 mmol/l, 100 nl) after CNQX (2 mmol/l, 100 nl) (G) into the CVLM on gallbladder pressure (GP). Microinjection of either NMDA or AMPA decreased GP. Microinjection of ketamine increased GP and reduced the effects of NMDA on GP. Microinjection of CNQX had no effects on GP and abolished the effects of AMPA on GP. ***$P < 0.001$ vs NS.

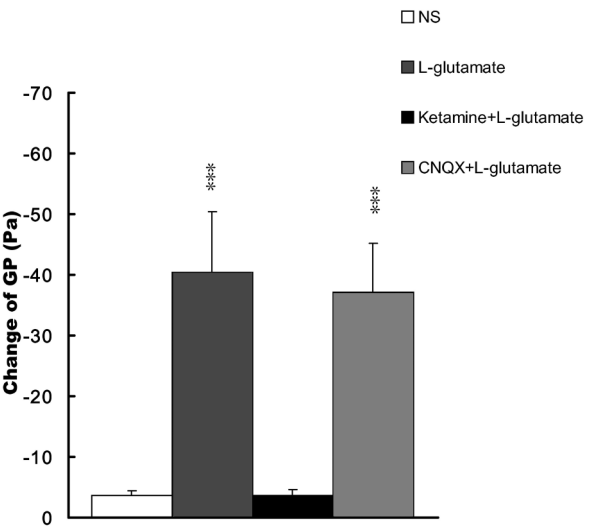

Fig. 6. Microinjection of L-glutamate (106 mmol/l, 100 nl) into the CVLM significantly decreased gallbladder pressure (GP). Preadministration with ketamine (90 mmol/l, 100 nl) markedly blocked the effects of L-glutamate (106 mmol/l, 100 nl) on GP. But 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 2mmol/l, 100 nl) could not block the effects exerted by L-glutamate (106 mmol/l, 100 nl). Values are means±S.D. ***$P < 0.001$ vs NS.

noncompetitive NMDA receptor antagonist, was microinjected into the CVLM and increaseed (+36 ± 6 Pa, $P < 0.001$) the basal GP (Fig. 2E and Fig. 5C).

In 14 rabbits, we first microinjected ketamine (90 mmol/l, 100 nl) into the CVLM. One or two min later, NMDA (0.5 mmol/l, 100 nl), was microinjected into the same site. Fig. 2F and Fig. 5D showed that ketamine completely abolished the effects of NMDA on GP.

Effects of AMPA Receptors on the Motility of Gallbladder

In 14 rabbits, AMPA (0.1 mmol/l, 100 nl), the specific AMPA receptor agonist, decreased GP (-41 ± 8 Pa, $P < 0.001$) after it was microinjected into the CVLM (Fig. 2G and Fig. 5E).

In 11 rabbits, CNQX (2 mmol/l, 100nl), a competitive AMPA receptor antagonist, was microinjected into CVLM, but no discernible changes were found on GP (Fig. 2H and Fig. 5F).

In 15 rabbits, the effects of preadministration with CNQX (2 mmol/l, 100 nl) were studied upon AMPA-induced responses. Fig. 2I and Fig. 5G showed that CNQX markedly reduced the effects of AMPA on GP.

Effects of NMDA and AMPA Receptor Antagonists on L-glutamate-Induced Responses

Two experiments were conducted to determine whether NMDA receptors or AMPA receptors were involved in L-glutamate-induced effects on gallbladder motility. Firstly, in 20 rabbits, L-glutamate (106 mmol/l, 100 nl) was microinjected into the CVLM 1-2 min after microinjection of ketamine (90 mmol/l, 100 nl). Fig. 2J and Fig. 6 showed that the effects of L-glutamate on GP were markedly reduced. Secondly, in 18 rabbits, L-glutamate (106 mmol/l, 100 nl) was microinjected into the CVLM 1-2 min after pretreatment with CNQX (2 mmol/l, 100 nl). CNQX did not affect the effects exerted by L-glutamate (Fig. 2K and Fig. 6).

