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Research report

Oxytocin microinjected into dorsal motor nucleus of the vagus excites gallbladder motility via NMDA receptor–NO-cGMP pathway

Chuan Yong Liu*, Dong Ping Xie, Ke Jing Liu, Yu Qin Zhou, Jing Zhang Liu

Institute of Physiology Medical School of Shandong University Jinan 250012 P. R. China

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Abstract

Our recent study indicated that, in the dorsal motor nucleus of the vagus (DMV), the *N*-methyl-D-aspartic acid (NMDA) receptor–nitric oxide (NO)–cGMP pathway participated in the regulation of gallbladder motility in rabbits. Oxytocin (OT) is involved as a neurotransmitter in autonomic regulation. The aim of the present experiments is to investigate the effect of OT microinjected into DMV on the gallbladder motility and the involvement of NMDA receptor–NO–cGMP pathway. A frog bladder connected with transducer was inserted into the gallbladder to record the gallbladder pressure. Microinjection of OT (10–50 nmol/L, 100 nl) dose dependently increased the strength of gallbladder phasic contraction. The excitatory effect of OT (10 nmol/L, 100 nl) was completely abolished by atosiban (10 mmol/L, 100 nl), the specific OT receptor antagonist, but was not influenced by [deamino-Pen^1, O-Me-Tyr^2,Arg^8]-vasopressin (10 mmol/L, 100 nl), the V_1 receptor antagonist. Pretreatment of ketamine (10 mmol/L, 100 nl), the NMDA receptor antagonist, suppressed the gallbladder motor response to OT; but pretreatment of 6-Cyaon-7-Nitroquinoxaline-2,3-(1H,4H)-Dione (CNQX; 10 mmol/L, 100 nl), the non-NMDA receptor antagonist, did not affect it. Pretreatment of L-NAME (10 mmol/L, 100 nl), the nitric oxide synthase (NOS) inhibitor, or methyl blue (10 mmol/L, 100 nl), the guanylyl cyclase inhibitor, inhibited the excitatory effect of OT on gallbladder motility. Hence, we deduced that the microinjection of OT into the DMV enhanced the gallbladder motility through binding specific OT receptors and activating the NMDA receptor–NO–cGMP pathway.

Theme: Neurotransmitters, modulators, transporters, and receptors
Topic: Interactions between neurotransmitters

Keywords: Oxytocin; Vasopressin receptors; Dorsal motor nucleus of the vagus; Gallbladder motility; Nitric oxide; *N*-methyl-D-aspartic acid receptor

1. Introduction

The long-descending oxytocinergic pathway from the hypothalamus to the nucleus tractus solitarius/dorsal motor nucleus of the vagus (NTS/DMV) area serves as a link between the two main neural controllers of the gastrointestinal tract. Electrical stimulation of the paraventricular nucleus (PVN) resulted in increased amounts of oxytocin (OT) released from the NTS/DMV area [4]. Gastric motility is inhibited by the microinjection of OT into the DMV in anesthetized rats [14,15], and the inhibition of gastric motility after electrical stimulation of the hypothalamic PVN is blocked by the microinjection of an OT receptor antagonist in DMV [14]. The gastric motility in unanesthetized, freely moving rats was reduced both by intracerebroventricular (i.c.v.) administration of OT and by electrical stimulation of the PVN, and both of these inhibitory effects were blocked by i.c.v. administration of an OT antagonist [3]. The OT antagonist alone administrated i.c.v. caused an increase in baseline gastric motility of the conscious rats, hence, it seems that oxytocinergic neurons exert a tonic inhibitory effect on gastric motility in rats [3]. There is a close relationship in humans between gallbladder motility and gastrointestinal motility during the fasting state, as well as in the postprandial period [13]. So far, no data about the effect of OT in DMV on gallbladder motility has been reported.

Our previous study indicated that the gallbladder motility is controlled by the central nervous system. In urethane anesthetized rabbits, microinjection of cholecystokinin

* Corresponding author. Fax: +86 531 8382502.
E-mail address: liucy@sdu.edu.cn (C. Yong Liu).

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octapeptide into PVN decreased the gallbladder pressure [19]; the microinjection of glutamate [9], thyrotropin-releasing hormone (TRH; [6,8]), and donor of nitric oxide (NO; [17]) increased the phasic contraction of gallbladder. The excitatory effect of glutamate on gallbladder motility is mediated by the *N*-methyl-D-aspartic acid (NMDA) receptor and the synthesis of nitric oxide in DMV [9]. There is an NMDA receptor–NO–cGMPpathway in DMV that controls the gallbladder motility [9].

The aim of the present study was to investigate the effect of exogenous administration of OT into DMV on gallbladder motility and to determine whether OT mediates this effect selectively through OT receptors. The possibility that NMDA receptor–NO–cGMPpathway participates in the effect of OT on gallbladder motility was also tested.

