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Oxytocin inhibited stress induced visceral hypersensitivity, enteric glial cells activation, and release of proinflammatory cytokines in maternal separated rats

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ABSTRACT

Visceral hypersensitivity (VH) is a significant contributor to irritable bowel syndrome (IBS). Oxytocin (OT) possesses analgesic effects on the central nervous system (CNS) and attenuates microglial activation, however, little is known about its peripheral effects and involvement in VH of IBS. Reactive enteric glial cells (EGCs) contributes to abnormal motility in gastrointestinal (GI) diseases. The aim of this study was to evaluate the peripheral use of OT to maintain VH and activation of EGCs through involvement of the Toll-like receptor (TLR) 4/MyD88/NF-κB signaling. After assessing a baseline visceromotor response (VMR) to colorectal distension (CRD), rats were exposed to a 1 h water avoidance stress (WAS) session. Before each WAS session, intraperitoneal injection of OT (1 mg/kg body weight, in phosphate-buffered saline (PBS)) atosiban (0.5 mg/kg body weight, in PBS) or PBS (as a vehicle control, 1 ml/kg body weight) was administered. Animas are killed 24 h after the last WAS session. EGCs activity, relative OT receptor expression, glial fibrillary acidic protein (GFAP) expression and TLR4/MyD88/NF-κB signaling were evaluated. Neonatal maternal separation (MS) significantly increased the OT receptor expression and enhanced VMR to CRD. WAS improved VMR to CRD only during neonatal MS. OT treatment prevented WAS-induced higher VMRs to CRD, which was reversed by an OT receptor antagonist administration. Compared to the vehicle, OT pre-treated rats reduced EGCs activation, GFAP expression and TLR4/MyD88/NF-κB signaling. We conclude that neonatal MS induces VH and visceral pain in rats. Furthermore, exogenous OT attenuated stress-induced VH and EGCs activation, which was mediated by TLR4/MyD88/NF-κB signaling.

1. Introduction

Irritable bowel syndrome (IBS), characterized by abdominal pain or discomfort associated with defecation or altered bowel habits, is one of the most common functional gastrointestinal disorders (FGID). It affects approximately 11% of the population worldwide and is a significant health care problem (Canavan et al., 2014). Although the exact pathophysiology of IBS has not been elucidated, visceral hypersensitivity (VH) is considered a significant contributor. Enhanced response to colorectal distension (CRD) contributes to the abnormal perception of pain and discomfort, is present in the majority of IBS patients (Bouin et al., 2002). Stress, an important predisposing factor for symptom development and exacerbation (Moloney et al., 2016), can be recapitulated in the neonatal maternal separation (MS) animal model. The separated animals are characterized by visceral hyperalgesia, exaggerated stress response, macromolecular permeability and anxiety-like behaviors, which are all evident in IBS (O'Mahony et al., 2011).

Oxytocin (OT) is widely used as a medication to facilitate childbirth, prevent postpartum hemorrhage and stimulate lactation. It plays a role in central nervous system (CNS) processes, such as learning, feeding, social bonding and memory (Lee et al., 2009). It comes to the fore that OT involves in brain disorders such as stress, anxiety (Koch et al., 2016; Lebowitz et al., 2015; Lee et al., 2015; Minhas et al., 2016), which is closely related to IBS/Inflammatory bowel disease (IBD). Additionally, OT functions as a gastrointestinal hormone, involved in motility modulation, the release of neurotransmitters, enteric neuronal activity, mucosal homeostasis, intestinal permeability, and inflammation (Welch et al., 2014).

Various studies suggest that OT possesses analgesic properties on CNS (Robinson et al., 2002; Russo et al., 2012), and its peripheral effects (Li et al., 2015; Qiu et al., 2014) are under investigation. However, little information is known about peripheral OT/OT receptor (OTR)

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signal involvement in VH of IBS. OTR is a G-protein-coupled receptor, which located on both enteric nervous system(ENS) and gastrointestinal epithelium (Monstein et al., 2004; Welch et al., 2009), has also attracted attention for the role in visceral pain.

Enteric glial cells (EGCs) are located together with enteric neurons on ENS and support the development, survival, and differentiation of enteric neurons. EGC network, connecting with neurons, immune cells, or other cells in the gut microenvironment, has been shown to play important roles in the maintenance of intestinal homeostasis (Yu and Li, 2014). It also modulates intestinal barrier function, mucosal immunity, and enteric neurotransmission via releasing various substances (Gulbransen and Sharkey, 2012). Currently, EGCs are emerging as a new frontier in neurogastroenterology and a potential therapeutic target (Ochoa-Cortes et al., 2016). Emerging evidence demonstrates that Toll-like receptor (TLR) 4 signaling participates in microglial activation and pathology of visceral pain. Microglial cells activation in hypothalamic paraventricular nucleus (PVN) (Zhang et al., 2016) and spinal (Chen et al., 2015) participates in the development of CRD-induced VH through TLR4/MyD88/NF-κB signaling pathway. Whether EGCs also participate in the pathology of visceral pain through TLR4 signaling is not well established.

In current study, we aimed to assess (1) whether stress-induced VH, in the form of neonatal MS stress, was associated with differences in OTR expression and EGCs activation in the colonic tissues. (2) Whether OT was capable of reversing post stress VH during neonatal MS in rats. (3) The efficacy of OT to EGCs activation through the involvement of the TLR4/MyD88/ NF-κB signaling.

