Pflugers Arch - Eur J Physiol (2010) 460:1063–1071
DOI 10.1007/s00424-010-0880-7

MUSCLE PHYSIOLOGY

CCK mediated the inhibitory effect of oxytocin on the contraction of longitudinal muscle strips of duodenum in male rats

Yinglian Lv · Mei Feng · Tongtong Che · Hui Sun · Yan Luo · Kejing Liu · Chuanyong Liu

Received: 12 June 2010 / Revised: 6 September 2010 / Accepted: 9 September 2010 / Published online: 5 October 2010

Abstract The aim of the present study was to investigate the effect of oxytocin (OT) on duodenum motility in rats and the possibility that cholecystokinin (CCK) was involved in this process. The isometric contraction of longitudinal muscle strips of duodenum was monitored by polygraph. ELISA was used to measure the concentration of CCK and OT in duodenum. CCK mRNA was assayed by RT-PCR. Oxytocin receptor (OTR) and CCK in duodenum were located by immunohistochemistry and immunofluorescence staining. OT (10^{-5} and 10^{-6} M) inhibited the spontaneous contraction of the muscle strips. On the contrary, atosiban (OT receptor antagonist), lorglumide (CCK_1 receptor antagonist), and tetrodotoxin (TTX, blocker of voltage-dependent Na^+ channel on nerve fiber) excited the contraction. The inhibitory effect of OT on duodenal motility was reversed by pretreatment of atosiban, lorglumide, or TTX. Exogenous OT did not influence the expression of OT mRNA in duodenum but increased the concentration of CCK in the culture medium of the cells isolated from longitudinal muscle myenteric plexus. The OTR and CCK were co-expressed in the neurons of the myenteric plexus in duodenum. We concluded that OT inhibited the contraction of the LD spontaneous contraction of rats in vitro. This effect was mediated by the CCK released from the neurons of the myenteric plexus in duodenum.

Keywords OT · Oxytocin receptor · Duodenum motility · CCK

Y. Lv · T. Che · H. Sun · Y. Luo · K. Liu · C. Liu (✉)
Department of Physiology,
Shandong University School of Medicine,
Jinan 250012, People's Republic of China
e-mail: liucy@sdu.edu.cn

M. Feng
Central Lab of Shandong Provincial Hospital,
Shandong University,
Jinan 250012, China

H. Sun
Department of Physiology, Binzhou Medical Collage,
Yantai 264003, China

C. Liu
Key Lab of Mental Disease of Shandong Province,
Shandong University School of Medicine,
Jinan 250012, China

Introduction

Oxytocin (OT) is a nonapeptide of the neurohypophyseal protein family. The well-known physiological function of OT was to induce uterine contraction and milk ejection reflex [14, 17]. In recent years, more and more studies indicated that OT might be involved in the regulation of gastrointestinal motility but the effect appeared to be species and region specific. Both OT and OT receptor (OTR) were expressed throughout the human gastrointestinal (GI) tract [6, 10]. In rats, exogenous OT accelerated gastric and colonic motility in human, rabbits, and rats [3, 9, 12, 13], inhibited the gastric emptying and intestinal transit in rats [20, 21], and excited the phasic contraction of gallbladder in rabbits [4]. Most of these effects were abolished by atosiban, a specific OTR antagonist [3, 4, 21]. Systemic administration of atosiban inhibited the spontaneous contraction of gallbladder in rabbits [4] and delayed gastric emptying in human [7]. In human, oxytocin is expressed in the myenteric and submucous ganglia and nerve fiber along the entire GI tract and was secreted following a fat meal [8, 10]. So it is possible that OT is an endogenous neuropeptide in ENS that is involved in the regulation of GI motility, but the underlying mechanism has not been clearly understood.

Cholecystokinin (CCK) is a hormone and neuropeptide in GI tract. Several studies indicated that CCK mediated the inhibitory effect of OT on GI motility in rats. Systemic administration of OT increased the plasma concentration of CCK [20]. The CCK_1 receptor antagonist, devazepide or lorglumide, reversed the inhibitory effect of OT on GI motility [13, 20]. As far as we know, the mechanism underlying the effect of OT on the secretion of CCK was unknown. Our recent study indicated that systemic OT administration induced an early transit decrease and followed by a later long-lasting increase of the intragastric pressure in rats [13]. Pretreatment with devazepide only reversed the early inhibition but did not influence the later excitation [13]. Exogenous OT exerted only excitatory effect on the contraction of muscle strips of stomach in vitro [13]. Because OTR was expressed in myenteric plexus in duodenum [19], we hypothesized that OT might induce the secretion of CCK in the myenteric neuron of duodenum.

In order to test this hypothesis, we investigated the effect of OT on the contraction of longitudinal muscle strips of duodenum of rats in vitro. The possibility that CCK was involved in the OT effect on muscle contraction was tested. The amount of CCK mRNA expression in duodenum was detected by RT-PCR analysis. The amount of CCK and OT secreted from the neurons of the myenteric plexus in duodenum was detected by ELISA. The expression of OTR and CCK in duodenum was localized by immunohistochemistry and immunofluorescence staining.

Materials and methods

Experimental animals

Male Wistar rats, weighing 280–300 g, were purchased from the Animal Center of Shandong University. They were fasted overnight with free access to water before experiments. All experimental procedures were approved by the Medical Ethics Committee for Experimental Animal, Shandong University School of Medicine.

Muscle strips preparation

The muscle strips of duodenum were prepared as described elsewhere [18] with some modification. Briefly, immediately after the rat was sacrificed by decapitation, a segment of duodenum (1 cm from pylorus) was removed, opened along the mesenteric border, and the resulting rectangular sheet was pinned flat (mucosa up) in a silica gel dish filled with oxygenated Krebs solution. Muscle strips (4 mm wide, 10 mm long) parallel to the long axis of the longitudinal fibers were cut.

