Endogenous Oxytocin Excites Phasic Contraction of Gallbladder in Rabbits through Oxytocin Receptor

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Abstract

These experiments were performed to study the effect of oxytocin (OT) and it's specific receptor on gallbladder motility in rabbits. The fasted New Zealand white rabbits (2.0-2.5 kg) were anaesthetized by urethane (1 g/kg). The gallbladder pressure was recorded continuously to monitor the gallbladder motility. Systemic OT (0.01, 0.02, 0.04 mg/kg, iv) did not affect the gallbladder pressure, but dose-dependently increased the frequency of phasic contraction. Five min after OT administration (0.04 mg/kg, iv), the strength of phasic contraction increased to 0.23 ± 0.08 mmHg/min ($P < 0.01$, n = 6). The gallbladder motility returned to normal 15 min later after OT treatment. Intravenous injection of atosiban (0.04 mg/kg, iv), an OT receptor antagonist, decreased the strength of gallbladder phasic contraction but did not affect gallbladder pressure. Pretreatment of atosiban (0.04 mg/kg, iv) completely abolished the systemic OT effect on gallbladder. Vasopressin (VP) (0.1 - 0.5 IU/kg, iv) dose-dependently decrease the gallbladder pressure but did not affect the phasic contraction. MK-329 (0.4 mg/kg, iv), the CCK-A receptor antagonist, L-365, 260 (0.4 mg/kg, iv), the CCK-B receptor antagonist and atropine (0.2 mg/kg, iv), the M receptor antagonist, did not affect the OT effect on gallbladder motility. We suggest that endogenous OT regulates gallbladder phasic contraction through specific OT receptor. This effect is independent of the peripheral CCK and M receptors.

Key Words: gallbladder, oxytocin, vasopressin, phasic contraction

Introduction

Oxytocin (OT), an abundant neuropeptide, regulates the physiological functions *via* OT receptor, which was widely distributed in many organs, such as brain, uterus, kidney, pancreas, vasculature and thymus (1, 5-9). It influences the gastrointestinal functions through both central and peripheral mechanisms. The paraventricular nucleus (PVN) of the hypothalamus modulates vagal digestive motor functions *via* oxytocinergic projections to the dorsal nucleus of the solitary tract (NST) and dorsal motor nucleus of the vagus (DMV) in rats (19, 21). Microinjection of OT into DMV inhibited the gastric motility (20). Many reports indicated that OT also regulated the digestive function through peripheral receptors. OT (1 mg/kg, s.c.) showed significant antisecretory and antiulcer activity in pylorus ligated rats (2). OT inhibited the tone and peristaltic contraction of stomach and small intestines *in vivo* and *in vivo* experiments (4, 17, 25). This effect may results from the activation of Ca^{2+}- sensitive K^+ conductivity (4). Our recent study also indicated that, *in vivo*, systemic OT inhibited the gastric emptying and intestinal transit through increasing the releasing of cholecystokinin (CCK) (24). *In vitro*, OT inhibited

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the contractile motility of proximal colon (25). So it is clearly that OT is one of the gastrointestinal function modulators. Gallbladder is an important organ in the digestive system. It stores and concentrates the bile during the interdigestive period and empties it after food intake, so to facilitate the digestion and absorption of fats. The motion of gallbladder, especially the phasic contraction during the fast state, is very important for the physiological function of gallbladder in the interdigestive period (16). But the effect of OT on gallbladder motility has not been reported.

Because of the structural similarity between OT and vasopressin (VP), the two hormones can activate not only their own but also each other's receptors (3, 27) There are three types of VP receptors (V1a-Vascular, V2-renal, and V1b-hypophyseal) and one type of OT receptors. They are all present in outer membranes of target cells and couple to G-proteins (22, 28). In higher concentrations, VP activates OT receptors and OT activates V1a receptors (3, 27). Although recent studies indicate that OT receptors exist in the gut (2, 4), the possibility that OT may regulate the gastrointestinal function by binding the VP receptors has not been excluded.

The aim of this study is to investigate the effect of systemic OT on gallbladder motility and the associate mechanism.

