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Testosterone Replacement Attenuates Haloperidol-Induced Catalepsy in Male Rats

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ABSTRACT

***Purpose*:** Parkinson's disease (PD) is a progressive neurodegenerative disease. Recent studies have indicated a higher prevalence of PD in male gender. Furthermore testosterone deficiency is more common among male parkinsonians in compare to healthy men. This study was aimed to investigate the effect of testosterone on catalepsy, in male rats.

***Methods*:** The study carried out on male Wistar rats. To induce catalepsy, haloperidol (1 mg/kg, i.p) as D2 antagonist was administered before testing animals via Bar test. Animals were gonadectomized to investigate testosterone elimination effect on catalepsy, and also the androgen receptor blocker, flutamide, and the aromatase inhibitor, letrozole, were administered in certain groups of animals. The bar test method was used to evaluate haloperidol-induced catalepsy.

***Results*:** Haloperidol 1 mg/kg, i.p, was able to induce catalepsy. Gonadectomy worsened the catalepsy and subchronic testosterone replacement could restore this effect to the level of normal animals. While low dose of flutamide administration represented an improvement in cataleptic symptoms, higher doses worsened catalepsy. Letrozole(4mg/kg,sc) administered animals represented nearly the same cataleptic symptoms as the control group.

***Conclusion*:** Testosterone deficiency increases catalepsy and testosterone replacement can significantly be effective in catalepsy remission. It seems that the anticataleptic effect of testosterone is exerted through affecting on androgenic receptors.

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease[1] caused by the destruction of dopaminergic neurons projecting from the substantia nigra pars compacta (SNc) to the striatum. Some of the most prominent motor symptoms are tremor, bradykinesia[1] and catalepsy.[2] Catalepsy is defined as inability to correct externally imposed postures, and the muscle stiffness comes towards this failure.[3] According several studies, there is a relation among PD and Sexual Hormones.[4,5] PD is common in 1% of people over 50 years[1] and at least in 2% of people over 65 years. Testosterone deficiency is common in20-25% of males over 65 years.[4] Recent studies shows that testosterone deficiency is 35% more common in parkinsonian subjects over 65 years.[4,5]

Many of non-motor symptoms of PD such as fatigue, depression, sleep disorders and sexual impotency are correlated with testosterone deficiency[4] and an appropriate therapeutic response was seen in these symptoms by testosterone therapy.[5] However, it is not yet clarified whether testosterone replacement can be influent directly on motor symptoms of PD or not.[4]

Human neurologic disorders can be modeled in animals through standardized procedures.[6] The two most common experimental models of PD in rats and mice are use of dopaminergic neurotoxins, 6-hydroxydopamine (6-OHDA) and N-Methyl-4-Phenyl-1, 2, 3, 4, Tetrahydropyridine (MPTP).[6-8] These toxins cause degeneration of nigrostrital dopaminergic neurons and subsequent striatal dopamine loss.[7,9] Moreover, PD and some extrapyramidal signs can be induced by neuroleptics and D_2 antagonists. Instance catalepsy can be induced in rats and other laboratory animals by haloperidol, through nigrostrital D_2receptor inhibition.[2]

The results of studies about testosterone influence on motor symptoms of PD are controversial. Results of a study shows that testosterone increases catalepsy in comparison to estrogen.[3] Furthermore, in another research, 7-day treatment of male rats with estradiol

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benzoate increased haloperidol-induced catalepsy; and 7-day treatment with medroxy progesterone acetateand acutely testosterone administration, decreasehaloperidol-induced catalepsy, which shows progesterone and testosterone's effect on increasing of dopaminergic tonus.[10]

According to the controversies about effects of testosterone on motor symptoms of PD, this study aimed to investigate effect of testosterone on haloperidol-induced catalepsy in male rats. Results of thisstudy, can suggest adjuvant therapeutic approaches in decreasing neuroleptic drugs' side effects or decreasing catalepsy of PD.

Materials and Methods

Chemicals

Haloperidol was obtained from Caspian Pharmacy Co. (Iran). Flutamide and letrozole, testosterone enanthate and Phenyl butazone (Vetanyl® 5%) were all obtained from Abureihan Pharmacy Co. (Iran). Ketamine and Xaylazine were obtained from Alfasan Co. (Holland).