2. Materials and methods

New Zealand white rabbits of both sexes, weighing 2.0–2.5 kg, were used in this study. After being fasted for 18–24 h, the rabbits were anesthetized by 20% urethane (1 g/kg, i.v.). All rabbits were paralyzed with gallamine trithiodine (2 mg/kg, i.v.) and artificially ventilated during the experiments. The abdomen was opened through a midline incision. The gallbladder was pulled out into the operating area from the hepatic bed. The liver tissue surrounding the gallbladder was protected by cotton from the contamination of biles. A small incision was done on the funds of the gallbladder, and a frog bladder filled with normal saline was inserted into the gallbladder through it. The frog bladder was connected with a force transducer (TP-200T, Nihon Kohden, Tokyo, Japan) by a tube (100 μm internal diameter and 200 μm external diameter) to record the gallbladder pressure. Femoral artery catheter connected with force transducer (TP-200T, Nihon Kohden) was placed into the right femoral artery to monitor blood pressure. Results (gallbladder and blood pressure) were recorded on a four-channel polygraph recorder (RM-6000, Nihon Kohden) at a paper speed of 1 mm/s. Animals were then placed prone in a sterotaxic instrument. The occipital bone was removed, and the dorsal surface of the medulla was exposed. According to Messen's Atlas [11], the injection region was located at the coordinates of −1.0 to 3.0 mm to the obex, 0.1–1.2 mm lateral to the midline, and 0.3–1.1 mm ventral to the medulla surface. A micropipette (30 μm internal diameter, 300 μm external diameter) filled with drug solution was used for the microinjection of chemicals into DMV. All drug solutions, in volumes of 100 nl, were microinjected into the left or right side of DMV within 1 min.

After the termination of the experimental procedure, a high concentration of L-glutamate (2 mol/L, 1 μl volume) was microinjected into the same position, destroying the neurons, to cause a small lesion in situ. Then, the rabbits were killed by air emboli. The medulla was removed and immersed in a solution of 6% formalin for 3 days. The bulbar region was frozen, serially sectioned at a thickness of 40 μm, and stained with Hemeloxylin and Eosin, to facilitate the identification of the lesion site (Fig. 1).

The chemicals used and their sources were as follows: Ketamine was produced by Shandong Provincial Biochemical Reagent Center (Jinan, Shadnong, China); L-NAME (N^G-nitro-L-arginine-emthyl) was purchased from Cayman (Ann Arbor, MI, USA); and Oxytocin, [deamino-Pen^1, O-Me-Tyr^2,Arg^8]-vasopressin (the V_1 receptor antagonist), NMDA (*N*-methyl-D-aspartic acid), 6-Cyaon-7-Nitroquinoxaline-2,3-(1H,4H)-Dione (CNQX) and methylene blue were purchased from Sigma (Saint Louis, MO, USA). Atosiban is produced by Ferring, Limhamn, Sweden. All agents used were freshly prepared and dissolved in the normal saline at the desired concentration.

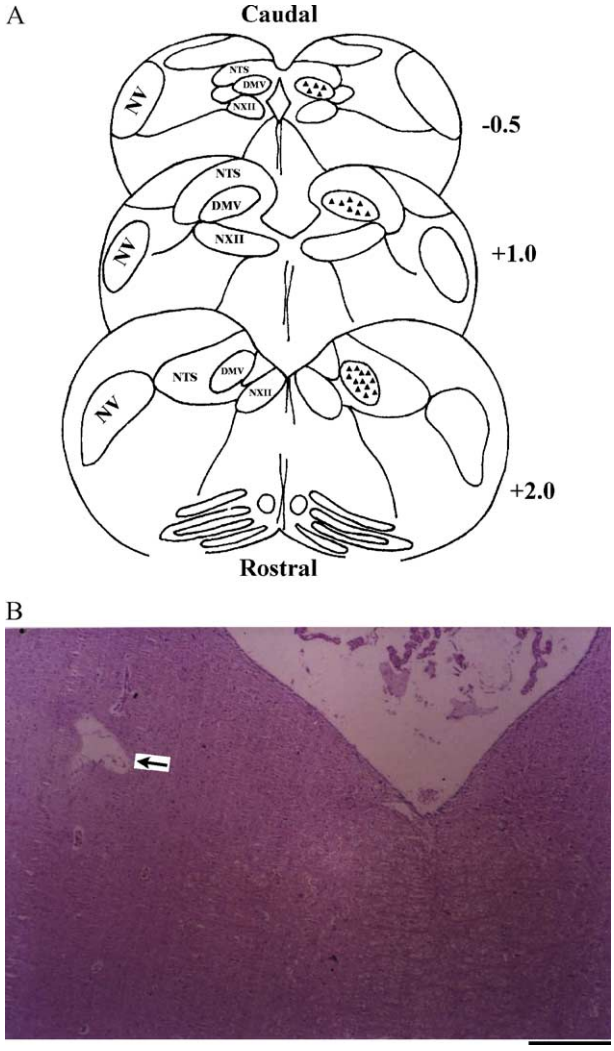

Fig. 1. (A) Schematic drawing of coronal sections of DMV made across the caudal, middle and rostral levels. Numerals on the right side of the drawing refer to the distance (mm) caudal (−) and rostral (+) to the obex. The triangles indicate effective injections points of glutamate. NTS: nucleus of solitary tract. NXII: nucleus of the hypoglossal nerve. NV: nucleus of the trigeminal nerve. (B) Coronal section of the brain stem passing through rostral DMV. Microinjection of glutamate (2 mol/l, 1 μl) into the rostral DMV caused a small lesion. The arrow indicates the injection site (bar = 0.5 mm).