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Oxytocin improves animal behaviors and ameliorates oxidative stress and inflammation in autistic mice

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ABSTRACT

Objective: Autism is a neurodevelopmental disorder which significantly impacts the quality of people's life. Oxytocin is a hormone impacting the social cognition and interpersonal trust. In this study, we aimed to explore the role of oxytocin in autism.

Methods: Autistic mice models were established by valproate. Animal behaviors were assessed by open field test, tail suspension test, marble burying test and three-chamber social interaction test. Oxidative stress was evaluated by the levels or activities of malondialdehyde, superoxide dismutase, glutathion peroxidase, reduced glutathione and reactive oxygen species. Inflammation was assessed by the levels of tumor necrosis factor-α, interleukin-1β and interleukin-6. The number of activated microglia was detected by immunofluorescence with an Iba-1 antibody.

Results: Our results showed that oxytocin improved the behaviors of autistic mice, with less anxiety, depression and repetitive behavior, and ameliorated social interaction. Further study showed that the elevated oxidative stress and inflammation in autistic mice were alleviated after treatment of oxytocin.

Conclusion: Our study demonstrates that oxytocin treatment ameliorates autism in a mouse model, maybe through its modulation on oxidative stress and inflammation. It is indicated that oxytocin may beneficial to autism.

1. Introduction

Autism is a kind of neurodevelopmental disorder, which is characterized by impaired social communication and social interaction, and restricted and repetitive behaviors [1]. These symptoms become apparent before 3 years old. Autism is a life-long burden with a prevalence of approximately 1%, and 2–3 times in males than females [2]. In recent years, the prevalence of autism is still increasing. There are numerous risk factors for autism, such as inherited factors [3], environmental factors [4] and epigenetic factors [5], impacting the development of nervous system, thus contributing to autism.

Oxytocin, a hormone produced by hypothalamus and released into circulation, has a variety of functions implicated in uterine contraction, maternal behaviors, and neuromodulation [6–8]. Oxytocin receptor, which is found in autonomic nervous system, mediates the roles of oxytocin [9]. By acitivating oxytocin receptor, oxytocin elevates intracellular calcium concentration in hypothalamus, resulting in the further release of oxytocin [10]. Through interaction with its receptor, oxytocin also cooperates with the contraction of uterine smooth muscles, resulting in a more powerful labor [11]. Oxytocin also has effects on the central nervous system. It is reported that oxytocin signaling enhances fear through its interaction with oxytocin receptor [12,13], and confers a neuroprotective effect through modulating γ-aminobutyric acid signaling [14].

Oxytocin also shows anti-inflammatory and anti-oxidant properties [15,16], and performs an alleviated role in various kinds of damages [17,18]. Oxytocin also excerts significant impacts on social cognition [19] and interpersonal trust [20,21]. A meta-analysis shows that variation in oxytocin receptor is associated with autism [22]. Autistic patients show lower oxytocin levels in plasma [23,24], and there is somewhat correlation between oxytocin levels and autism severity [25,26]. However, the effects of oxytocin on autism remain unclear.

In recent years, due to its high prevalence and poor therapeutic outcomes, autism gets more and more attention in the world. It seems

Abbreviations: DAPI, 4,'6-diamidino-2-phenylindole; GSH, reduced glutathione; GSH-Px, glutathion peroxidase; IL, interleukin; LPS, lipopolysaccharide; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF, tumor necrosis factor; VPA, valproate

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that oxytocin restores the social behaviors in autistic mice models [27], thus more and more researchers pay attention to oxytocin as a promising candidate therapeutic for autism. In the present study, the effects of oxytocin on autism were explored.

2. Materials and methods

2.1. Animal experimental protocol

Experiments in this study were carried out according to the Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of China Medical University (KT2018058). C57Bl/6J mice were obtained from Huafukang Bioscience (Beijing, China) and fed in a standard environment (22 ± 2 °C, 50%–60% humidity, 12 h/12 h-light/dark cycles). Then the mice (female: male = 2:1) were put into cages overnight. The next morning, mouse with a vaginal plug was identified as a pregnant mouse, and this day was defined as embryoic day 0.5 (E0.5). The pregnant mice were randomly divided into 2 groups: the control group and valproate (VPA) group. At E12.5, pregnant mice in the VPA group received VPA (Aladdin, Shanghai, China; 600 mg/kg; intraperitoneal injection) to induce autism of offsprings. Pregnant mice in the control group received equal amounts of normal saline. Male offsprings were used for subsequent experiments. After identification of autism, the offsprings were divided into 3 groups: the control group, autism group and autism + oxytocin group. At postnatal day 30 (P30), offsprings in autism + oxytocin group received oxytocin (GL Biochem, Shanghai, China; 200 μg/kg; intranasal administration). 30 min later, animal behavior tests were performed.