Recording of the contraction of muscle strips

The muscle strips were suspended in tissue chambers containing 5 ml Krebs solution (37°C), which bubbled continuously with 95% O_2 and 5% CO_2. One end of the strip was tied to a hook at the bottom of the chamber. The opposing end was connected to an external isometric force transducer (JH-2B, Instrument Company of Chengdu, Chengdu, China). The temperature of the Krebs solution in the chamber was kept at 37 ± 0.5°C. Tension of spontaneous contraction of duodenum strips (under preload of 1 g) was recorded using a polygraph system (ML785-PowerLab, ADI, Sydney, Australia). After the spontaneous contraction of duodenum strips had been stable, OT was administrated to the chambers. Each muscle strip was exposed to OT only once, and tension was recorded continuously for 15 min after OT administration. In some experiments, the strips were pretreated with atosiban, lorglumide, or TTX for 10 to 30 min before application of OT.

RT-PCR

Whole thick duodenal tissue (50–100 mg) were frozen in liquid nitrogen and stored at −80°C. The frozen specimens were homogenized with an electrokinetic homogenizer in Trizol (Invitrogen, Carlsbad, USA) for total RNA extraction. RNA reverse transcription was conducted using the high-capacity cDNA reverse transcription kit (4368814; Applied Biosystems, Warrington, UK). Oligonucleotide primers were designed on the basis of the DNA sequence of rat CCK. The primer pair of CCK was (forward: 5-CAAGATCTA TGAAGTGCGGCGTGT-3′; reverse: 5′-GGCGGATCCAC TACGATGGGTA-3′) and that of β-actin was (forward: 5′-TCTACAATGAGCTGCGTGTGG-3′; reverse: 5;-GGAG TCCATCACAATGCCAGT-3′). The PCR was conducted at 94°C for 5 min, followed by 26 cycles for 30 s at 94°C, 30 s at 58°C, 40 s at 72°C, and the final step was for 10 min at 72°C. The PCR-amplified fragment size of CCK was 350 bp. The products were separated by electrophoresis on a 1.5% agarose gel.

Preparation of LMMP, cells isolation, and ELISA

Male Wistar rats, weighing 280–300 g, were fasted overnight with free access to water before experiments. After the rats were anesthetized with amobarbital sodium (0.056 g/kg), a segment of duodenum (1 cm from pylorus) was removed immediately, opened along the mesenteric border, and the resulting rectangular sheet was pinned flat (mucosa up) in a silica gel dish filled with oxygenated Krebs solution. The sheet was transferred to a germ-free Krebs solution, the mucosa, submucous layer, and circular muscle was torn with microinstrument under anatomical lens. The left sheet

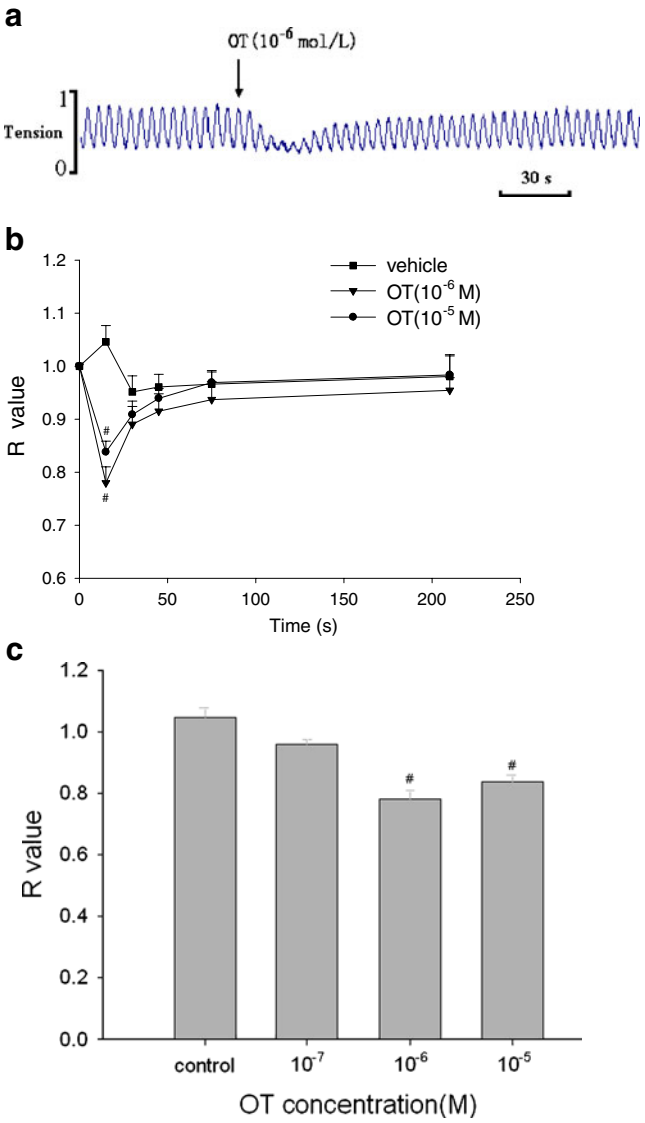

Fig. 1 Effect of OT on the contraction of longitudinal muscle strips of duodenum of rats in vitro. **a** Representative recording of the effect of OT (10^{-6} M) on the spontaneous contraction of muscle strips. The contraction of muscle strips was inhibited immediately after the administration of OT. *Arrow* indicates the treatment of OT. **b** The time course of effect of OT (10^{-6} and 10^{-5} M) on the contraction of muscle strips. It shows that the *R* value of the muscle strips reached the nadir within 30 s following OT administration and returned to normal at 60 s. $^{\#}P<0.05$ vs. the baseline. **c** The effect of three doses of OT on the *R* value of muscle strips. The data in every group was taken at 30 s following OT or vehicle administration. Lower dose of OT (10^{-7} M) did not change the *R* value but higher doses of OT (10^{-6} and 10^{-5} M) exerted significant inhibition. $^{\#}P<0.05$ vs. control

