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Amelioratory effects of testosterone propionate supplement on behavioral, biochemical and morphological parameters in aged rats

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ABSTRACT

Testosterone has been shown to affect motor behavior and nigrostriatal dopaminergic (NSDA) system in young and adult male rats. However, it is not known whether exogenous testosterone intervention to aged male rats can ameliorate age-related motor impairment. Thus, in the present study, the open field motor behavior and adhesive tape removal motor performance as well as the expression of tyrosine hydroxylase (TH) and dopamine transporter (DAT) of NSDA system were examined in aged male rats following chronic subcutaneous injections of testosterone propionate (TP). Aged rats showed significantly reduced open field motor behavior and adhesive tape removal motor performance compared to adult rats. Chronic TP supplement significantly ameliorated the age-related motor deficits. The expression of TH and DAT of NSDA system was significantly enhanced in TP-treated aged rats revealed by RT-PCR, Western blot and immunohistochemistry analysis respectively. The results imply that chronic TP treatment may favorably improve the declined motor behavior and motor performance with aging. Testosterone propionate supplement that facilitated NSDA system may influence the maintenance of motor behavior and performance in aged rats.

1. Introduction

The motor behavior and the motor performance involving balance, coordination, and strength decline significantly in aged rats, which is the hallmark of aging (Emerich et al., 1993; Jänicke et al., 1983; Sanchez et al., 2008). The nigrostriatal dopaminergic (NSDA) system, which is involved in motor control, also accordingly undergoes a progressive decline during normal aging (Kish et al., 1992; Meng et al., 1999), such as a decrease in tyrosine hydroxylase (TH) and its mRNA (De La Cruz et al., 1996; Emborg et al., 1998; McCormack et al., 2004; Sanchez et al., 2008; Tümer et al., 1997), as well as in dopamine transporter (DAT) and its mRNA (Cruz-Muros et al., 2009), which implicates the impairment of dopaminergic system in aged animals. The impaired NSDA system (Cruz-Muros et al., 2009; McCormack et al., 2004; Salvatore et al., 2003; Sanchez et al., 2008) may contribute to the significant decline in motor behavior and motor performance in aged animals (Emborg et al., 1998; Emerich et al., 1993; Irwin et al., 1994; Jänicke et al., 1983; McCormack et al., 2004).

Androgenic steroids exert pleiotropic effects in the nervous system (Patchev et al., 2004). The influence of anabolic androgenic steroids (AAS) on central dopaminergic activity has been reported in animal studies (Vermes et al., 1979). Chronic administration with nandrolone decanoate to male rats has been shown to increase DAT density in the striatum visualized in vivo by positron emission tomography and in vitro by autoradiography (Kindlundh et al., 2002, 2004). Relatively high doses of AAS increase dopaminergic metabolism in male rat brain (Thiblin et al., 1999). Castration reduces TH activity in the striatum of male rats (Abreu et al., 1988). The administration of testosterone to castrated rats completely prevents the castration-induced reduction of striatal TH activity (Abreu et al., 1988).

A number of studies have shown that AAS can influence behaviors of organisms (Frye and Seliga, 2001; Lambadjieva, 1999; Perry et al., 2003). Subcutaneous administration of testosterone increases anti-anxiety behavior and enhances cognitive performance in aged intact male mice (Frye et al., 2008). Androgen-treated rats have shown significantly more exploratory behavior in the open field (Edinger and Frye, 2005). In our latest study, intranasal administration of testosterone increases immobile-sniffing, exploratory behavior, motor behavior and grooming behavior in rats (Zhang et al., 2011). Supplement of testosterone restores the open field activity (Adler et al., 1999) and the copulatory behaviors in castrated male rats (Putnam et al., 2001, 2003). Testosterone restoration of copulatory behavior correlates with medial preoptic dopamine release (Putnam et al., 2001). Long term testosterone replacement may favorably alter the decline in

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the process of sexual activity with aging and the restoration by testosterone replacement of dopaminergic activity in the medial preoptic area may be involved in the maintenance of sexual function in aged rats (Sato et al., 1998).

To date, it is not known whether exogenous testosterone intervention to aged male rats ameliorates the impaired motor behavior and motor performance and whether NSDA system in aged rats still remains responsive to the intervention. Alternative interventions that act on NSDA system have been reported to affect locomotor activity in aged rats. For example, injections with glial cell line-derived neurotrophic factor can increase locomotor activity of aged rats through enhancing TH activity in the substantia nigra (SN) and striatum of aged rats (Lapchak et al., 1997). Based on the effects of AAS on dopaminergic neurons and the fact that aged rats have the lower level of testosterone (Ghanadian et al., 1975; Sato et al., 1998), we presume that chronic androgen supplement might ameliorate the declined motor behavior and motor performance (Emborg et al., 1998; Emerich et al., 1993; Irwin et al., 1994; Jänicke et al., 1983; McCormack et al., 2004) of aged rats by altering NSDA system. Therefore, in the present study, the motor-related behaviors of aged rats after testosterone propionate (TP) treatment were observed by open field test as well as by adhesive tape removal test and the effect of chronic administration of TP on aged rats was investigated by analyzing the altered expression of TH and DAT in NSDA system.