Materials and Methods

Studies were preformed on New Zealand White rabbits of both sexes (2.0-2.5 kg). After fasted for 18-24 h, the rabbits were anaesthetized by 20% urethane (1 g/kg, i.v.). The rabbits were paralysed 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 funds to record the gallbladder motility. The right femoral artery was catheterized to monitor blood pressure. The changes of gallbladder pressure (GP) and blood pressure (BP) were recorded on a four-channel polygraph recorder (RM-6000, Nihon Kohden, Tokyo, Japan) at a paper speed of 10 mm/min. Anal temperature and BP of all animals were monitored during the experiment and the temperature was kept at 37.5-38.5 °C.

OT and VP are from SIGMA company; MK-329 and L-365,260 are products of ML Laboratories PLC, Liverpool, U.K.; Atosiban from Ferring AB, Limhamn, Sweden; Atropine is product of Jinan Dongfeng Pharmacy Company. OT, VP, atosiban and atropine were dissolved into normal saline; MK-329 and L-365, 260 were dissolved into 20% dimethyl sulfoxide (DMSO, Wako Pure Chemical Industries, Ltd, Japan).

All values are expressed as mean ± SEM. The change of gallbladder pressure was obtained by subtracting the value of baseline, which was regarded as 0 during the statistic analysis. If the gallbladder pressure rose above 0.05 mmHg and returned to the baseline within 20 sec, it was regarded as phasic contraction. The strength of phasic contraction (mmHg/min) was the summation of the amplitude of all phasic contractions in one min. The paired *t* test was used, $P < 0.05$ was regarded as significant difference.

Results

Effect of Systemic OT on Gallbladder Motility

OT (0.01-0.04 mg/kg, iv) dose-dependently enhanced the phasic contraction of gallbladder. One min after OT administration (0.02 mg/kg, iv), the strength of gallbladder pressure increased from 0.0375 ± 0.0072 mmHg/min (baseline) to 0.118 ± 0.0277 mmHg/min ($P < 0.05$, n = 4). The response reached to the peak at 3 min (0.1750 ± 0.0144 mmHg/min, $P < 0.05$, n = 4). The strength of gallbladder phasic contraction began to decrease at 5 min (0.1625 ± 0.0239 mmHg, $P < 0.05$) and returned to normal level at 20 min (0.1000 ± 0.0204 mmHg/min, $P > 0.05$). Administration of all the three doses of OT (0.01, 0.02, 0.04 mg/kg, iv) did not affect the gallbladder pressure (Fig. 1).

Effect of Atosiban, the OT Receptor Antagonist, on Gallbladder Motility

Administration of atosiban (0.01-0.04 mg/kg, iv) did not influence the gallbladder pressure, but dose-dependently inhibited the spontaneous phasic contraction. Atosiban (0.02 mg/kg, iv) decreased the strength of gallbladder contraction from 0.0420 ± 0.0153 mmHg/min (baseline) to 0.0150 ± 0.0090 mmHg/min ($P < 0.05$, n = 6). The strength of phasic contraction reached the lowest at 5 min (0.0120 ± 0.0046 mmHg/min, $P < 0.05$, n = 6) and returned to normal at 20 min (0.0420 ± 0.0153 mmHg/min, $P > 0.05$, n = 6) (Fig. 2).

Effect of VP on Gallbladder Motility

Low dose of VP (0.1-0.2 IU/kg, iv) did not influence the gallbladder motility. One min after the treatment of high dose of VP (0.5 IU/kg, i.v.), the gallbladder pressure decreased by 0.2375 ± 0.0554 mmHg ($P < 0.05$, n = 6). At 15 min, when the response reached to the peak, the gallbladder pressure decreased by 0.3625 ± 0.0473 mmHg ($P < 0.01$, n = 6) and the gallbladder pressure began to return at 20 min

