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Deficits in coordinated motor behavior and in nigrostriatal dopaminergic system ameliorated and VMAT2 expression up-regulated in aged male rats by administration of testosterone propionate

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ABSTRACT

The effects of testosterone propionate (TP) supplements on the coordinated motor behavior and nigrostriatal dopaminergic (NSDA) system were analyzed in aged male rats. The present study showed the coordinated motor behavioral deficits, the reduced activity of NSDA system and the decreased expression of vesicular monoamine transporter 2 (VMAT2) in 24 month-old male rats. Long term TP treatment improved the motor coordination dysfunction with aging. Increased tyrosine hydroxylase and dopamine transporter, as well as dopamine and its metabolites were found in the NSDA system of TP-treated 24 month-old male rats, indicative of the amelioratory effects of TP supplements on NSDA system of aged male rats. The enhancement of dopaminergic (DAergic) activity of NSDA system by TP supplements might underlie the amelioration of the coordinated motor dysfunction in aged male rats. TP supplements up-regulated VMAT2 expression in NSDA system of aged male rats. Up-regulation of VMAT2 expression in aged male rats following chronic TP treatment might be involved in the maintenance of DAergic function of NSDA system in aged male rats.

1. Introduction

The coordinated motor behavior significantly declines during normal aging (Cunningham et al., 2011; Emerich et al., 1993; Emborg et al., 1998; Janicke et al., 1983; Shukitt-Hale et al., 1998; Spangler et al., 1994; Wallace et al., 1980; Yurek et al., 1998), which is related to the dysfunction of dopaminergic (DAergic) activity (Emborg et al., 1998; Kish et al., 1992; McCormack et al., 2004; Yurek et al., 1998). Nigrostriatal dopaminergic (NSDA) system plays a crucial role in maintaining normal coordinated motor behavior (Goren et al., 2005; Vaugoyeau et al., 2003; Wolff et al., 1989). Declined DAergic activity in NSDA system with advancing age results in coordinated motor behavioral deficits (Emborg et al., 1998; Kish et al., 1992; McCormack et al., 2004; Yurek et al., 1998). Enhancing DAergic activity in the aged improves the coordinated motor dysfunction (Esteban et al., 2010; Hurley et al., 2011; Razgado-Hernandez et al., 2015).

The endogenous male hormone testosterone and structurally related synthetic compounds (Hoberman and Yesalis, 1995) can influence the behaviors of organisms (Frye and Seliga, 2001; Lambadjieva, 1999; Perry et al., 2003). Androgen significantly increased exploratory behavior (Edinger and Frye, 2005; Zhang et al., 2011) and motor behavior (Zhang et al., 2011). The open field activity was reduced in male rats by gonadectomy (GDX) and the supplements of androgens recovered the open field activity in GDX rats (Adler et al., 1999; Zhang et al., 2011). Androgen replacement improved the spatial learning and memory in GDX adult male rats (Spritzer et al., 2011) and increased anti-anxiety behavior in aged male mice (Frye et al., 2008).

There are evidences that DAergic activities in the brain are influenced by androgen administration (de Souza Silva et al., 2009; Mitchell and Stewart, 1989; Thiblin et al., 1999). The manipulation of the DAergic activity via D1 and D2 receptor antagonist blocked the conditioned place preference effects of chronic testosterone treatment in rats (Schroeder and Packard, 2000). The application of testosterone to intact male rats led to an increase in dopamine (DA) levels in both the neostriatum and nucleus accumbens (de Souza Silva et al., 2009). Gonadectomy decreased DA and 3,4-dihydroxyphenylacetate (DOPAC) in

Abbreviations: CPu, caudate putamen; DA, dopamine; DAergic, dopaminergic; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; NSDA, nigrostriatal dopaminergic; SN, substantia nigra; TH, tyrosine hydroxylase; TP, testosterone propionate; VMAT2, vesicular monoamine transporter 2.

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the nucleus accumbens (Mitchell and Stewart, 1989). Testosterone replacement to GDX male rats restored DAergic activity (Mitchell and Stewart, 1989) and modulated striatal dopamine storage/uptake mechanisms in GDX mice (Shemisa et al., 2006).

With advancing age, the males experience a decrease in the circulating level of testosterone (Basaria, 2013; Cunningham et al., 2011; Garban et al., 1995; Ghanadian et al., 1975). Meanwhile, NSDA system undergoes a progressive decline during normal aging (Emborg et al., 1998; McCormack et al., 2004; Meng et al., 1999; Yurek et al., 1998). Aging induced a decrease in both striatal DA and homovanillic acid (HVA) levels in males (Dorce and Palermo-Neto, 1994). The supplements of testosterone ameliorated the motor function in Parkinson's disease (Mitchell et al., 2006) and motor behavioral defects in GDX rats and aged rats (Cui et al., 2012; Zhang et al., 2011; Zhang et al., 2013), as well as enhanced the cognitive performance of aged male mice (Frye et al., 2008), which were associated with androgen-enhanced DAergic activity in rats (Abreu et al., 1988; Cui et al., 2012; de Souza Silva et al., 2009; Thiblin et al., 1999; Zhang et al., 2013).