2. Materials and methods

2.1. Animals and testosterone propionate supplement

Thirty five male Wistar rats were supplied by the Experimental Animal Center of Hebei Medical University. Thirty of them were divided into three groups consisting of rats of 6-month-old group (6Mon), those with TP supplement group (24Mon-TP) and aged vehicle control group (24Mon). Each group included ten rats. For 24Mon-TP group, the rats received subcutaneous TP injection (2 mg/kg per day at 5:00 PM to 6:00 PM) at the age of 21 months. The supplement of TP was continued for 12 weeks (84-day). The rats both in 6Mon group and in 24Mon group were subjected to the same treatment using sesame oil as vehicle. Another five naive rats, which were the same age as rats in 24Mon group and received handling as well as exposure to the chamber on the habituation and training days but did not receive drug or vehicle, were used as pairfed control to behavioral studies. Following behavioral tests, five rats in each group were used for RT-PCR and Western blot analysis, and another five used for immunohistochemical analysis.

The rats 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 of the experimental procedures followed 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.

2.2. Open field test

All of the animals were tail-marked and handled for 5 days prior to the behavioral test. Open field test was performed between 8:00 AM and 1:00 PM. Each rat was placed in an open field chamber ($100 \times 100 \times 40$ cm) according to the procedure used in our previous study (Zhang et al., 2011). Illumination of open field was set to 20 lx. No background noise was provided. A digital video camera (Canon HF100, Japan) was installed above the apparatus. To neutralize odors, the arena was cleaned with 70% ethanol before each rat was tested. On the 82nd day and 83rd day of TP injection, the rats were accustomed to the open field apparatus for 5 min. On the 84th day of TP injection and the next day, each rat was individually placed in the center of the open field apparatus and allowed to explore the field. Five-minute open field behavior was recorded based on the studies (Glenn et al., 2008; Sanchez et al., 2008). The 2-day open field behavior was recorded and analyzed post hoc. Grooming behavior and motor behavior were analyzed in our study (Zhang et al., 2011). The 2-day continuous behavior parameters were scored by the observers blind to the experimental plan and registered by shorthand. We observed that the data was consistent for both days. Since there were no substantial differences in most behavior parameters in the last 2 days (ANOVA), the results are averaged for each rat. The averaged amount of individual behavior parameters was presented for each rat in the results.

2.3. Adhesive removal test

Adhesive removal test was performed between 1:00 PM and 6:00 PM in the home cage on the day when last open field test ended. The method was developed by Schallert et al. (1982). For group-caged animals, the cage mates were removed during testing. For tactile stimulation of the snout, strips of adhesive paper, which are commonly used as labels for file folders (Avery), were cut into 1.9 cm × l.4 cm rectangular pieces. This piece was placed lengthwise (horizontal to the ground) along left or right side of the snout, with the forward edge about 6 mm behind the nose. The vibrissae were lightly pressed against the snout. Latency to remove stimulus was recorded. Each trial ended when the label was removed, or after 3 min elapsed. Three trials were given, but only the first trial was used in the data analyses according to the method by Schallert et al. (1982). The tactile stimulation of the forelimbs was provided by round pieces of adhesive paper (dot) measuring l.3 cm in diameter. The dot was placed on the radial aspect of the rats' wrist. The distal part of the dot covered about 1 mm of the hairless part of the forepaw. Latency to remove the dot from the wrist was recorded. The trial ended when the animal removed the dot, or after 5 min elapsed. To minimize possible variations in adhesiveness caused by lipids in the fur and skin, the forelegs of the rats were washed with a 50% ethanol solution and allowed to dry for at least 15 min before testing. The dots were put on following the wrists and paws were wiped with clean, dry cotton gauze. The first of total three trials was used in data analyses.

2.4. Sample preparation for RT-PCR and Western blot

Following the behavioral tests, the rats in each group used for RT-PCR and Western blot were sacrificed by decapitation. The brains were removed quickly. The SN (between 3.00 and 4.00 mm rostral to the interaural axis; Paxinos and Watson, 1998) of ventral midbrain and caudate putamen (CPu; between 10.00 mm and 8.60 mm rostral to the interaural axis; Paxinos and Watson, 1998) were dissected on ice-cold plate, using a scalpel for ophthalmic surgery and a stereomicroscopy. Dissected tissue pieces were frozen in liquid nitrogen and stored at −80 °C until further utilization. Bilateral CPu blocks were pooled from individual rats for Western blot. Unilateral SN was processed into a plastic tube for RT-PCR or Western blot separately.

2.5. RT-PCR analysis

Total RNA was extracted from SN using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Frozen tissue was weighed and immediately homogenized in TRIzol Reagent using a pellet pestle cordless motor (Kimble kontes LLC) on ice. Add 1 ml of TRIzol Reagent per 50–100 mg of tissue. After incubation at room temperature for 5 min, 0.2 ml of chloroform was added per 1 ml of TRIzol Reagent. After vigorous shaked by hand for 15 s and further incubated at room temperature for 3 min, the samples were centrifuged at $12{,}000 \times g$ for 15 min at 4 °C. The upper aqueous phase was transferred to a fresh tube and 0.5 ml of isopropyl alcohol