(longitudinal muscle myenteric plexus, LMMP) was incubated for 50 min in papayotin solution (6 mg/ml) at 37°C, incubated in collagenase II solution (1 mg/ml) for 50 min, blown for 30 times with suction pipe, centrifuged at 2,000 rpm at room temperature for 10 min, and the sediment, including the isolated cells, was suspended in 1 ml Neurobasal A Medium (Invitrogen). Thirty seconds following OT administration, the culture medium of the isolated cells was centrifuged at 1,000 rpm at room temperature for 10 min, and the supernatants were stored at −20°C until assayed.

CCK and OT levels in the supernatants of culture medium were measured by ELISA using a CCK ELISA commercial kit (C033-80, GBD, San Diego, USA) (sensitivity, 1 ng/ml; slope value of the standard curve, 0.014; R^2, 0.947) and a OT ELISA commercial kit (F16373, Westang, Shanghai, China) (sensitivity, 1 ng/ml; ID50, 420 ng/g; slope value of the standard curve, 0.53; R^2, 0.940).

Immunohistochemistry

Whole thickness of duodenal tissue was fixed in 4% paraformaldehyde, rinsed, dehydrated, cleared, and immersed in wax. Then, the tissue was sectioned with 4-μm thickness. Sections were stained using a two-step protocol. After deparaffinization, rehydration, rinse, antigen restoration, and blockade of the endogenous peroxidase by 3% hydrogen

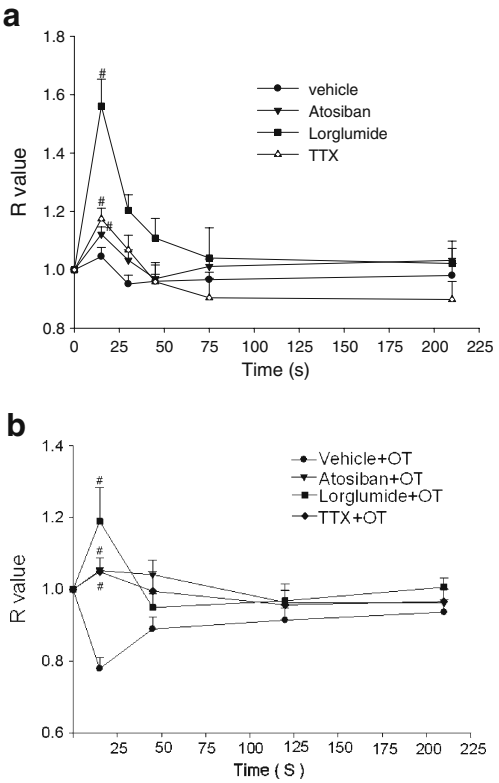

Fig. 2 Time course of the effect of several chemicals on the spontaneous contraction of longitudinal muscle strips of duodenum in rats. **a** The effect of atosiban (10^{-6} M), lorglumide (3×10^{-6} M), and TTX (10^{-5} M) on the contraction of the muscle strips. All these three chemicals transiently accelerated the contraction of muscle strips. The *R* value reached the highest value at 15 s ($^{\#}P<0.05$ vs. vehicle) and returned to normal at 45 s. **b** The effect of three chemical pretreatment, including atosiban (10^{-6} M), lorglumide (3×10^{-6} M), and TTX (10^{-5} M) on the inhibitory effect of OT (10^{-6} M) on the contraction of the longitudinal muscle strips of duodenum of rats. All the three chemicals reversed the inhibition exerted by OT on the muscle contraction. $^{\#}P<0.05$ vs. NS+vehicle

peroxide for 15 min, the sections were incubated with primary goat anti-OT receptor antibody (1:50; Santa Cruz Biotechnology, Santa Cruz, USA) and primary rabbit anti-CCK antibody(1:150; Millipore, Bedford, USA) overnight in a humid chamber at 4°C. After three rinses in PBS, the sections were incubated with polymer peroxidase goat anti-rabbit serum (ZSGB-BIO, Beijing, China) for 30 min at room temperature. Then, the sections were washed and treated with HRP-labeled streptavidin complex (ZSGB-BIO) for 30 min at room temperature. After three rinses, peroxidase was revealed by a 3, 3′-diaminobenzidine tetrahydrochloride substrate kit (ZSGB-BIO). In negative controls, the sections were incubated with PBS instead of primary antibody.

Co-localization of OTR and CCK by immunofluorescence in LMMP

The procedure is similar to immunochemistry above with some modification. After pre-incubation in 5% donkey serum for 1 h, the LMMP preparation were incubated with the primary antibody mixture composed of primary goat anti-OTR antibody (1:50; Santa Cruz Biotechnology) and primary rabbit anti-CCK antibody (1:150 in PBS) overnight in a humid chamber at 4°C. After rinsing three times in PBS, the LMMP were incubated with the secondary antibody mixture composed of Alexa Fluor 488-labeled donkey anti-goat (A11055, invitrogen) and RB-200-labeled donkey anti-rabbit IgG (sc-2095, Santa Cruz Biotechnology) for 1 h at room temperature. In negative controls, the sections were incubated with PBS instead of primary antibody.