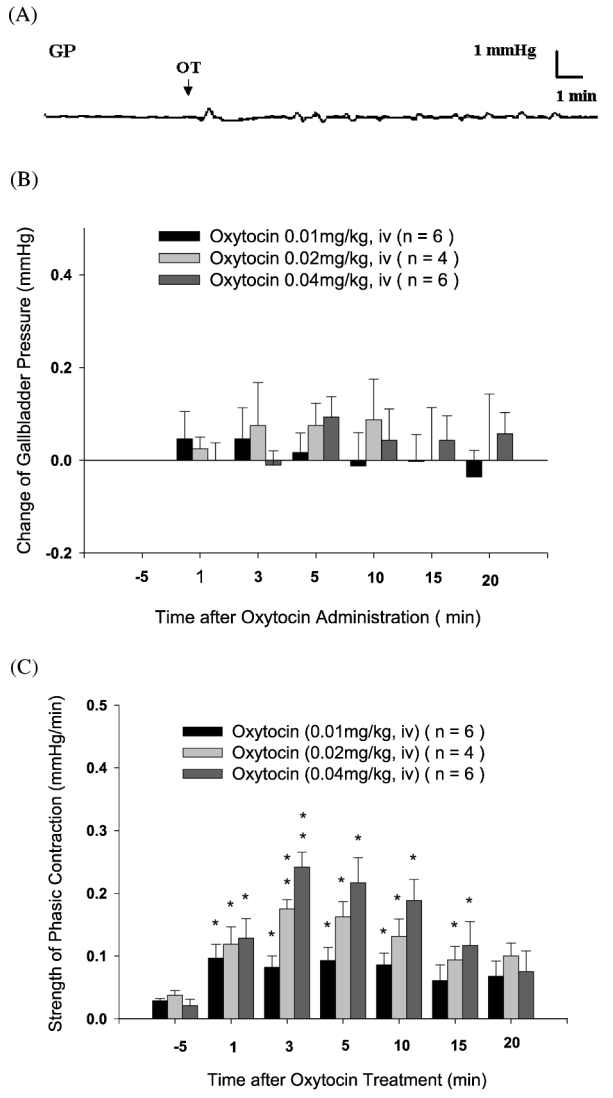

Fig. 1. Dose-dependent effect of systemic administration of OT (0.01-0.04 mg/kg, iv) on gallbladder pressure. A. Representative of the recording of the gallbladder motion. GP, gallbladder pressure; OT, oxytocin (0.02 mg/kg, iv). The arrow indicates the treatment. B. Statistic analysis of the OT effect on gallbladder pressure. C. Statistic analysis of the OT effect on gallbladder phasic contraction. **P* < 0.05, ***P* < 0.01 compared with the data prior to OT administration.

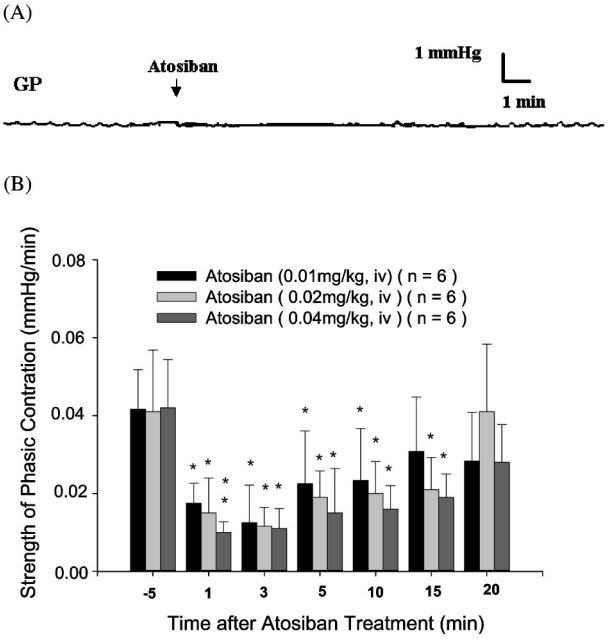

Fig. 2. Does-dependent effect of atosiban administration (0.01-0.04 mg/kg, iv) on gallbladder phasic contraction. A. Representative of the recording. GP, gallbladder. The arrow indicates the treatment (atosiban, 0.02 mg/kg, iv). B. Statistic analysis. **P* < 0.05, ***P* < 0.01 compared with the data prior to atosiban administration.

