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

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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-Pen1, O-Me-Tyr2,Arg8]-vasopressin (10 mmol/L, 100 nl), the V1 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
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–cGMP pathway 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–cGMP pathway 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 funder 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 Hemelxylin 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, Shandong, 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 Ferining, 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).
A series of experiments were conducted to test the hypothesis about the effect of OT in DMV on gallbladder motility.

(1) Several doses of OT (5–50 nM, 100 nl) microinjected into the DMV to investigate the dose-dependent effect of OT.

(2) Control experiment. Microinjection of normal saline (100 nl) into the DMV or microinjected OT outside DMV.

(3) To investigate the mechanism involved in the effect of OT on regulating the gallbladder motility, several receptor blockers or antagonists of some agonists were microinjected into the DMV 3 min before OT was administrated in the same way.

3.1. Pretreatment of Atosiban, the specific OT receptor antagonist

3.2. Pretreatment of [deamino-Pen1, O-Me-Tyr2, Arg8]-vasopressin, the specific V1 receptor antagonist

3.3. Pretreatment of ketamine, the NMDA receptor blocker

3.4. Pretreatment of CNQX, the non-NMDA receptor antagonist

3.5. Pretreatment of L-NAME, the blocker of NOS

3.6. Pretreatment of methyl blue, the inhibitor of guanylyl cyclase

The original recording was written on paper by an ink-writing recorder system (RM-6000, Nihon Kohden), and the original data were manually obtained. If the gallbladder pressure rose above 0.05 mm Hg and returned to the baseline within 20 s, it was regarded as phasic contraction. The strength of phasic contraction (mm Hg/min) was the summation of the amplitude of all the phasic contractions in 1 min. The statistical analysis was conducted by SigmaStat (Version 3.2). All values are present as mean ± S.E.M. Student’s t test, or one-way ANOVA, followed by Dunnet’s t test, which is applicable, was used. P < 0.05 (n = 7–15) was regarded as significant difference.

3. Results

3.1. Normal gallbladder motion

In the interdigestive period in rabbits, two kinds of motion of the gallbladder was recorded, the tonic and phasic contractions. Tonic contraction, or tone of the smooth muscle, maintained the gallbladder pressure at a stable level (10–12 mm Hg). Phasic gallbladder contraction, with a duration of 5–20 s and frequency of 1–3 times/min, raised gallbladder pressure by 0.05–0.2 mm Hg.

Microinjection of normal saline (NS) into the DMV or of OT (10 nmol/L, 100 nl) 0.5 mm lateral to the DMV did not influence the gallbladder motility.

3.2. Effect of OT microinjected into DMV increase the gallbladder phasic contraction

The microinjection of OT (5 nmol/L, 100 nl) did not initiate any change of the gallbladder motility. High concentration of OT (10–50 nmol/L, 100 nl) dose-dependently increased the strength of gallbladder phasic contraction. In the group of 10 nmol/L, the gallbladder motility increased 1 min after the OT administration, from 0.031 ± 0.01 to 0.15 ± 0.03 mm Hg (P < 0.05), reached the apex at 3 min (0.29 ± 0.13 mm Hg, P < 0.05) and began to decline at 5 min. Twenty minutes after OT microinjection, the strength of gallbladder motility was 0.05 ± 0.01 mm Hg/min (P < 0.05; Fig. 2A and B).

3.3. Pretreatment of receptor antagonists of OT and vasopressin (VP)

The pretreatment of atosiban (10 mmol/L, 100 nl), the specific oxytocin receptor antagonist, into DMV did not affect normal gallbladder motility but completely abolished the excitatory effect of OT (10 nmol/L, 100 nl) on gallbladder motility. Three minutes after OT administration, the strength of gallbladder phasic contraction is 0.07 ± 0.03 mm Hg/min, which did not differ significantly from that before the OT administration within the same group (0.05 ± 0.02, P > 0.05), but was lower than that of the OT group at the same time point (P < 0.001). One to 20 min after OT administration, the strengths of the gallbladder phasic contraction in the atosiban pretreatment group were not significantly different from data of that before OT administration (P > 0.05; Fig. 3A and B).

The pretreatment of [deamino-Pen1, O-Me-Tyr2,Arg8]-vasopressin (10 mmol/L, 100 nl), the V1 receptor antagonist, into DMV did not affect the normal gallbladder motion or excitatory effect of OT (10 nmol/L, 100 nl) on gallbladder motility. One to 20 min after OT administration, the strength of gallbladder phasic contraction were not significantly different between the two groups ([deamino-Pen1, O-Me-Tyr2,Arg8]-vasopressin + OT vs. OT group; Fig. 3A and B).

3.4. Pretreatment of ketamine and CNQX

The pretreatment of ketamine (10 mmol/L, 100 nl), the NMDA receptor antagonist, did not change the normal gallbladder motion but completely reversed the excitatory effect of OT (10 nmol/L, 100 nl) on gallbladder motility. In the ketamine pretreatment group, from 1 to 20 min after OT administration, the strength of gallbladder phasic contraction are not significantly different from that before OT microinjection (P > 0.05; Fig. 4A and B).

The pretreatment of CNQX (10 mmol/L, 100 nl), the non-NMDA receptor antagonist, did not influence the normal gallbladder motion or the effect of OT (10 nmol/L, 100 nl) on gallbladder motility. Three minutes after OT
administration, the strength of the gallbladder phasic contraction increased from $0.04 \pm 0.01$ to $0.20 \pm 0.02$ mm Hg/min ($P > 0.05$). These data were not different from that of the OT group ($P > 0.05$; Fig. 4A and B).