Haloperidol was dissolved in normal saline 0.9%. Testosterone enanthate, flutamide and letrozole were dissolved in Castor oil. All drugs solutions were prepared freshly on the days of experimentation and were injected intraperitonealy (i.p) except for letrozole which was administered subcutaneously (s.c).

Animals

The experiments were carried out on male Wistar rats weighing 220-230g and each group included eight rats. Animals were housed in standard polypropylene cages, four per cage, under a 12:12 h light/dark schedule at an ambient temperature of 23±2 °C and had free access to food and water. All experiments were carried out under ethical guidelines of the Tabriz University of MedicalSciences, for the care and use of laboratory animals (National Institutes of Health Publication No 85-23, revised 1985).

Surgical procedure

The rat was anesthetized by ketamine (i.p, 50mg/kg) and xylazine (i.p, 5mg/kg). Then scrotum was cut about 1.5 cm to expose gonads and vas deferens. Gonads were tied from epididymis part by silk suture string and then were cut from down the tied part. This method is used to eliminate testosterone hormone production source in male mature rats in which one month after surgery the amount of testosterone is nearly zero.[11]

Motor impairment study

Motor impairment was induced with haloperidol (1 mg/kg, i.p) and catalepsy was measured by using of standard bar test, in which the rat was maintained in an imposed position with both front limbs extended and resting on a 9-cm high wooden bar (0.9 cm in diameter). The end point of catalepsy was considered the time that one of the front paws was removed from the bar or if the rat moved its head in an exploratory manner. The cut-off time was 720 seconds.Haloperidol-induced catalepsy was measured by bar test, in 5, 60,120, 180 min after haloperidol injection.[1] All observations were made between 9:00 AM and 16:00 PM in a quiet room by an observer who was blind to treatments.

Data analysis

Descriptive analysis and comparison of differences between each data set were calculated by use of InStat 2 software. The data were expressed as mean ± SEM, and were analyzed by ANOVA in each experiment. In the case of significant variation ($p<0.05$), the values were compared by Tukey test. Statistical significance was accepted at the level of $p<0.05$.

Results

Haloperidol-induced catalepsy and effect of castration on it

In order to induce catalepsy 8 ratswere treated by haloperidol 1 mg/kg, i.p. and were tested under bar test (Hal group). In the Intact group 8 rats were treated by haloperidol vehicle, normal saline 1ml/kg (INT group).Haloperidol 1mg/kg, i.p. was able to induce significant catalepsy in all four times of 5, 60, 120 and 180 minutes with intact group ($p<0.0001$) (Figure 1).

Then, a group of animals were castrated by gonadectomy procedure (GNX group).[11] Four weeks after surgery, the castrated animals were tested under bar test after haloperidol 1 mg/kg, i.p administration.Results in Figure 1 show that castration was able to increase haloperidol-induced catalepsy in GNX group in comparison withshamsurgery group in 60 and 180 minutes after haloperidol injection ($p< 0.05$).

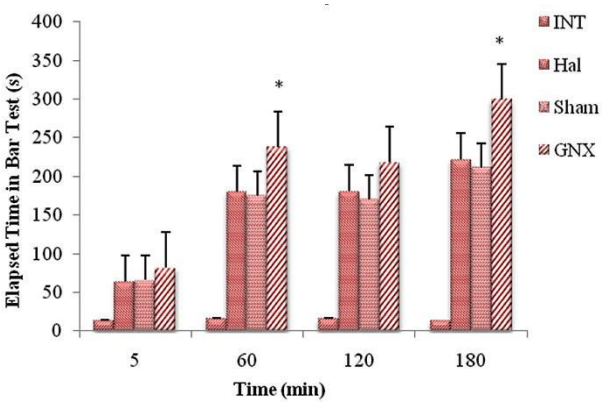

Figure 1. Effect of castration on haloperidol (1 mg/kg, i.p.)-induced catalepsy. Each bar represents the mean±SEM of elapsed time (s) in bar test, n=8 rats for each group. *: $p<0.05$ vs Hal. INT= Intact, Hal=Haloperidol, Sham= Sham surgery, GNX= Gonadectomy.