(- 0.35 ± 0.0866, *P* < 0.05). All the three doses of VP (0.1, 0.2, 0.5 IU/kg, i.v.) did not affect the strength of phasic contraction (Fig. 3).

Block of the Peripheral Oxytocin Receptor

In this group, pretreatment of atosiban (0.02 mg/kg, iv) was conducted 5 min before OT administration (0.02 mg/kg). As described in result 2, the spontaneous phasic contraction was significantly inhibited after the treatment of atosiban. In this group, the strength of gallbladder contraction did not change within 20 min after OT injection (*P* > 0.05, n = 6) (Fig. 4).

Block of the Peripheral Muscarine Receptors

Pretreatment of atropine (0.2 mg/kg, iv), which was used to block the peripheral muscarine receptors, did not influence the effect of OT (0.02 mg/kg, iv) on gallbladder motility. Five min after OT administration, the strength of phasic contraction of gallbladder increased from 0.0313 ± 0.0193 mmHg/min (baseline) to 0.1388 ± 0.0297 mmHg/min (*P* < 0.05, n = 6). It reached the peak at 5 min (1.762 ± 0.0425 mmHg/min, *P* < 0.01) and returned to normal at 20 min (0.0950 ± 0.0347, *P* > 0.05, n = 6). There is no significant difference between the value of this group with that of the control (*P* > 0.05) (Fig. 5).

Inhibition of CCK Receptors

Both of MK-329 (0.4 mg/kg, iv)(CCK-A receptor antagonist), L-365, 260 (0.4 mg/kg, iv), CCK-B receptor antagonist did not affect the systemic OT effect on the strength of gallbladder phasic contraction. At any time points, the value of CCK receptors antagonist groups were not significantly different

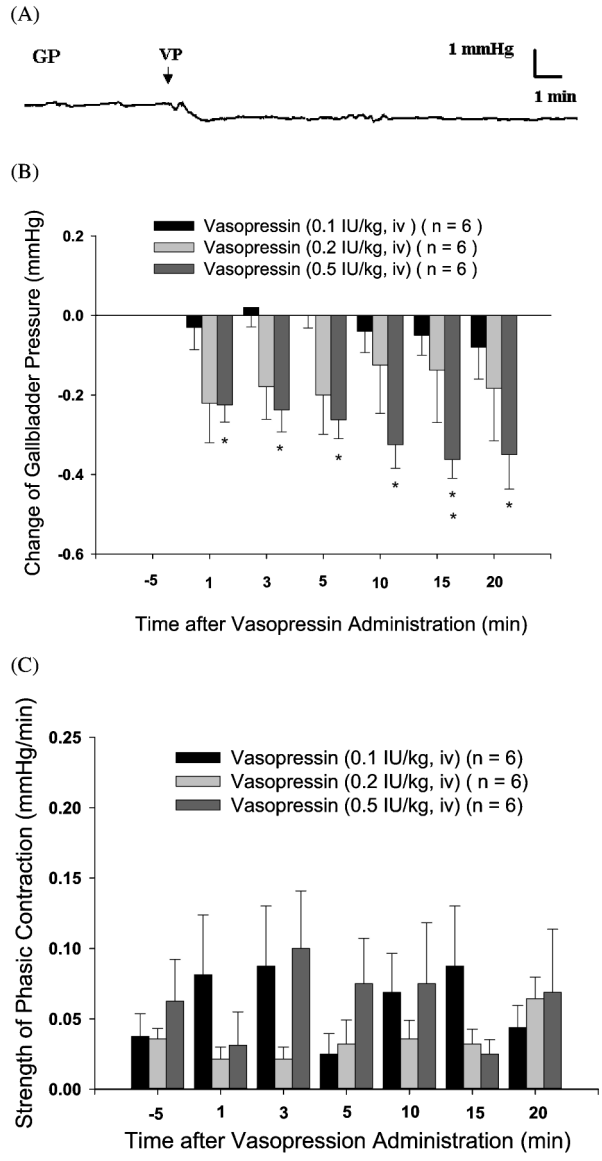

Fig. 3. Effect of VP (0.1-0.5 IU/kg, iv) on gallbladder motility. A. Representative of the recording. GP, gallbladder pressure; VP, vasopressin (0.2 IU/kg, iv). The arrow indicates the treatment. B. Statistic analysis of the VP effect on gallbladder pressure. $*P < 0.05$, $**P < 0.01$ compared with the data prior to VP administration. C. Statistic analysis of the VP effect on gallbladder phasic contraction.