3.5. Pretreatment of L-NAME and methyl blue

The pretreatment of L-NAME (nitric oxide synthase inhibitor; 10 mmol/L, 100 nl) or methyl blue (10 mmol/L,
100 nl; guanylyl cyclase inhibitor) in DMV did not influence normal gallbladder motility but completely inhibited the effect of OT. Within 20 min, the microinjection of OT (10 nmol/L, 100 nl) did not affect the gallbladder motility if DMV was pretreated with L-NAME or methyl blue ($P > 0.05$; Fig. 5A and B).

### 4. Discussion

The present study indicated that the microinjection of oxytocin dose-dependently increases the phasic contraction of gallbladder, and this effect was blocked by atosiban, the specific OT receptor antagonist. Hence, it is clear that the
long-descending oxytocinergic pathway from the hypothalamus to the nucleus tractus solitarius/dorsal motor nucleus of the vagus (NTS/DMV) area plays an important role in regulating the gallbladder motility.

Contrary to the inhibitory effect of OT in DMV on the gastric motility, the microinjection of OT into the same position increased the gallbladder phasic contraction. Although the gallbladder is one part of the gastrointestinal tract, the motor activity of this organ has some special physiological importance. In the interdigestive period, the phasic contraction of the gallbladder stirs the bile in the gallbladder and thus facilitates the concentration of the bile and prevents the formation of bile stones [10]. During our experiment, we found that longer periods of food deprivation (> 18 h) increased the spontaneous phasic contraction of the gallbladder (unpublished data). Hence, during the fasting state, the motion of the stomach and the intestine is intermittent (migrating motor complex), but that of the phasic contraction of gallbladder is continuous. Our series study indicated that this kind of motion is controlled by the brain and the peripheral vagus nerve [6,8,17,7]. According to the present data, we believe that OT in DMV participates in the regulation of gallbladder motility in the interdigestive period.

OT and VP are cyclic nonapeptides whose actions are mediated by the activation of specific G-protein-coupled receptors currently classified into V1a-vascular, V2-renal and V1b-pituitary VP and OT receptors [16,20]. Both of these two peptides are involved as neurotransmitters in autonomic regulation.[4] Because of the structural similarity between OT and VP, the two agents can activate not only their own receptors but also each other’s receptors [2,18]. In high concentrations, VP activates OT receptors, and OT activates V1a receptors [2,18]. But they preferentially act on their own receptors if the concentration is relatively low. Both vasopressin and oxytocin induced a vasoconstriction with a similar maximum effect through V1 receptor. The EC50 was $0.44 \pm 0.02$ and $25 \pm 3.1$ nmol/L for vasopressin and oxytocin, respectively. Thus, vasopressin was 57-fold more potent than was oxytocin in exciting the V1 receptor [2]. On the other hand, OT was 75 and 57 times more potent than lysine vasopressin in increasing myometrial contractility in pregnant and nonpregnant tissue sows, respectively, through oxytocin receptor [18].

In vitro, the application of OT (10$^{-6}$ mol/L) and VP (10$^{-6}$ mol/L) increased the firing rate of neurons on the DMV. The VP induced excitatory responses was completely blocked by V1 receptor antagonist, and that of OT was suppressed by a selective OT receptor antagonist [12]. In the present study, the excitatory effect of OT administrated into DMV was blocked by atosiban, the selective OT receptor antagonist, but was not affected by V1 receptor antagonist. Hence, it is clear that, in DMV, OT activates the gallbladder motility through the OT receptors, but not the VP receptors.

In a recent study, we reported that the NMDA receptor–NO–cGMP pathway existed in the DMV and exerted an excitatory effect on gallbladder motility [9]. In present experiments, pretreatment of ketamine, the NMDA receptor antagonist (but not CNQX, the non-NMDA receptor blocker), abolished the excitatory effect of OT on gallbladder motility. Both that of l-NAME (the NOS inhibitor) and methyl blue (the cGMP antagonist) also suppressed the effect of OT. Hence, we deduced that both NMDA receptors and NO are involved in the effect of OT on gallbladder motility. The excitatory effect of OT in DMV is through the NMDA receptor–NO–cGMP pathway.

As the most important excitatory neurotransmitter, glutamate is widely distributed in the central nervous system [5]. In DMV, glutamate exerted influence on gallbladder mainly by activating the NMDA receptor [9]. There are three possibilities through which the OT activates the NMDA receptor. One is that OT directly binds NMDA receptors; another possibility is that OT increases the release of glutamate from nerve terminals through activating the presynaptic receptor; and the third possibility is by depolarizing the membrane potential, OT increases the excitability of NMDA receptors. The first and second possibilities could be excluded by the data from two research groups. Mo et al. [12] reported that the excitatory effect of OT still persisted after blocking the synaptic transmission in a low-Ca$^{2+}$ or Ca$^{2+}$-free, high-Mg$^{2+}$ solution, indicating the direct action of OT on the postsynaptic membrane. Alberi et al. [1] reported that OT-induced inward current in vagal neurons of the rat is mediated by G-protein activation. G-protein coupling is the property of OT receptor; thus, the only possible mechanism of the correlation between OT and NMDA receptor is through G-protein. The activated OT receptor opens some cation channels, and the inward current depolarizes the membrane potential, which, in turn, increases the activity of the NMDA receptor.

In conclusion, the present data indicate that, in DMV, OT exerts excitatory influence on the phasic contraction of gallbladder by activating the specific oxytocin receptors, but not the V1 receptors. The NMDA receptor–NO–cGMP pathway is involved in this effect of OT.

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References