2. Materials and methods

2.1. Animals

All experimental procedures involving animals were approved by the Institutional Animal Care and Utilization Committees of Xi'an Jiaotong University (Xi'an, China). Pregnant female Sprague-Dawley rats ($n=10$, primiparous) were purchased from Xi'an Jiaotong University (Xi'an, China), on gestational days 14–16. Animals were caged individually with their litters (40 × 25 × 20 cm Plexiglas boxes with a metal top and sawdust bedding) on a 12:12 H light-dark cycle (light on at 8:00 a.m.), maintained in an air-conditioned room (temperature 21 ± 1 °C, relative humidity 60 ± 10%), and had access to food and water ad libitum.

2.2. Maternal separation

Birth was considered as postnatal day (PND) 1. Entire litters were randomly assigned to either the neonatal MS protocol or nonseparation (NS) protocol. Pups were stressed as described previously (Gosselin et al., 2010). Briefly, the separated pups were removed from the home cage, placed in an individual cage (kept at temperature 30–33 °C) for 180 min (9:00 a.m. − 12:00 a.m.) daily from PND 2 to PND 12. Non-separated pups were maintained in their home cage with the dams. After PND 12, all pups remained with dams until weaned on PND 23. At PND 30, 4–5 pups of the same gender were housed in similar-condition pairs through adolescence and into adulthood (8–10 weeks).

2.3. Measurement of the visceromotor response and data analysis

During distension protocols, rats were placed in Bollmann cages, and then, electrode leads were connected to a custom-made electromyogram (EMG) amplifier. EMG signals were amplified, filtered (3000 Hz), digitized by a Power Lab system (AD Instruments, Colorado Springs, CO, USA), and acquired using computer software (Lab Chart 7, AD Instruments, CO, USA). A latex balloon (Ultra-cover 8F; International Medical Products, Zutphen, The Netherlands) was inserted into the colorectal cavity and connected to an air-filled 2 ml syringe. Data were analyzed as reported previously (Welting et al., 2005). Briefly, after correction for movement and respiration, the area under the curve (AUC) of absolute responses was obtained by subtracting its preceding 20 s of baseline recording from each 20 s distension result. Relative responses were given as normalized data sets, which were calculated from the absolute data by setting the 1.2 ml value of the first (pre-stress) distension at 100%.

2.4. Colorectal distension protocol and water avoidance stress

On PND 56–70, rats were exposed to CRD as reported previously (O'Mahony et al., 2009). After electrodes had been placed in abdominal muscles, rats were fasted overnight prior to the first CRD. After adaptation to the cage, rats were submitted to CRD caused by inflation of a graded volume of water (0.4 ml, 0.8 ml, and 1.2 ml) using a manual syringe. After each 20 s distension period, water was quickly removed and followed by a 2 min resting period. Rats were subjected to 1 h of WAS session during which they were positioned on a pedestal surrounded by water. Experiments (WAS and CRD) were performed in the morning, between 9 a.m. to12 a.m. each day to avoid variations due to the circadian rhythm.

2.5. Drug administration

A total of 36 male rats were used, including 6 non separated rats as control, and 30 MS rats that were randomly divided into 5 groups (n = 6 rats per group), and received the following treatments:

1. none.
2. water avoidance stress.
3. water avoidance stress and vehicle.
4. water avoidance stress and oxytocin.
5. water avoidance stress and oxytocin and atosiban.

After obtaining the baseline VMR, MS rats were exposed to a 1 h WAS session. Before each WAS session, intraperitoneal (IP) injections of OT (1 mg/kg body weight, in PBS), OT + atosiban (0.5 mg/kg body weight, in PBS) or PBS (as a vehicle control, 1 ml/kg body weight) were administered. Animals were killed 24 h after the last WAS session; the distal colon was rapidly dissected and stored at −80 °C.

2.6. Protein extraction and Western blot analysis

For protein extraction, the distal colon was homogenized in RIPA lysis buffer containing protease and phosphatase inhibitors (Solarbio, Beijing, China). Lysates were incubated on ice for 30 min and centrifuged at 10,000*g* for 10 min at 4 °C. The supernatant fluid was collected and used as whole tissue lysate for protein assays. Samples were stored at −80 °C until further use. The protein concentration was determined by BCA assay (Solarbio, Beijing, China). An equal amount of protein (10 μg) from whole tissue lysates were separated by 10% SDS-PAGE and transferred to PVDF transfer membranes (Bio-Rad, Hercules, CA, USA), which were blocked at room temperature for 1 h in 5% BSA in TBST. Membranes were incubated overnight at 4 °C with primary antibodies including goat anti-OTR (Abcam, Hong Kong, 1:2000 dilution), rabbit anti-TLR4 (Proteintech, Chicago, IL, USA, 1:1000 dilution), rabbit anti-MyD88, mouse anti- NF-κB p65, rabbit anti- IκBα and mouse anti-GAPDH (all from Santa Cruz, CA, 1:1000 dilution) overnight at 4 °C. After washing, membranes were incubated with the HRP-conjugated secondary antibody (rabbit anti-mouse/goat, mouse anti-rabbit, Proteintech, Chicago, IL, USA, 1:8000 dilution) for 1 h at room temperature. Protein bands were visualized using with a chemiluminescence substrate kit (Millipore, Amsterdam, The Netherlands). Images were captured using imaging software (Bio-Rad, Hercules, CA, USA). Quantification of bands was performed using Gel-Pro analyzer 4.0 software and results of target protein were normalized to GAPDH.