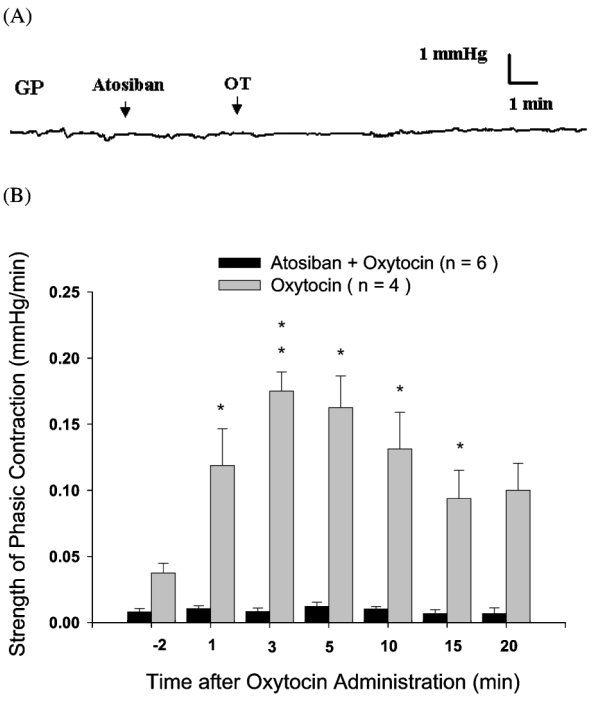

Fig. 4. Atosiban (0.02 mg/kg, iv) abolished the effect of OT (0.02 mg/kg, iv) on gallbladder phasic contraction. A. The representative of the recording. GP, gallbladder pressure; OT, oxytocin. The arrows indicate the treatment. The statistic analysis. $*P < 0.05$, $**P < 0.01$ compared with the data prior to OT administration.

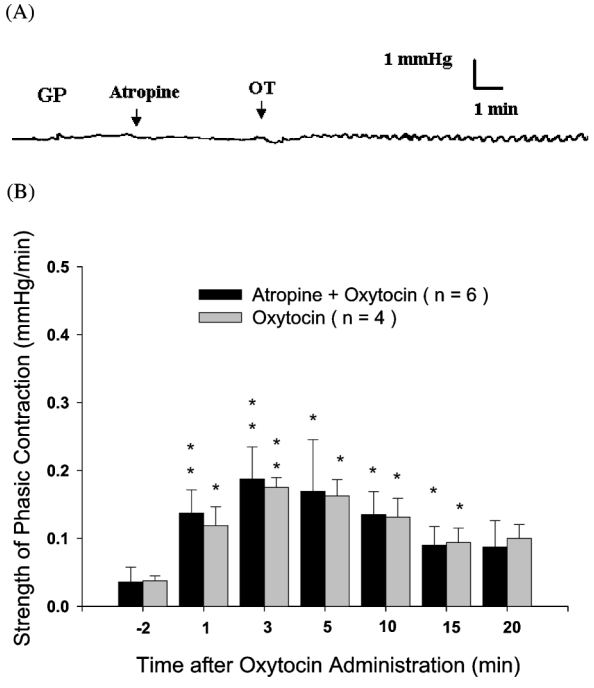

Fig. 5. Atropine (0.2 mg/kg, iv) did not affect the OT (0.02 mg/kg, iv) effect on gallbladder phasic contraction. A. Representative of the recording. GP, gallbladder pressure; OT, oxytocin. The arrows indicate the treatment B. Statistic analysis. $*P < 0.05$, $**P < 0.01$ compared with the data prior to OT administration.

from that of the control group respectively (Fig 6).