Solutions and chemicals

Oxytocin and lorglumide were purchased from Sigma-Aldrich Corp (St. Louis, MO, USA). Atosiban was purchased from Ferring AB (Malmoe, Sweden). TTX was purchased from RuiFang Biotechnology limited company (Dalian, China).

The Krebs solution was composed of the following reagents (in millimoles per liter), NaCl 120.6, KCl 5.9, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 15.4, and glucose 11.5. Oxytocin, lorglumide, TTX, and atosiban were diluted into Krebs solution.

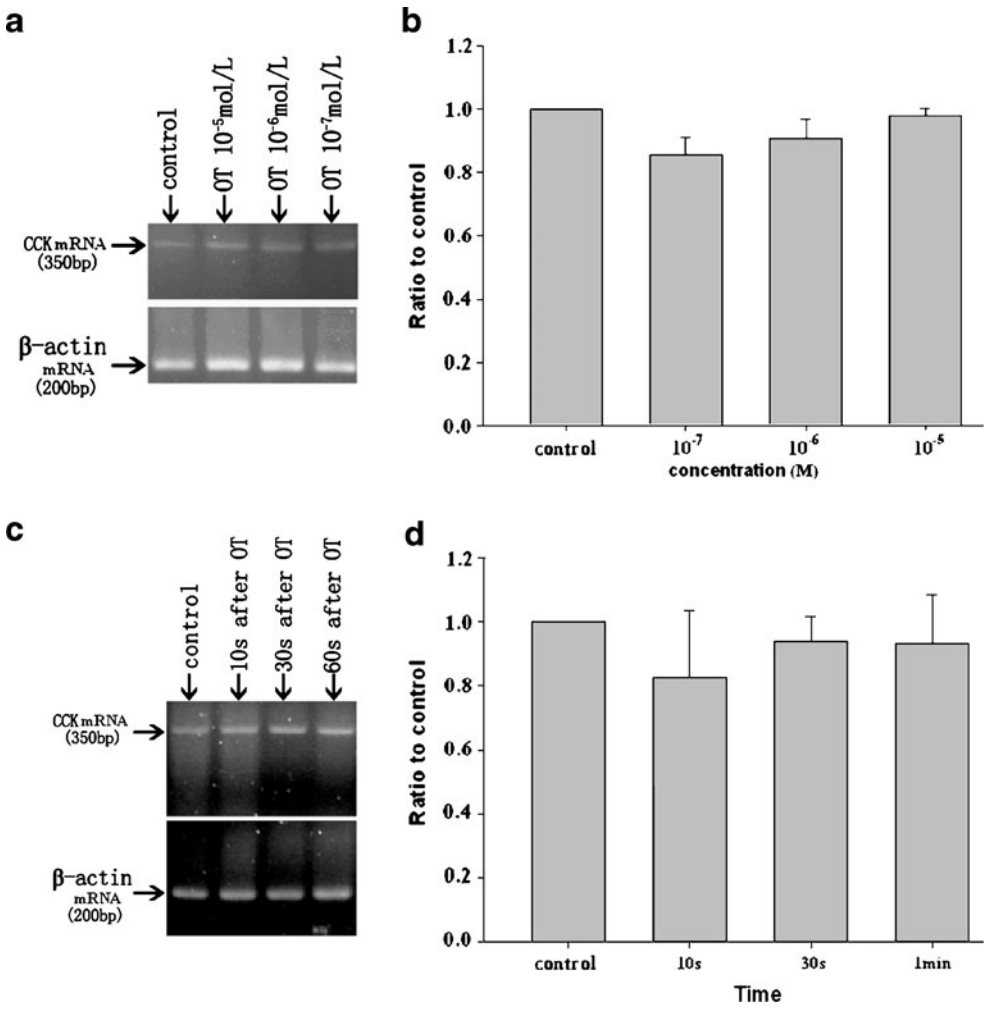

Fig. 3 The amount of CCK mRNA expression in duodenum of rats with and without the treatment of OT. **a** The representative bands of CCK mRNA 30 s following three doses of OT (10^{-7}, 10^{-6}, and 10^{-5} M) or same volume of vehicle (control) administration. β-actin acted as internal control. **b** The summarized data. It is clear that OT (10^{-7}–10^{-5} M) did not influence the expression of CCK mRNA ($P>0.05$ vs. control, $n=5$). **c** The representative bands of mRNA in duodenum at 10, 30, and 60 s following OT (10^{-6} M) administration. β-actin acted as internal control. **d** The summarized data. It is clear that the amount of CCK mRNA did not change with 60 s following OT (10^{-6} M) administration ($P>0.05$ vs. control, $n=5$)

Data analysis

Muscle strips contraction The mean value of the average tension for 3-min period before treatment with OT was taken as the baseline. The average tension for 10 s, 30 s, and 1 min after each OT treatment was divided by that of baseline, and the ratio to control is signed *R*, which is regarded as the change of contraction of the muscle strips induced by OT. The *R* value of baseline was equal to 1.

RT-PCR The value of ratio to baseline is the ratio of grayscale division between the rats with and without OT administration.

All the data were presented as mean ± SEM. One-way analysis of variance (ANOVA) followed by Dunnett's test or Student's *t* test was used to determine the difference between the means of different groups. $P<0.05$ was considered to be significantly different. The *n* means the number of the rats in the group.

Results

Effect of OT on the spontaneous contraction of the muscle strips

OT (10^{-5} and 10^{-6} M) inhibited the spontaneous contraction of the muscle strips. The contraction of the muscle strips decreased immediately after OT administration, reached the lowest level at 30 s, and returned to normal 1 min later. Thirty seconds following the two doses of OT administration (10^{-5} and 10^{-6} M), the *R* value of the muscle strip decreased from 1 (baseline) to 0.838 ± 0.021 ($P<0.05$, $n=6$) and 0.780 ± 0.030 ($P<0.05$, $n=6$). Lower dose of OT (10^{-7} M) did not significantly influence the contraction of the muscle strips (Fig. 1).

