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Tropisetron attenuated the anxiogenic effects of social isolation by modulating nitrergic system and mitochondrial function☆

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ABSTRACT

Background: Early social isolation stress (SIS) is associated with the occurrence of anxiety behaviors. It seems interaction between the nitrergic system and mitochondrial function plays a role in mediating the anxiety-like behaviors. In this study, we aimed to investigate the anxiolytic effects of tropisetron in animal model of SIS and we try to illustrate the possible role of nitrergic system and mitochondrial function.
Methods: We applied early social isolation paradigm to male NMRI mice. Animals treated with various doses of tropisetron, nitric oxide agents or their combination and anxiety-like behaviors of animals were assessed using valid behavioral tests including elevated plus maze (EPM), open-field test (OFT) and hole-board test (HBT) in their adulthood. Effects of housing conditions and drug treatments on the mitochondrial function were investigated in the hippocampus by assessing the ATP, GSH, ROS and nitrite levels.
Results: Anxiogenic effects of early SIS were assessed in the EPM, OFT, and HBT. Also, SIS disrupted mitochondrial function and caused oxidative stress in the hippocampus of stressed animals. Tropisetron showed an anxiolytic effect in the stressed mice. Also, these effects were mediated by nitrergic system by affecting mitochondrial function and modulating the oxidative stress. L-arginine, a nitric oxide precursor, abolished the anxiolytic effects of tropisetron in the behavioral tasks and blocked the protective effects of it against mitochondrial and oxidative challenge.
Conclusions and general significance: Our results demonstrated tropisetron attenuated the anxiogenic effects of SIS by mitigation of the negative effects of nitric oxide on mitochondrial function.

1. Introduction

Anxiety and stress disorders are common mental illnesses with high prevalence and comorbidity [1,2]. Experiencing aversive events in early stages of life negatively affects the behavior and brain development and also, is regarded as a putative risk factor for vulnerability to psychiatric disorders such as affective disorders [3,4]. A substantial body of evidence indicates that applying social isolation stress (SIS) to rodents induces a variety of long-lasting behavioral disturbances relevant to stress such as anxiety-like behaviors [5,6]. In this regard, underlying mechanisms through which SIS induces anxiety-like behaviors are not clearly understood. Increasing lines of evidence indicate that mitochondrial dysfunction [7], oxidative and nitrosative stress (O&NS) also contribute to pathogenesis of anxiety-like disorders [8–10]. Evidence indicates that SIS-induced O&NS contributes to behavioral and neurochemical alterations in rodents [11,12]. Under stressful conditions, mitochondria generate excessive amounts of reactive oxygen species (ROS), which correlates with glutathione (GSH) and ATP depletion, and consequently oxidative damage [13–15]. Additionally, it has been reported that anxiolytic drugs decrease the O&NS in stressed animals [9]. Moreover, overproduction of nitric oxide (NO) in the stressful conditions has been reported to induce anxiety-like behaviors [16] that administration of aminoguanidine (specific inhibitor of inducible

Abbreviations: SIS, social isolation stress; O&NS, oxidative and nitrosative stress; AG, aminoguanidine; HBT, hole-board test; OFT, open-field test; EPM, elevated plus maze

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nitric oxide synthase or iNOS) or L-NAME (non-specific inhibitor of NOS) reversed the anxiety-like responses [17,18].Also, NO-induced O&NS causes mitochondrial dysfunction and cell injury [19].

On the other hand, a number of studies have reported that impairment in neurotransmitter systems (mostly serotonergic system) plays a role in development of aggression, anxiety and fear in socially isolated rodents [20–22]. Evidence suggests that 5-hydroxytryptamine3 (5-HT3) receptors, as ligand gated ion channels, are involved in development and maturation of the brain mostly formation of the inhibitory networks. Also, 5-HT3 receptors have been reported to contribute to pathophysioliogy of anxiety and mood disorders that mice lacking these receptors exhibit reduced anxiety-like behaviors [23]. In this regard, several lines of research have demonstrated that tropisetron, a 5-HT3 antagonist, exhibits anxiolytic effects in both clinical and preclinical studies [24,25]. According to our recent studies, we found that tropisetron possesses protective properties against O&NS in pathologic conditions. In this context, we showed that tropisetron is able to attenuate O&NS as well as inflammatory responses in animal models of Alzheimer's disease and stroke [26,27]. In addition, recent studies have reported the antidepressant-like properties of 5-HT3 antagonists (including tropisetron) in both non-stressed and stressed animals [28–30]. Considering that mitochondrial performance and O&NS were reported as underlying mechanisms involved in pathogenesis of anxiety disorders, we tested the hypothesis that whether tropisetron is able to decrease anxiogenic effects of early SIS via regulating the mitochondrial performance. In this study, we applied early SIS paradigm because it has been suggested as a reliable and valid animal model to investigate the negative impacts of social environment (such as chronic stress) on neurobehavioral and neurochemical changes which similarly were observed in psychiatric disorders in humans [31,32].