Discussion

More and more evidence indicate that OT regulates gastrointestinal function through peripheral receptor. Milenov K (18) *et al*. reported that, on wakeful and anaesthetized dogs, systemic OT administration decreased the tone and abolished the peristaltic contractions and the spike-potentials in the

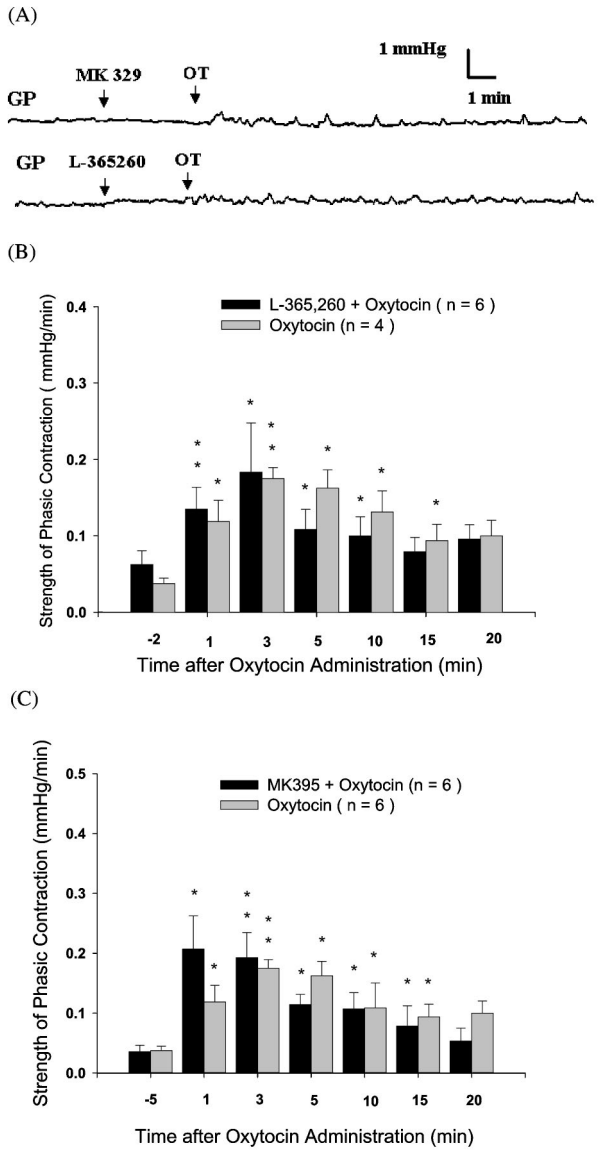

Fig. 6. MK-329 (0.4 mg/kg, iv) or L-365, 260 (0.4 mg/kg, iv) did not influence the OT effect on gallbladder phasic contraction. A. Representatives of the recordings. GP, gallbladder pressure; OT, oxytocin. The arrows indicate the treatment. B and C. The statistic analyses of the MK-329 (B) and L-365, 260 (C) on the excitation of phasic contraction caused by systemic OT (0.02 mg/kg, iv). $*P < 0.05$, $**P < 0.01$ compared with the data prior to OT administration.

stomach and intestine muscle for 8 to 15 min. In wake dogs, OT reduced two or three times the frequency of the basic electric rhythm (BER) in the stomach and increased the propagation of BER in the small intestine for 10 sec to 10 min. The effect of OT on the gastrointestinal motility was not eliminated by adrenolytics, cholinolytics and gangliolytics, but that on BER was eliminated by cholinolytics and gangliolytics (18). Duridanova *et al*. (1995) reported that, on the gastric smooth muscle cells on the guinea-pig antrum, physiological concentrations of OT (10^{-12} mol/l to 10^{-9} mol/l) dose-dependently suppressed the tetrodotoxin- and atropine- resistant spontaneous phasic contraction. This OT-related relaxation resulted from the activation of Ca^{2+}-sensitive K^+ conductivity *via* activation of IP_3-induced release of Ca^{2+} from the submembrane located cisternae of the sarcoplasmic reticulum Ca^{2+} stores (4). Our recent studies indicated that, in wakeful rats, systemic OT inhibited the gastric emptying and intestinal transit (24). Pretreatment of progesterone potentiated this effect (12). The inhibition of gastrointestinal motility was reversed by the pretreatment of MK-329, the CCK_A receptor antagonist. The plasma CCK concentration was elevated after OT administration (24). So it seems that the inhibitory effect of OT on the gastric and intestine muscle is partly through the secretion of CCK and partly through the myenteric receptor on the smooth muscle.

