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Enhancement of dopaminergic activity and region-specific activation of Nrf2-ARE pathway by intranasal supplements of testosterone propionate in aged male rats

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ABSTRACT

The potential influence of intranasal testosterone propionate (InTP) supplements on mesodopaminergic system in aged male rats was investigated by analyzing the exploratory and motor behaviors as well as dopamine neurobiochemical indices. Meanwhile, oxidative stress parameters and pathway of nuclear factor erythroid 2-related factor 2 (Nrf2)-binding antioxidant response elements (Nrf2-ARE) were examined to check whether the Nrf2-ARE pathway was involved in the InTP-induced alteration of mesodopaminergic system in aged male rats. The exploratory and motor behavioral deficits, as well as the reduced expression of dopamine, tyrosine hydroxylase, and dopamine transporter, which indicated the declined activity of mesodopaminergic system, were ameliorated in rats administered with 12-week InTP. The results indicated that chronic InTP supplements could effectively influence the brain function activity in a way opposite to the effect of aging on the mesodopaminergic system of rats. The increased levels of Nrf2, heme oxygenase-1, and NAD(P)H:quinone oxidoreductase-1 in the substantia nigra and ventral tegmental area, but not in the hippocampus of InTP-administered aged male rats, indicated that the ameliorative effect of InTP supplements on mesodopaminergic system might be related to the region-specific activation of the Nrf2-ARE pathway.

Introduction

In normal aging, mesodopaminergic system undergoes a progressive decline (Cabello et al., 2002; Gerhardt et al., 2002; Haycock et al., 2003; Marshall and Rosenstein, 1990), such as a decrease in the number of mesodopaminergic neurons (Cabello et al., 2002; Gerhardt et al., 2002) and dopamine (DA) content (Marshall and Rosenstein, 1990; Haycock et al., 2003), which leads to aging-related behavioral disturbances. Thus, amelioration of declined dopaminergic systems has been suggested as therapeutic strategy for the behavioral deficits.

Oxidative stress is one of the mechanisms implicated in the progression of dopaminergic degeneration (Chen et al., 2008; Dias et al., 2013; Hwang, 2013). Aging is usually associated with a progressive disruption of redox balance, leading to recurrent damage resulting from oxidative stress (Bokov et al., 2004; Kregel and Zhang, 2007). Manipulation of brain oxidative stress constitutes an effective neuroprotective strategy against the aging-related risk of depressed dopaminergic function (Villar-Cheda et al., 2014). An alternative approach for limiting oxidative stress is the activation of nuclear factor erythroid 2-related factor 2 (Nrf2) (Li et al., 2013; van Muiswinkel and Kuiperij, 2005). Nrf2 activation induces a series of antioxidant genes through the antioxidant response element (ARE), such as heme oxygenase-1 (HO-1) and NAD(P)H:quinone oxidoreductase-1 (NQO1), to protect cells against oxidative stress (Li et al., 2013). The activation of the Nrf2-ARE pathway might be an effective way to prevent the dopaminergic neuronal death (Williamson et al., 2012).

Testosterone induces neuroprotection from oxidative stress (Ahlbom et al., 1999; Meydan et al., 2010). Androgen treatment of neonatal rats decreased the susceptibility of neurons to oxidative stress (Ahlbom et al., 1999). Administration of testosterone propionate (TP) suppressed orchiectomy-induced oxidative damage and neuronal morphological changes in adult rats (Meydan et al., 2010). With aging, male rats experience a decrease in the circulating level of testosterone (Basaria, 2013; Ghanadian et al., 1975) and reduction of self-defense ability (Suh et al., 2004). Administration of testosterone to aged male rats might attenuate aging-induced oxidative damage and increase the antioxidant response.

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Testosterone is typically administered *via* oral medication, injectable testosterone esters, surgically implanted testosterone pellets, transdermal patches, and gels. However, these types of testosterone administration were shown to be problematic (Jordan, 1997; Nieschlag et al., 2004) and seem not suitable for chronic testosterone supplements in old male rats. Currently, intranasal testosterone delivery offers a potentially effective option for targeting the central nervous system (Banks et al., 2009; de Souza Silva et al., 2009; Zhang et al., 2011). Intranasally administered testosterone reversed the open-field behavioral deficits in adult gonadectomized rats (Zhang et al., 2011) and quickly activated the mesodopaminergic neurons (de Souza Silva et al., 2009).

To date, the fact that intranasal testosterone supplements could ameliorate the declined mesodopaminergic systems and the DA-related behavioral deficits of aged male rats is not well known. Therefore, on the basis of the influence of androgenic steroids on the dopaminergic system (de Souza Silva et al., 2009; Mitchell and Stewart, 1989; Thiblin et al., 1999), the lower testosterone levels in aged male rats (Basaria, 2013; Ghanadian et al., 1975), and intranasal testosterone delivery to efficiently target the brain (Banks et al., 2009; de Souza Silva et al., 2009; Zhang et al., 2011), leading to a quick DA increase in adult male rats (de Souza Silva et al., 2009), the potential influence of intranasal TP supplements on the mesodopaminergic system of aged male rats was investigated in this study. DA-related behaviors, neurochemical indices, and the expression of tyrosine hydroxylase (TH) and dopamine transporter (DAT) (the marker of mesodopaminergic neurons) were analyzed in aged male rats. Meanwhile, in order to evaluate whether the Nrf2-ARE pathway was involved in the InTP-induced alteration of dopaminergic system in aged male rats, oxidative stress parameters and the Nrf2-ARE pathway were examined after chronic InTP supplements. Subcutaneous injection was also included in this study for comparison.