Effect of atosiban, TTX, and lorglumide on the inhibitory effect of OT on the contraction of duodenal muscle strips

Both atosiban (10^{-6} M), a competitive antagonist of OTR, and lorglumide (3×10^{-6} M), a potent CCK_1 receptor antagonist, transiently increased the spontaneous contraction of the muscle strips. The *R* value reached the maximal at 15 s and returned to normal at 45 s. At 15 s, the *R* value of the atosiban group was 1.122 ± 0.023, significantly higher than that of vehicle control ($P<0.05$, $n=6$). Lorglumide exerted a similar effect. At 15 s following lorglumide administration, *R* value was increased from 1.028 ± 0.033 (vehicle group, $n=6$) to 1.561 ± 0.093 ($P<0.05$, $n=6$). Similar to the two agents, TTX also transiently increased the spontaneous contraction of the muscle strips. At 15 s following TTX administration, *R* value was increased from 1.028±0.033 (vehicle group, $n=6$) to 1.175±0.036 ($P<0.05$, $n=6$) (Fig. 2a).

Pretreatment with atosiban (10^{-6} M), lorglumide (3×10^{-6} M), or TTX (10^{-5} M), significantly reversed the inhibitory effect of OT at 10^{-6} M on the contractions of the muscle strips. At 15 s following OT administration, the *R* value in the group of atosiban + OT, lorglumide + OT, and TTX + OT was 1.053 ± 0.009, 1.190 ± 0.094, and 1.049 ± 0.040, all significantly higher than that of the NS + OT group (Fig. 2b).

Amount of CCK mRNA expressed on duodenum

The amount of CCK mRNA expressed on duodenum did not change within 3 min following OT (10^{-7}–10^{-5} M) administration (Fig. 3).

Concentration of CCK and OT

The concentration of CCK in culture medium of the cells isolated from LMMP of duodenum increased significantly

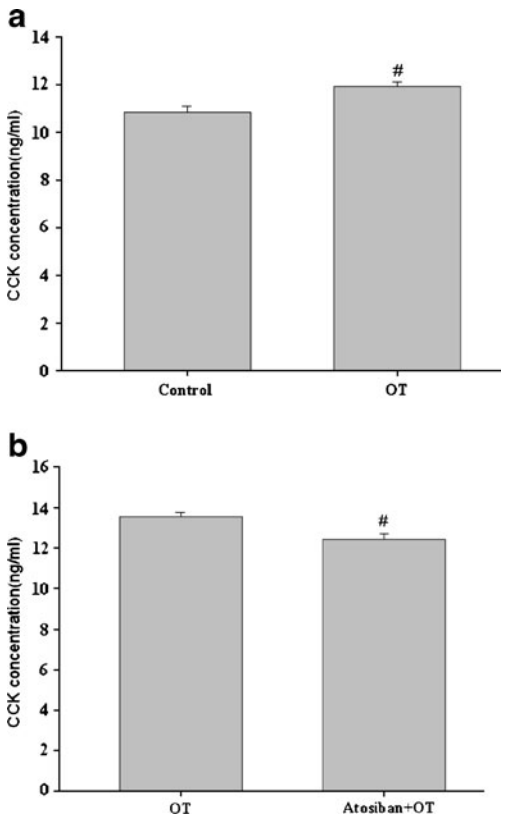

Fig. 4 The concentration of CCK in the culture medium of the cells isolated from LMMP of duodenum. **a** The concentration of CCK increased significantly ($^{\#}P<0.05$ vs. vehicle control), 30 s following OT (10^{-6} M) administration. **b** Pretreatment of atosiban (10^{-6} M) significantly attenuated the increase of CCK concentration in the culture medium following OT (10^{-6} M) administration. $^{\#}P<0.05$ vs. OT