2.2. Open field test

The open field test was performed to assess the anxiety-like behaviors in a novel environment using an open field apparatus with a transparent box (30 cm × 30 cm × 21 cm). Mice were put in the corner of arena and allowed to explore freely for 10 min. The total travel distances were recorded.

2.3. Tail suspension test

Mice were hung upside down in the box. The time of immobility in 5 min was recorded.

2.4. Marble burying test

Mice were put in the cages for 10 min to pre-adapt. Animal cages were filled with clean bedding (3 cm). 20 marbles (1.5 cm) were put onto the surface of bedding (4 × 5 arrangement). Mice were put back to the cages in order to explore for 20 min. Then the mice were removed and the number of marbles buried in the bedding (> 50%) was recorded.

2.5. Three-chamber social interaction test

Three-chamber social interaction test was conducted for 30 min. Mice were put into the center area of the box (42.5 cm × 19.1 cm × 22.2 cm) for 10 min to pre-adapt. Then a novel mouse was introduced into the right chamber (stranger zone 1), and the subject mouse was allowed to explore the three chambers for 10 min. The behaviors of the subject mouse were recorded. Thereafter, social preference test was carried out. The subject mouse was put back to the center area. Another novel mouse was introduced into the left chamber (stranger zone 2). Then the subject mouse was allowed to explore for 10 min, and the behaviors of the subject mouse were recorded. The social index and social preference index were calculated as follows: social index = time in strange zone 1/ time in empty area; social preference index = time in strange zone 2/ time in strange zone 1.

2.6. Detection of malondialdehyde (MDA), superoxide dismutase (SOD), glutathion peroxidase (GSH-Px), reduced glutathione (GSH) and reactive oxygen species (ROS)

Hippocampus and amygdale tissues of mice in each group were collected and made into homogenates. After measuring protein concentration using a BCA protein assay kit (Beyotime, Shanghai, China), the activity of GSH-Px and SOD were detected with a GSH-Px detection kit (JianchengBio, Nanjing, China) and a total SOD detection kit (JianchengBio), respectively. The contents of GSH, MDA were measured with a GSH detection kit (JianchengBio) and a MDA detection kit (JianchengBio), respectively. The level of ROS was detected with a ROS detection kit (Beyotime).

2.7. Quantitative real-time PCR

Total RNA in hippocampus and amygdale tissues of mice in each group was extracted using TRIpure (BioTeke, Beijing, China) according to the manufacturer's instruction. Then the total RNA was reversely transcribed to cDNA using MMLV- reverse transcriptase (BioTeke). The levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 in hippocampus and amygdale tissues were measured using quantitative real-time PCR (SYBR Green method). The SYBR Green reagent was obtained from Solarbio (Beijing, China). The primers used were listed in Table 1. Relative mRNA levels were normalized to β-actin and calculated using $2^{-\Delta\Delta Ct}$ method.

2.8. Immunofluorescence

Hippocampus and amygdale tissues of mice in each group were obtained, fixed in 10% neutral formalin, dehydrated in a graded series of ethanol, embedded in paraffin and cut into 5 μm-slices. After dewaxing in xylene and rehydrating in a graded series of ethanol, the slices were maintained in antigen retrieval buffer for 10 min. The slices were then rinsed with PBS and blocked with normal goat serum. Thereafter, the slices were incubated with primary antibody against Iba-1 (1: 100, Abcam, Cambridge, UK) at 4 °C overnight. The slices were then rinsed with PBS and incubated with Cy3-labelled secondary antibody (1:200, Beyotime) in the dark at room temperature for 60 min. The slices were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (Biosharp, Hefei, China) and observed with a fluorescence microscope (OLYMPUS, Tokyo, Japan). Images were captured (400× magnification) and the number of Iba-1-positive cells was calculated.

Table 1
Primers for quantitative real-time PCR.

Gene name	Forward primer	Reverse primer
TNF-α	5'-AGAAAGCATGATCCGCGAC-3'	5'-TTGTGAGTGTGAGGGTCTGG-3'
Il-1β	5'-TTGGGCCTCAAAGGAAAGAAT-3'	5'-TGCTTGTGAGGTGCTGATGTA-3'
IL-6	5'-AATGATGGATGCTACCAAACTG-3'	5'-AGGACTCTGGCTTTGTCTTTC-3'
β-actin	5'-CTGTGCCCATCTACGAGGGCTAT-3'	5'-TTTGATGTCACGCACGATTTCC-3'