Materials and methods

Animals and housing

A total of 90 male Wistar rats supplied by the Experimental Animal Center of Hebei Medical University were housed in an air-conditioned room (22 ± 2 °C) on a 12-h light–dark cycle (lights on 06:00 h). Food and water were available *ad libitum*. All experimental procedures were conducted in accordance with the rules in the "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research" and were approved by the Committee of Ethics on Animal Experiments at Hebei Medical University.

TP supplements

TP was administered to rats either *via* nostrils or by subcutaneous injection. For intranasal experiment, 45 rats were randomly assigned to one of the following three groups: 6-month-old rats (6Mon-in, $n = 15$), 24-month-old rats (24Mon-in, $n = 15$), and 24-month-old rats that were intranasally supplemented with TP (24Mon-in.TP, $n = 15$). For subcutaneous injection experiment, 45 rats were randomly divided into three groups consisting of 6-month-old rats (6Mon-sc, $n = 15$), 24-month-old rats (24Mon-sc, $n = 15$), and 24-month-old rats that received subcutaneous TP injection (24Mon-sc.TP, $n = 15$). TP (SERVA Electrophoresis, Cat. No.: 35,805, Germany; 2 mg/kg per day at 5:00–6:00 PM) was intranasally administered or subcutaneously injected to 21-month-old rats. Sesame oil (Sigma, MKBH4400V, S3547-250ML, USA) as a vehicle was intranasally delivered or subcutaneously injected to rats beginning at the age of 3 months or at the age of 21 months. The supplement of TP or vehicle was continued for 12 weeks (84 days) (Cui et al., 2012; Mostafa et al., 2012). All the rats were weighed every week.

Open-field test

A total of 10 rats in each group were used for open-field test. For the TP-treated rats, open-field test was performed after 12 h on the 83rd day and 84th day of TP treatment. This test followed the procedure used in our previous study (Cui et al., 2012; Zhang et al., 2011). Open-field behavior was recorded for 5 min and analyzed post hoc. The results presented the averaged amount of 2-day exploratory behavior and motor behavior parameters for each rat.

Sample preparation

Following open-field test, the 10 rats selected from each group were sacrificed by decapitation. The brains were removed quickly. The block containing substantia nigra (SN) or ventral tegmental area (VTA) of ventral midbrain (between 2.96 and 3.70 mm), hippocampus (Hip) (between 5.40 and 6.20 mm), as well as caudate putamen (CPu) or accumbens nucleus (Acb) (between 9.70 and 10.60 mm) rostral to the interaural axis (Paxinos and Watson, 1998), was dissected on an ice-cold plate using a scalpel for ophthalmic surgery and stereomicroscope. The tissue blocks from five rats in each group were processed for western blot analysis or liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) assay, and those from the other five rats were chosen for malondialdehyde (MDA) and reduced glutathione (GSH) assay immediately by spectrophotometry or prepared for real-time fluorescence quantitative polymerase chain reaction (PCR) analysis based on the study purposes.

MDA and GSH assay

The SN, VTA, or Hip block was homogenized separately with 10 times (*w*/*v*) ice-cold 0.1 M phosphate buffer (PB) at pH 7.4. The homogenates were used to assess reduced GSH and lipid peroxidation product. GSH and MDA were measured spectrophotometrically using detection kits, following the manufacturer's instruction (GSH: 016,835; MDA: 022,446, Nanjing Jiancheng Bioengineering Institute, China).

Quantitative real-time PCR analysis

Total RNA (2 μg) from the SN, VTA, or Hip block was subjected to reverse transcription using random primer to obtain the first-strand cDNA template. Real-time fluorescence quantitative PCR was performed with 0.8-μl cDNA (diluted 1:10), 2-μl specific primers, and 2X GoTaq® Green Master Mix (Promega, USA) with a final volume of 20 μl. PCR was performed as follows: an initial cycle at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s, 58 °C for 20 s, and 72 °C for 27 s. The products were then analyzed by melting curve to confirm the specificity of amplification. Expression of TH, DAT, Nrf2, HO-1, and NQO1 genes was analyzed using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control. The sets of primers were as follows: TH (5′-GCTTCTCT GACCAGGTGTATCG-3′ and 5′-GCAATCTCTTCCGCTGTGTAT-3′), DAT (5′-ACTCTGTGAGGCATCTGTGTG-3′ and 5′-TGTAACTGGAGAAGGCAA TCAG-3′), Nrf2 (5′-GACCTAAAGCACAGCCAACACAT-3′ and 5′-CTCAAT CGGCTTGAATGTTTGTC-3′), HO-1 (5′-TGTCCCAGGATTTGTCCGAG-3′ and 5′-ACTGGGTTCTGCTTGTTTCGCT-3), NQO1 (5′-GGGGACATGAAC GTCATTCTCT-3′ and 5′-AGTGGTGACTCCTCCCAGACAG-3′), GAPDH (5′-TGAACGGGAAGCTCACTG-3′ and 5′-GCTTCACCACCTTCTTGATG-3′).

Western blot analysis

The tissue blocks for detection of TH and DAT protein levels were homogenized in radioimmunoprecipitation assay (RIPA) buffer containing 1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate, and protease inhibitors (100-μg/ml phenylmethanesulfonyl fluoride, 30-μg/ml aprotinin, and 1-mM sodium orthovanadate), and then sonicated for 4 × 10 s. After centrifugation at 12,000×*g* for 20 min at 4 °C, the