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Exogenous oxytocin reverses the decrease of colonic smooth muscle contraction in antenatal maternal hypoxia mice via ganglia

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ABSTRACT

Oxytocin (OT) has been reported to have a potential protective effect on stress-induced functional gastrointestinal disorders. This study determined whether colonic contraction in adults was affected by antenatal maternal hypoxia, and whether OT is involved in antenatal maternal hypoxia induced colonic contraction disorder. Isometric spontaneous contractions were recorded in colonic longitudinal muscle strips in order to investigate colonic contractions and the effects of exogenous OT on the contraction in antenatal maternal hypoxia and control mice. Both high potassium and carbachol-induced contractions of proximal colon but not distal colon were reduced in antenatal maternal hypoxia mice. Exogenous OT decreased the contractions of proximal colonic smooth muscle strips in control mice, while it increased contractions in antenatal maternal hypoxia mice. OT increased the contractions of distal colonic smooth muscle strips in both antenatal maternal hypoxia and control mice. Hexamethonium blocked the OT-induced potentiation of proximal colon but not distal colon in antenatal maternal hypoxia mice. These results suggest that exogenous oxytocin reverses the decrease of proximal colonic smooth muscle contraction in antenatal maternal hypoxia mice via ganglia.

1. Introduction

The stress of antenatal maternal hypoxia (AMH) can lead to a number of physiological and pathological changes in both mother and fetus. Irritable bowel syndrome (IBS) and constipation are related to functional colonic motility. Childhood events are thought to be a risk factor for IBS in women [1]. It is not known whether antenatal maternal hypoxia stress affects the gastrointestinal system of the fetus so as to affect gastrointestinal function in adults.

There are reports that oxytocin (OT) has a protective effect on stress-induced functional gastrointestinal disorders. For example, OT was reported to ameliorate oxidative colonic inflammation [2]. Central OT attenuates augmented gastric postprandial motility induced by restraint stress in rats [3]. OT treatment alleviates stress-aggravated colitis [4]. OT has inhibitory effect on accelerated colonic motility induced by water-avoidance stress in rats [5]. Combined administration of secretin and OT inhibits chronic colitis in rats [6]. However, it is unknown whether exogenous OT is involved in hypoxia-induced colonic motility disorder.

OT has been reported to effect colonic smooth muscle contraction via OT receptors [7,8]. OT receptor transcripts and protein have been detected in the nodose ganglia of enteric nervous system [9], leading to the hypothesis that OT might act on colonic smooth muscle via ganglia.

The present study was undertaken to investigate (1) whether prenatal exposure to hypoxia causes changes in colonic smooth muscle contraction, (2) to determine the effects of exogenous OT on the contraction of colonic smooth muscle in antenatal maternal hypoxia mice, and to determine the mechanisms involved.

2. Materials and methods

2.1. Animals

The use and treatment of animals followed the International Animal Care and Use Committee of the Tongji University. All animals were cared for in compliance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals, published by the National Science Council, China.

Experiments were performed using pregnant BALB/c mice and their offspring. All mice were given a standard rodent chow and water ad libitum, and kept on a 12:12-h light–dark cycle in room air. Timed matings of adults were carried out by pairing strain-matched males and nulligravida females for one night, with the morning after mating designated as embryonic day 0.5 (E0.5). Pregnancy was assumed based on a 10% weight gain on E10.5. Gravid females were kept under constant conditions until day E13.5, when exposure to hypoxia was initiated. Mice were then allocated to one of two groups:

1) Hypoxia: in which mothers were exposed to intermittent $FiO_2 = 12\%$ [10] between E13.5 and E17.5.

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2) Normoxia: control mothers that were continuously exposed to room air, 21% O_2.

The mice were housed in the chambers of an environment system (CYS-1 digital Hyp Oxyc system, Xinfei Analyzer Instrument Manufacture Inc., Nanjing, China). Three cycles of hypoxia were applied each day between E13.5 and E17. A single cycle of hypoxia lasted for two minutes, with the first minute being the hypoxia exposure phase, and the following minute being the reoxygenation phase. During the hypoxia phase, ambient O_2 concentration in the chamber was rapidly decreased to 12% at nadir by controlling the flush time and flow rate. The nadir O_2 lasted for 20–25 s per cycle. During the reoxygenation phase, the O_2 concentration was restored to 21% by rapid flushing with room air.

Post birth, pups were weaned during postnatal week 4 (postnatal days 25–27). They were group housed with same sex littermates. At 16 weeks of age, age-matched female animals from both the hypoxia and normoxia (control) groups were fasted overnight and sacrificed.