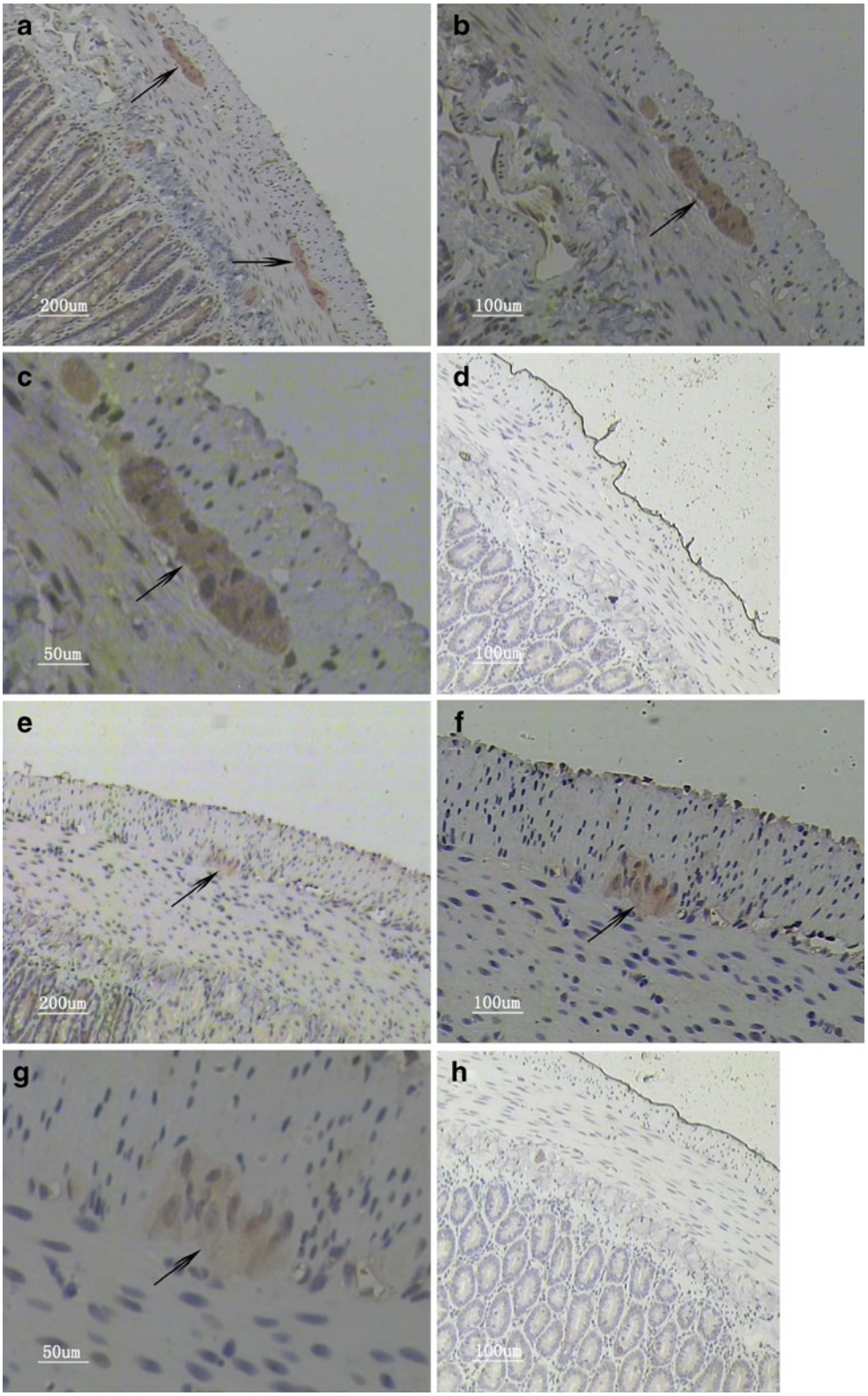

Fig. 5 Localization of CCK (**a**–**d**) OTR (**e**–**h**) in the whole thickness tissue of duodenum by immunohistochemistry staining. Photo **a** shows that CCK immunoreactive cells were located in myenteric plexus. Photos **b** and **c** were the same photo slices with higher magnification. Photo **d** shows the negative control, in which the primary antibody was replaced by PBS. In photo **e**, OTR immunoreactive cells were located in myenteric plexus. Photos **f** and **g** were the same photo slices shown in photo **e** with higher magnification. Photo **h** was the negative control, in which the primary antibody was replaced by PBS. The *arrows* indicated the CCK (**a**–**d**) or OTR (**e**–**g**) immunoreactive cells in the myenteric plexus

following OT (10^{-6} M) administration. The CCK concentration in culture medium increased from 11.124 ± 0.340 (vehicle control, n=6) to 12.086 ± 0.232 (P<0.05, n=6). Pretreatment with atosiban inhibited this effect of OT. With the pretreatment of atosiban (10^{-6} M), the CCK concentration in culture medium decreased from 13.530 ± 0.217 (OT) to 12.438 ± 0.263 (atosiban + OT) (P<0.05, n=6) (Fig. 4).

The concentration of OT in LMMP was 11.385 + 0.217 ng/g tissue (n=12).

Location of OTR and CCK in duodenum

Both CCK immunoreactive cells and OTR immunoreactive cells were located at the enteric nerve plexus of duodenum. No immunoreactive cell was detected in control tissues in which the primary antibody was replaced by PBS (Fig. 5).

OTR and CCK were co-expressed on cells of myentric plexus of LMMP from duodenum (Fig. 6).

Discussion

In this study, we found that exogenous OT inhibited the spontaneous contraction of longitudinal muscle strips of duodenum. This result was consistent with the report of Wu et al., which indicated that systemic administration of OT inhibited the intestinal transit in rats [20]. Because this effect was reversed by pretreatment of atosiban, the specific OTR antagonist, we believe that the inhibitory effect of OT on duodenal motility was mediated by OTR. Lorglumide, the specific CCK_1 receptor antagonist, also blocked this effect, so it seemed that in this study, CCK was involved in the inhibition of longitudinal muscle contraction induced by OT.

There are two kinds of cells that synthesize and release CCK, including endocrine I cells in the duodenal wall and peptidergic nerves in ENS and central nervous system (CNS) [16]. Although there were several studies indicated that OT inhibited gastric and intestinal motility by increasing the release of CCK [13, 20], the origin of CCK secretion following OT administration was not clarified. In order to exclude the possibility that OT induced the secretion of CCK from CNS, this study was conducted in the duodenum in vitro. The result of this study was consistent to that in vivo [20, 21], so we believe that the CCK which mediated the inhibition of OT on duodenal contraction might be released from duodenum but not from the brain.