2. Materials and methods

2.1. Animals

Male NMRI mice (Pasteur Institute, Tehran, Iran), weighing 10–12 g and in the postnatal day (PND: 21–23) were housed for 4 weeks under two different conditions: 1) social condition (SC) and 2) isolated condition (IC). Socially conditioned animals were housed in groups (6 mice per cage: 25 × 25 × 15 cm) while IC mice were housed individually in Plexiglas boxes (24 × 17 × 12 cm) under standard laboratory conditions (free access to food and water, temperature: 22 ± 2 °C, and 12-h light–dark cycle). All procedures in this work were carried out in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication #80-23) and institutional guidelines for animal care and use (Department of Pharmacology, School of Medicine, TUMS). Each experimental animal group consists of 6–9 in behavioral assessments, and 3–6 in molecular evaluations.

2.2. Drugs

The following drugs were used in this study: L-arginine (L-arg, a NO precursor), NG-nitro-L-arginine methyl ester (L-NAME, a non-selective NOS inhibitor), aminoguanidine (AG, a selective iNOS inhibitor) (Sigma, UK) and tropisetron (a selective 5-HT3 antagonist) (Sigma, St. Louis, MO, USA). All drugs were dissolved in saline and were administered intraperitoneally (i.p.) in the volume of 5-ml/kg animal weights. Doses of drugs were chosen according to the previous studies as well as our pilot studies (described below), we treated mice with L-arg (30 min), AG and L-NAME (45 min) and tropisetron (60 min) prior to behavioral or molecular experiments [17,33].

2.3. Experimental design

After 4 weeks of housing under isolation or social conditions, animals (PND: 52) were subjected to behavioral tests. In the first part of the experiment, effects of housing conditions on the anxiety-like behaviors were investigated using behavioral tests which are considered as valid methods for assessing anxiety in rodents including open field test (OFT), hole board test (HBT), and elevated plus maze test (EPM) [34–36].

In the next step, we investigated the possible effect of tropisetron as well as NO agents in mediating the anxiety-like behaviors of animals. In this regard, different sets of SC and IC mice were treated with different doses of following drugs: tropisetron (1, 3, and 5 mg/kg), L-NAME (5, 10 mg/kg), AG (20, 50 mg/kg), and L-arg (50, 100 mg/kg). Doses of each drug were chosen according to the pilot treatments, which were published in previous studies [31,33,37]. After administration of drugs, SC animals and IC animals were subjected to mentioned behavioral tasks. To exclude the possible effect of saline administration on animal behavior, SC and IC groups were injected with 5-ml/kg physiological saline. In order to investigate the role of nitrergic system in mediating the anxiolytic effect of tropisetron, subeffective doses of L-NAME and AG co-administered with subeffective dose of tropisetron to both SC and IC mice. Also, we co-administered subeffective dose of L-arg with effective dose of tropisetron. In the next step, we investigated the effects of different housing conditions and drug treatments on the hippocampal levels of glutathione (GSH), nitrite, and ATP and ROS production in the different sets of animals.

2.4. Behavioral tests

2.4.1. Open-field test (OFT)

The OFT was used to evaluate the anxiety-like behaviors of mice in response to various treatments and housing conditions [38]. The OFT apparatus was made of white opaque Plexiglas (50 cm × 50 cm × 30 cm) which was dimly illuminated. Mice were placed individually on the central zone of OFT box (30 cm × 30 cm) and spent time in the central area was recorded by a camera for a 5 min period. The apparatus was cleaned with 70% ethanol after testing each mouse.

2.4.2. Elevated plus maze (EPM)

The elevated plus maze (EPM) is an appropriate test to assess the effects of both anxiogenic and anxiolytic agents in rodents [36]. The apparatus was made of black opaque Plexiglas and consisted of two open (30 × 5 cm) and closed (30 × 5 × 15 cm) arms, which were connected by a platform area (5 × 5 cm). Testing room was dimly illuminated and animals were individually placed in the center of the EPM facing to closed arm and each behavioral session was videotaped for a 5 min period. The apparatus was cleaned with 70% ethanol after testing each mouse. The total time spent in the open arms, and number of entries into the open arms were recorded over a period of 5 min and reported as percentages.

2.4.3. Hole-board test (HBT)

Hole-board test is a reliable test to determine the anxiogenic/anxiolytic state in mice [38]. The apparatus was made of a white Plexiglas square (50 cm × 50 cm) with 16 equally sized holes (3 cm in diameter) and was positioned 50 cm above the floor. Mice were placed in the center of the board, and the number of head-dips was counted in a 5 min period. Decrease in number of head-dips was considered as anxiety-like behavior in animals. The apparatus was cleaned with 70% ethanol after testing each mouse.

2.5. Molecular assessments

2.5.1. Glutathione (GSH) measurement

Animals were decapitated under mild anesthesia, and then the hippocampi were dissected on ice-cold surface and immediately immersed in liquid nitrogen. Samples were centrifuged at 3000 g for 10 min at 4 °C, and the supernatant were collected. Glutathione levels were determined using 5, 50-dithiobis-(2-nitrobenzoic acid) or DTNB as the