Vesicular monoamine transporter 2 (VMAT2), mainly localized on synaptic vesicular membranes in DAergic neurons, transports free intracellular DA into the synaptic vesicles and maintains the normal activity of DAergic system (Fon et al., 1997; Hall et al., 2014; Vergo et al., 2007). VMAT2 is reduced during aging (Cruz-Muros et al., 2008; Goettl et al., 2003; Scherman et al., 1989). Reduced VMAT2 leads to the decreased DA in synaptic vesicles and the increased free DA in cytoplasm (Caudle et al., 2007; Fon et al., 1997; Mooslehner et al., 2001; Pifl et al., 2014; Vergo et al., 2007). Free DA is metabolized by related enzymes into DOPAC and HVA. Reactive oxygen species generated in this process impair mitochondrial function (Hastings et al., 1996a; Montine et al., 1997; Rabinovic et al., 2000) and cause the degeneration of DAergic neurons (Caudle et al., 2007; Hastings et al., 1996b; Pifl et al., 2014; Rabinovic et al., 2000; Takahashi et al., 1997). In contrast, up-regulation of VMAT2 expression showed a curative or protective effect on the DAergic system and the impaired motor behaviors of rats (Lohr et al., 2014, 2015; Sun et al., 2004).

In terms of the effects of NSDA system on the coordinated motor behavior (Goren et al., 2005; Vaugoyeau et al., 2003; Wolff et al., 1989), the roles of VMAT2 in DAergic neurons (Caudle et al., 2007; Fon et al., 1997; Hall et al., 2014; Pifl et al., 2014; Vergo et al., 2007), and low levels of testosterone of aged rats (Cunningham et al., 2011; Garban et al., 1995; Ghanadian et al., 1975), in the present study, the coordinated motor behavior and the DAergic activity, as well as the expression levels of VMAT2 in NSDA system were analyzed in aged male rats following testosterone propionate (TP) supplements to observe the amelioratory effects of TP supplements on NSDA system and the involvement of VMAT2 in the amelioratory effects.

2. Materials and methods

2.1. Animals and housing

Male Wistar rats, supplied by the Experimental Animal Center of Hebei Medical University, were housed in an air-conditioned room (22 ± 3 °C) on a 12 h light–dark cycle (lights on 6:00 AM). All the rats had free access to food and water. The experimental procedures were 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.

2.2. Testosterone propionate supplements

All of the rats were randomly divided into the following 3 groups: 6-month-old rat group (6Mon, n = 75), 24-month-old rat group (24Mon, n = 75) and 24-month-old rats with TP treatment group (24Mon-TP, n = 75). TP was purchased from SERVA Electrophoresis (Cat. NO.: 35,805, Germany). For 24Mon-TP, the rats at the age of 21 months were treated by subcutaneous TP injection (2 mg/kg per day from 5:00 PM to 6:00 PM), which was continued for 12 weeks (84 days) (Cui et al., 2012). The rats in both 6Mon and 24Mon received the same treatment using sesame oil (from Sigma, MKBH4400V, S3547-250ML, USA) (Cui et al., 2012).

2.3. Coordinated motor behaviors

Seventy five rats in each group were divided into 3 subgroups and 3 subgroups were used for tapered beam walking test, adhesive removal test and cylinder test respectively.

2.3.1. Tapered beam walking test

Tapered beam walking test in the present study was described in detail by Strome E.M. and colleagues (Strome et al., 2006). Briefly, 2 cm below the beam, there was a 2.5 cm-wide beam that provided a platform for rats to step on. The beam became narrower during walking, gradually increasing difficulty. Based on the degree of difficulty, the beam was divided into wide, medium and narrow sections for scores, individually. The day before test day, rats walked on tapered beam for training 10 times. The following day, each rat was tested 5 times, which was recorded using digital video camera (Canon HF100, Japan). Data were collected based on the following. Taking a step with 1 or 2 toes on the main surface of the beam but the other 4 or 3 toes overhanging the beam was scored as a half foot fault; stepping with the entire foot on the ledge rather than on the main surface of the beam was scored as a full foot fault. Eq. 1 for standardization process (Zar, 2009) was used to calculate the data. The mean value of the scores of five-time tapered beam walking tests from the narrow section of the beam was used for statistical analysis.

$$p' = {}^{1}/_{2}\left[\arcsin\left(\sqrt{{}^{X}/_{(n+1)}}\right) + \arcsin\left(\sqrt{{}^{(X+1)}/_{(n+1)}}\right)\right] \quad (1)$$

Note: X = the number of errors and n = the number of steps.

2.3.2. Adhesive removal test

Adhesive removal test was performed in home cage as described by Schallert T. and colleagues (Schallert et al., 1982). With respect to group-caged rats, the cage mates were removed to an empty cage prior to testing. After adaptive training, the 1.9 cm × l.4 cm rectangular strips of adhesive paper (Avery) was placed lengthwise along the left or right side of the snout and the forward edge was about 6 mm behind the nose. Latency to remove stimulus was documented with a stopwatch. The trial ended when the animal removed the stimulus or when 3 min had elapsed. Subsequently, forelimbs of rats were washed with 50% ethanol to minimize potential variations in adhesiveness due to presence of lipids and dried for at least 15 min. Round pieces of adhesive paper (dot), l.3 cm in diameter, were placed on the radial side of the rats' wrist, with the distal part covering about 1 mm of the hairless part of the forepaw. Latency to thoroughly remove the dot from the wrist was documented. The trial ended when the dot was removed, or after 5 min had elapsed. Three trials were given, but only the data from the first trial was used for analysis.

2.3.3. Cylinder test

Cylinder test was performed as described in the studies (Gharbawie et al., 2004; Schallert et al., 1997; Urakawa et al., 2007). The apparatus was a cylinder made of clear Plexiglas and measured 20 cm in diameter and 30 cm in height (Schallert et al., 1997). Video records were made using digital video camera (Canon HF100, Japan). Animals, which had been previously handled for about 10 min per day for two weeks and were naïve to the apparatus, were used in the experiment. Each animal was individually placed in the cylinder and recorded for five minutes (Gharbawie et al., 2004). The number of rearing and the number of