Contrary to the inhibition of the stomach and intestine muscle, from this study, it is clear that systemic OT excited gallbladder motility, especially the phasic contraction. Because this effect was not influenced by pretreatment of atropine, MK-329 and L-365,260, so we believe that the excitatory effect of OT on gallbladder motility is independent of the cholinergic pathway and the secretion of CCK.

OT and VP are cyclic nonapeptides whose actions are mediated by activation of specific G protein-coupled receptors currently classified into V1a-vascular, V2-renal and V1b-pituitary VP receptors and OT receptors (22, 28). Besides activating their own receptors, OT and VP could also bind with the receptor of each other. It was reported that both of VP ($3 \times 10^{-9} \sim 10^{-6}$ mol/l) and OT ($3 \times 10^{-11} \sim 10^{-8}$ mol/l) dose-dependently increased the porcine myometrial contractility (27). Both of these two peptides also cause potent and long-lasting vasoconstriction of uterine arteries (10).

Despite the overlapping effect of these two hormones on the receptors of each other, using pharmacological method, we can still differentiate the specific receptor that was activated by them. OT was 75 and 57 times more potent than lysine VP in increasing myometrial contractility in pregnant and nonpregnant sows (27). L-366,948, a highly selective OT receptor antagonist, inhibited lysine VP and OT induced myometrial contraction. Although d(CH2)5 [D-Tyr(Me)2]-vasopressin, the V1a receptor antagonist, also inhibited the effect of OT and VP, higher concentration was required to achieve the antagonism. So it is clear that both VP and OT excite the porcine myometrial contractility through OT receptor (27). On the other hand, VP was 57-fold more than OT in the effect of constricting rat isolated

uterine resistance arteries. SR 49509, a selective V1a antagonist, reversed the effect of OT and VP on the arterial muscle, but atosiban, the OT receptor antagonist, did not. So the excitatory effect of these two peptides on the isolated uterine resistance arteries is mediated by V1a receptor (3).

In this study, we also investigated specific receptor that medicates the OT effect on gallbladder motility. OT (0.01 mg-0.04 mg/kg, iv) dose-dependently increased the phasic contraction of gallbladder, but did not influence the gallbladder pressure. On the other hand, high dose of VP lowered the gallbladder pressure but did not affect the phasic contraction. Atosiban (0.02 mg/kg, iv) completely abolished the effect of OT (0.02 mg/kg, iv) on the gallbladder motility. So it is clear that systemic OT and VP administration affected gallbladder motility through different mechanisms. The effect of OT was mainly through activating specific OT receptor, the effect of VP may *via* VP receptors.

In the interdigestive period, the phasic contraction of gallbladder is believed to be important in stirring the bile, facilitating the bile concentration, and preventing the gallstone formation in gallbladder (16). The phasic contraction is controlled by neuronal and humoral mechanism. We have reported that, microinjection of thyrotropin-releasing hormone (11, 15), glutamate (14) and nitric oxide (23) into DMV excited the gallbladder phasic contraction through vagus nerve and peripheral muscarine receptors. Nucleus raphe obscurus modulates the gallbladder motility through the similar pathway (26). Intravenous injection of erythromycin increased gallbladder phasic contraction through the cholinergic pathway (13). From this experiment, systemic OT administration also enhanced the gallbladder motility. This effect was mediated by specific OT receptor and was independent of peripheral cholinergic pathway and the secretion of CCK. Administration of atosiban, the OT receptor antagonist, decreased the spontaneous contraction. That is to say, during the interdigestive period, endogenous OT exerts tonic and physiological regulation on gallbladder phasic contraction.

In conclusion, the results of this study indicate that during the interdigestive period in rabbit, the endogenous OT regulates the phasic contraction *via* specific OT receptor. This effect is independent of peripheral cholinergic pathway and the secretion of CCK.

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