TTX is the specific blocker of voltage-dependent Na^+ channels on nerve fibers. Pretreatment of this chemical abolished the inhibition of the duodenal contraction following OT administration. This result was consistent with our hypothesis that OT induced the secretion of CCK from ENS. To further testify this hypothesis, two other

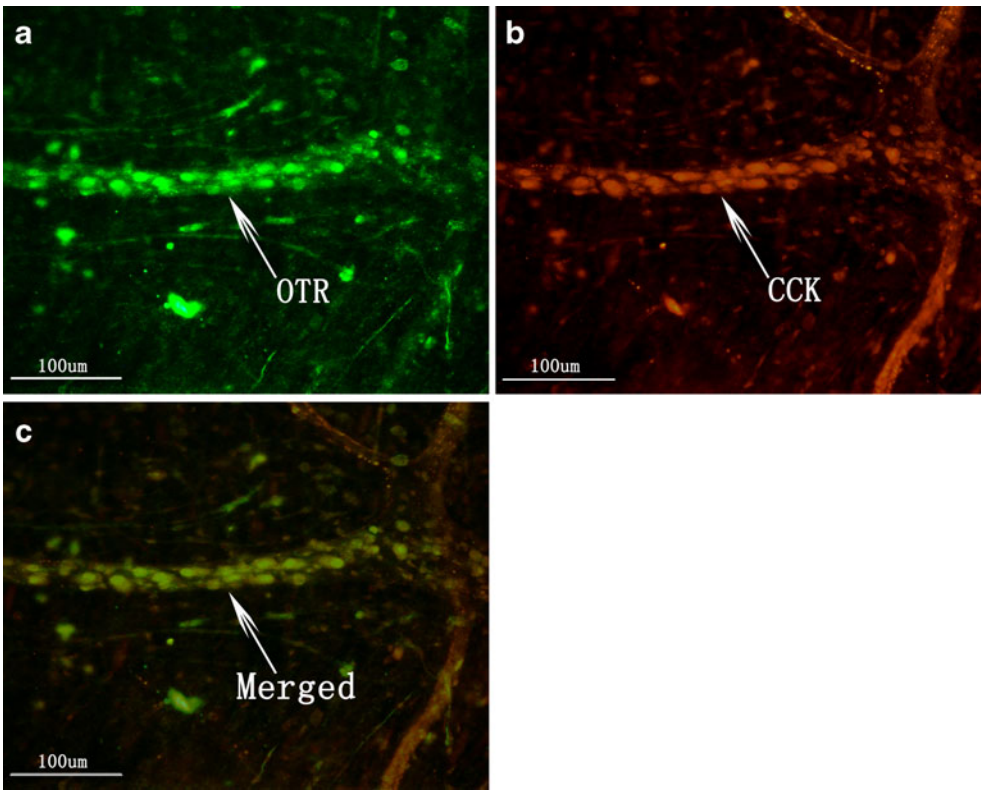

Fig. 6 Co-localization of OTR and CCK on the neurons of myenteric plexus in duodenum in rats. Photos **a**–**b** were the immunofluorescence staining for OTR (**a**) and CCK (**b**) on the specimen of longitudinal muscle myenteric plexus (LMMP) from duodenum in rats. Both OTR (**a**) and CCK (**b**) immunoreactive cells were located in the plexus. They were co-expressed on some cells in myenteric plexus. The *arrow* indicated the immunoreactive cells

experiments were conducted. In the first experiment, the effect of OT on the secretion of CCK from myenteric plexus was testified. Exogenous OT significantly increased the concentration of CCK in the culture medium of the isolated cells from LMMP, and atosiban abolished this effect. So it is clear that OT increased the secretion of the CCK from the neurons of myenteric plexus. In the second experiment, the OTR and CCK were localized in duodenum by immunohistochemistry and immunofluorescence staining. The immunohistochemistry staining in duodenum showed that both OTR and CCK were located in the myenteric plexus, and the immunofluorescence staining on LMMP showed that OTR and CCK were co-expressed on the neurons of the myenteric plexus. All these results supported our hypothesis that OT induced the release of CCK from neurons of myenteric plexus in duodenum.

Because administration of OT did not influence the expression of CCK mRNA in duodenum, it is possible that OT did not influence the synthesis of CCK in the neurons, at least at the time points (10–60 s) analyzed in this study.

It is noteworthy that both atosiban and lorglumide inhibited the spontaneous contraction of the muscle strips, and the concentration of OT in LMMP was about 10 ng/g tissue. These results indicated that endogenous CCK and OT exerted a tonic inhibition on the spontaneous contraction of longitudinal muscle strips. Like CCK, OT might be also a physiological regulator for GI motility. This result was also supported by our recent study and that of other groups, which indicated that systemic administration of atosiban decreased gallbladder contraction in rabbits and the gastric emptying in human [4, 7].

Although it has been widely recognized that OT induced the contraction of the smooth muscle on the uterus and mammary gland, the effect on the GI tract was controversial. Both excitatory and inhibitory effects were reported [2, 4, 7, 13, 20, 21]. This might be attributed to the different location of OTR in different organs and the same organ in different animals.

As the member of superfamily of G-protein coupled receptors, OTR activates phospholipase C, thereby increasing inositol trisphosphate production, leading to increases in intracellular calcium, triggering muscle contraction [1]. So OT induces the contraction of smooth muscle if OTR is expressed on the membrane of smooth muscle, such as that of uterus, mammary gland, and stomach [13]. On the other hand, OT also indirectly regulates muscle contraction if the OTR was not expressed on the membrane of smooth muscle. It induced the relaxation of vascular muscle via stimulation of the nitric oxide pathway in endothelial cells [15]. In this study, OT induced the relaxation of duodenum muscle strips via the released CCK in myenteric neurons. This is the other pathway by which OT inhibits the smooth muscle contraction. So the controversy of the effect of OT on GI motility in different species and regions might be attributed to the different location of OTR and the different underlying mechanisms. OT regulates GI motility via both direct and indirect pathways.