Analysis of the Innervation Pathway

To determine whether the CVLM regulates the motility of gallbladder *via* the sympathetic nerve or the vagus nerve, we performed some experiments to block the innervation pathway. In 8 rabbits, induction of peripheral α-adrenergic receptor blockade by the intravenous administration of phentolamine (1.5 mg/kg), significantly reduced the L-glutamate-induced responses (Fig. 2L). In 5 rabbits, intravenous administration of propranolol (1.5 mg/kg) could not block the L-glutamate-induced responses (Fig. 2M). These results suggest that the effects of the CVLM on

GP are normally due to the regulation of sympathetic nerve activity. In support of this interpretation, in 6 rabbits, transection of the spinal cord completely abolished the changes in GP normally observed after microinjection of L-glutamate (106 mmol/l, 100 nl) into the CVLM (Fig. 2N). In addition, in several rabbits, intravenous administration of atropine (0.2 mg/kg) or bilateral vagotomy did not affect the L-glutamate-induced responses (Fig. 2O).

Control Experiments

Normal saline (NS) was microinjected into the CVLM in an equivalent volume as the drugs under study (i.e., L-glutamate) (Fig. 2). L-glutamate (106 mmol/l, 100 nl) was also microinjected into an area outside the CVLM. These control microinjections did not result in discernible changes in gallbladder motility.

Discussion

The CVLM has been regarded as an important centrum in regulating the visceral activity. There were many transmitters in the CVLM, such as noradrenaline, GABA, serotonin and substance P (4). Neurons in the CVLM projected to many areas such as the RVLM, the hypothalamus and the nucleus accumbens (1, 20, 23). The comprehensive fiber connections may be the basis for the CVLM to participate in the regulation of many visceral functions.

In the present study, microinjection of L-glutamate into the CVLM decreased GP, while microinjection of GABA into the CVLM increased GP. The GP changes after microinjections of these agents strongly suggest that the CVLM may contain a pool of neurons which regulate the motility of gallbladder. L-glutamate excited these cells, and reduced GP, GABA inhibited these cells, and increased GP. Hence, the changes of GP were dose-related, consistent with the interaction between L-glutamate and its receptors.

L-glutamate and its receptors were involved in mediating synaptic transmission in the central nervous system (7). L-glutamate receptors were subdivided into ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs). The iGluRs have three types of receptors: NMDA, AMPA and the kainate receptors. In the present study, microinjection of either NMDA or AMPA decreased GP. Microinjection of ketamine totally inhibited the effects of NMDA, and microinjection of CNQX totally inhibited the effects of AMPA. These findings suggest that there are at least two types of L-glutamate receptors in the CVLM, NMDA and AMPA receptors.

L-glutamate may be involved in the activation of the NMDA receptors or AMPA receptors or both of them in the CVLM, since L-glutamate is a major excitatory synaptic neurotransmitter substance and can activate them in the vertebrate central neurons system (8). In the present study, microinjection of L-glutamate into the CVLM decreased GP, while ketamine, rather than CNQX, inhibited the effects of L-glutamate on GP. These data clearly demonstrate that the effects of L-glutamate are mainly mediated by NMDA receptors. Microinjection of ketamine increased GP, but microinjection of CNQX had no significant effects on GP. These findings indicate that endogenous L-glutamate in the CVLM physiologically regulated the motility of gallbladder through combination with the NMDA receptors.

Anatomical studies have shown that the CVLM neurons provided inhibitory GABA-ergic projections to the RVLM neurons which projected to sympathetic preganglionic neurons in the spinal cord (11). The physiological importance of this pathway was mainly associated with the modulation of the cardiovascular activities (3, 11). The pathway of CVLM-RVLM-sympathetic nerve might participate in the regulation of gallbladder motility. In our present experiment, a sharp decrease of gallbladder was recorded immediately after the pretreament with phentolamine (blocking the peripheral α receptor) and transecting the spinal cord (interrupting the connection between the CVLM and the originating neurons of the sympathetic nerve that innervate the gallbladder). It is consistent with our previous report. Sympathetic fiber exerted tonic excitatory influence on gallbladder smooth muscle (15). Blocking peripheral α-receptor or cutting of the spinal cord eliminated this tonic regulation. In this study, both of these pretreatments could cut the connection between CVLM and gallbladder through sympathetic nerve, but they did not affect the pathway through vagus nerve. Intravenous injection of phentolamine or transection of the spinal cord completely abolished the inhibitory effect of glutamate on gallbladder motility, but injection of propranolol or by atropine and bilateral vagotomy did not affect it. These results indicate that CVLM regulates the motility of gallbladder *via* the sympathetic nerve and α-adrenergic receptors.

Based on the results of the present study, we conclude that [1] the CVLM does participate in the regulation of gallbladder motility; [2] endogenous L-glutamate in CVLM is involved in the regulation mediated by NMDA receptors, the output of which is sent through sympathetic nerve and α-adrenergic receptors.

Acknowledgments

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