2.2. Tissue culture

Both the proximal colon (1 cm from the ileocecal sphincter) and the distal colon (above the pelvic brim) were taken as an approximately 4 cm segment and put in Krebs solution (composition in mM: NaCl 118.5, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.9, $NaHCO_3$ 25.0 and glucose 10.1). The segment of colon was opened along the mesenteric border and pinned mucosa side up. The mucosa was removed by sharp dissection and four full-thickness muscle strips (2×8 mm) were cut along the longitudinal axis. Silk thread was attached to both ends of the muscle strips, and the strips were mounted in 5 ml organ baths. The organ baths contained aerated (5% CO_2, 95% O_2) Krebs solution maintained at 37 °C. Strips were adjusted in length to an initial tension of 1 g, and were allowed to stabilize for 60 min before the experimental procedures were initiated. Isometric tension was measured using external force transducers (JH-2B, Peijing, China). Force signals were amplified with a SMUP-PC amplifier (Fudan University, Shanghai, China), and recorded on the MFlab system.

2.3. Experimental protocols

2.3.1. Contractions elicited by high potassium Krebs solution

The colonic strips from both the antenatal maternal hypoxia (hypoxia offspring) and gender/age-matched normoxia mice (normoxia offspring/control mice) were stabilized for 60 min in normal Krebs solution. The colonic strips were then exposed to high potassium Krebs solution (composition in mM: NaCl 34.6, KCl 90, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.9, $NaHCO_3$ 25.0 and glucose 10.1) while contractile force was recorded.

2.3.2. Effect of carbachol on colonic motility in mice

The colonic strips were exposed to carbachol (10 μM) for 10 min while contractile force was recorded.

2.3.3. Dose–response effect of oxytocin (OT) on colonic motility in mice

Colonic strips were exposed to OT (1, 3, 10, 30, 100 nM) in an ascending order of concentration. Each strip was exposed to each OT concentration for 3 min. During this period the chamber contained normal Krebs solution. The measurement of contractile force was begun 10 s after application of OT. Force was then measured continuously for 2.5 min, and the mean value of force during that period was calculated by the MF lab system.

2.3.4. Effect of hexamethonium on OT-induced response

To investigate whether OT acts directly on ganglion or not, we examined the effect of hexamethonium (1 μM) on OT-induced responses. The strips were either nontreated or treated with hexamethonium for 20 min. Increasing concentrations of OT (1–100 nM) were then applied, and OT-induced contractions were measured.

2.4. Chemicals

Chemicals used in the present study including oxytocin (oxytocin acetate salt hydrate, minimum 97% HPLC), carbachol (carbamoylcholine chloride, minimum 98% titration), and hexamethonium (hexamethonium bromide), were obtained from Sigma (St. Louis, MO, USA).

2.5. Data analysis

In experiments using high potassium Krebs solution and carbachol, analyses were based on the maximal values of contractions. In the experiments using OT, analyses were based on mean values of force, recorded starting 10 s after OT administration. Data are presented as means ± SEM, with n indicating the number of mice. Statistical analysis was performed by means of Student's paired t-test for comparisons between two groups and repeated-measures comparison on the same specimen with SigmaStat 3.5 software (SPSS Inc., Chicago, IL, USA). A probability level of $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Effect of high potassium Krebs solution on colonic motility in mice

High potassium (90 mM) Krebs solution evoked contractions of proximal and distal colonic strips from both the antenatal maternal hypoxic and normoxia mice (Fig. 1A, B, C). The force of contraction was significantly greater than background force ($P<0.05$, $n=6$). The force of proximal colonic strips observed in hypoxia samples was significantly less than the force in normoxia (control) strips (Fig. 1A, B, C, $P<0.05$).

3.2. Effect of carbachol on colonic motility

Carbachol (10 μM) caused contractions of proximal and distal colonic strips in both the hypoxia and normoxia mice (Fig. 2A, B, C). Contractile force was significantly ($P<0.05$, $n=6$) greater than the background force level. Carbachol-induced contractions of proximal colonic strips in hypoxia mice were smaller than observed in antenatal maternal normoxia mice (Fig. 2A, B, C, $P<0.05$, $n=6$).

3.3. Dose–response effect of oxytocin (OT) on colonic motility in mice

3.3.1. Proximal colonic muscle

In antenatal maternal normoxia mice, low concentrations of OT (1, 3 nM) failed to elicit effect on the strips of proximal colonic smooth muscle strips (Fig. 3A, B, $P>0.05$ compared with the data prior to OT administration, $n=6$). OT (10, 30 nM) decreased the contractions of proximal colonic smooth muscle strips (Fig. 3A, B, $P<0.05$ compared with the data prior to OT administration, $n=6$). But at dose of 100 nM, OT failed to elicit decrease (Fig. 3A, B, $P>0.05$ compared with the data prior to OT administration, $n=6$).

In antenatal maternal hypoxia mice, low concentrations of OT (1, 3 nM) failed to elicit effect on the strips of proximal colonic smooth muscle strips (Fig. 3A, B, $P>0.05$ compared with the data prior to OT administration, $n=6$). OT (10–100 nM) increased the contractions of proximal colonic smooth muscle strips (Fig. 3A, B, $P<0.05$ compared with the data prior to OT administration, $n=6$). From dose of 3 to 100 nM, OT reversed the contractions of proximal colonic smooth muscle strips in antenatal maternal hypoxia mice (Fig. 3A, B, $P<0.05$ compared with the data of antenatal maternal normoxia mice, $n=6$).