The result of the present study are consistent with the research of Martins et al., which indicated that endogenous CCK inhibited the spontaneous contraction of duodenal longitudinal muscle via V_1 receptors on myenteric neurons [5]. The CCK_1 receptors were located on vagal afferent neurons [22], myenteric neurons, and interstitial cell of Cajal [11]. So it is possible that CCK is an endogenous inhibitor of duodenal motility. The tonic inhibitory effect of CCK on duodenal muscle contraction is regulated by OT.

In conclusion, the results of this study indicated that OT inhibited the contraction of longitudinal muscle strips in rats in vitro. This effect was mediated by CCK secreted from the neurons of myenteric plexus. These findings demonstrated an indirect pathway that mediated the inhibitory effect of OT on GI motility.

Acknowledgement This work was supported by the Natural Scientific Foundation of China (NSFC 30971074), Specialized Research Fund for the Doctoral Program of Higher Education (SRFDP, No. 20090131110046), and the Key Project of Chinese Ministry of Education (No 109095).

References

1. Blanks AM, Thornton S (2003) The role of oxytocin in parturition. Bjog 110(Suppl 20):46–51
2. Feng M, Qin J, Wang C, Ye Y, Wang S, Xie D, Wang PS, Liu C (2009) Estradiol upregulates the expression of oxytocin receptor in colon in rats. Am J Physiol Endocrinol Metab 296:E1059–E1066
3. Li L, Kong X, Liu H, Liu C (2007) Systemic oxytocin and vasopressin excite gastrointestinal motility through oxytocin receptor in rabbits. Neurogastroenterol Motil 19:839–844
4. Liu CY, Liu JZ, Xie DP, Liu PY, Wang PS (2003) Endogenous oxytocin excites phasic contraction of gallbladder in rabbits through oxytocin receptor. Chin J Physiol 46:95–101
5. Martins SR, Oliveira RB, Ballejo G (2006) Activation of neural cholecystokinin-1 receptors induces relaxation of the isolated rat duodenum which is reduced by nitric oxide synthase inhibitors. Braz J Med Biol Res 39:271–275
6. Monstein HJ, Grahn N, Truedsson M, Ohlsson B (2004) Oxytocin and oxytocin-receptor mRNA expression in the human gastrointestinal tract: a polymerase chain reaction study. Regul Pept 119:39–44
7. Ohlsson B, Bjorgell O, Ekberg O, Darwiche G (2006) The oxytocin/vasopressin receptor antagonist atosiban delays the gastric emptying of a semisolid meal compared to saline in human. BMC Gastroenterol 6:11
8. Ohlsson B, Forsling ML, Rehfeld JF, Sjolund K (2002) Cholecystokinin stimulation leads to increased oxytocin secretion in women. Eur J Surg 168:114–118
9. Ohlsson B, Ringstrom G, Abrahamsson H, Simren M, Bjornsson ES (2004) Oxytocin stimulates colonic motor activity in healthy women. Neurogastroenterol Motil 16:233–240
10. Ohlsson B, Truedsson M, Djerf P, Sundler F (2006) Oxytocin is expressed throughout the human gastrointestinal tract. Regul Pept 135:7–11

11. Patterson LM, Zheng H, Ward SM, Berthoud HR (2001) Immunohistochemical identification of cholecystokinin A receptors on interstitial cells of Cajal, smooth muscle, and enteric neurons in rat pylorus. Cell Tissue Res 305:11–23
12. Petring OU (1989) The effect of oxytocin on basal and pethidine-induced delayed gastric emptying. Br J Clin Pharmacol 28:329–332
13. Qin J, Feng M, Wang C, Ye Y, Wang PS, Liu C (2009) Oxytocin receptor expressed on the smooth muscle mediates the excitatory effect of oxytocin on gastric motility in rats. Neurogastroenterol Motil 21:430–438
14. Sala NL, Luther EC, Arballo JC, Cordero Funes JC (1974) Oxytocin reproducing reflex milk ejection in lactating women. J Appl Physiol 36:154–158
15. Thibonnier M, Conarty DM, Preston JA, Plesnicher CL, Dweik RA, Erzurum SC (1999) Human vascular endothelial cells express oxytocin receptors. Endocrinology 140:1301–1309
16. Varga G, Balint A, Burghardt B, D'Amato M (2004) Involvement of endogenous CCK and CCK1 receptors in colonic motor function. Br J Pharmacol 141:1275–1284
17. Wakerley JB, Dyball RE, Lincoln DW (1973) Milk ejection in the rat: the result of a selective release of oxytocin. J Endocrinol 57:557–558
18. Wang SL, Xie DP, Liu KJ, Qin JF, Feng M, Kunze W, Liu CY (2007) Nitric oxide mediates the inhibitory effect of ethanol on the motility of isolated longitudinal muscle of proximal colon in rats. Neurogastroenterol Motil 19:515–521
19. Welch MG, Tamir H, Gross KJ, Chen J, Anwar M, Gershon MD (2009) Expression and developmental regulation of oxytocin (OT) and oxytocin receptors (OTR) in the enteric nervous system (ENS) and intestinal epithelium. J Comp Neurol 512:256–270
20. Wu CL, Hung CR, Chang FY, Pau KY, Wang JL, Wang PS (2002) Involvement of cholecystokinin receptor in the inhibition of gastric emptying by oxytocin in male rats. Pflugers Arch 445:187–193
21. Wu CL, Hung CR, Chang FY, Pau KY, Wang PS (2003) Pharmacological effects of oxytocin on gastric emptying and intestinal transit of a non-nutritive liquid meal in female rats. Naunyn Schmiedebergs Arch Pharmacol 367:406–413
22. Zhao H, Sprunger LK, Simasko SM (2010) Expression of transient receptor potential channels and two-pore potassium channels in subtypes of vagal afferent neurons in rat. Am J Physiol 298:G212–221