Effect of testosterone replacement therapy on haloperidol-induced catalepsy

As it is shown in Figure 2 four groups of animals were compared: Haloperidol group (Hal group), castrated group (GNX group), castrated group which was treated

by 7-day administration of testosterone 1mg/kg, i.p four weeks after surgery (GNX+T group) and group of animals which were treated by 7-day administration of testosterone 1mg/kg, i.p (T). All these groups were tested under bar test following haloperidol 1 mg/kg i.p administration in the certified day (four weeks after surgery for GNX group; one day after 7-day administration of testosterone for GNX+T and T groups). Positive effect of testosterone on haloperidol-induced catalepsy was seen in all time intervals after haloperidol injection in the 5th minute ($p< 0.05$) and in the 60th, 120th and 180th minutes ($p< 0.001$).

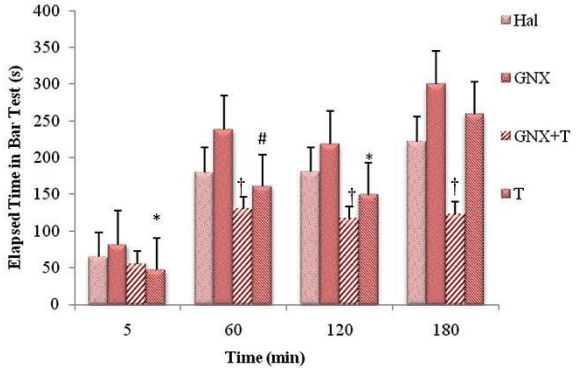

Figure 2. Effect of testosterone (1mg/kg, i.p. for 7days) replacement therapy on haloperidol-induced catalepsy. Each bar represents the maen ± SEM of elapsed time (s) in bar test. n=8 rats for each group. *: $p < 0.05$, #: $P < 0.01$, †: $p < 0.001$ vs GNX group. Hal= Haloperidol, GNX= Gonadectomy, T= Testosterone.

Effect of Flutamide on haloperidol-induced catalepsy

In order to make known the androgen receptor blocking effect of different doses of flutamide on haloperidol-induced catalepsy, four groups of animals were compared: flutamide Vehicle treated rats (group V), and three groups of non-gonadectomized rats which were received intraperitoneal doses of flutamide, 10, 20 and 30 mg/kg, for 7 days respectively (F10, F20 and F30 groups). All these groups were tested under bar test following haloperidol 1 mg/kg, i.p administration one day after 7-day administration of flutamide. As represented in Figure 3, catalepsy improvement was seen in 60th minutes after haloperidol injection in F20 group and 180th minutes after haloperidol injection in F30 group ($p< 0.01$). Also in 60th minute after haloperidol injection in F10 group, 180th minutes after haloperidol injection in F10 and F20 group improvement was seen ($p< 0.001$).

Effect of Letrozole and Testosterone on haloperidol-induced catalepsy in castrated animals

To clarify aromatase inhibitory effect of letrozole on haloperidol-induced catalepsy, a group of animals were castrated and four weeks after surgery were treated with letrozole 4mg/kg, s.c and testosterone 1 mg/kg, i.p for 7 days (GNX+T+L) and finally were tested under bar test after injection of haloperidol 1 mg/kg, i.p. Bar test results of mentioned group were compared with Hal, Hal+GNX and Hal+GNX+T groups. As represented in Figure 4, there was no significant difference between GNX+T+L group and GNX+T group.

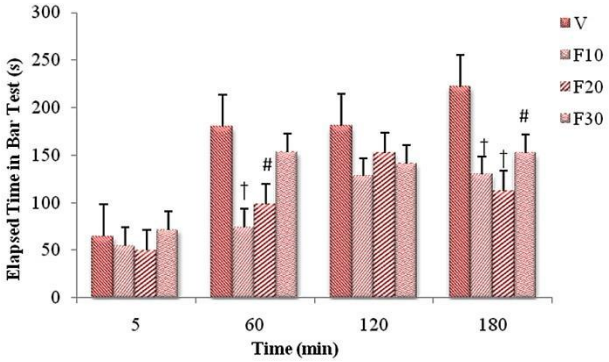

Figure 3. Effect of flutamide (10, 20 and 30 mg/kg, i.p. for 7 days) on haloperidol-induced catalepsy (1 mg/kg, i.p.). Each bar represents the mean±SEM of elapsed time (s) in bar test. n=8 rats for each group.#: $p<0.01$,†: $p<0.001$ vs CNT. V= Vehicle of flutamide, F= Flutamide.

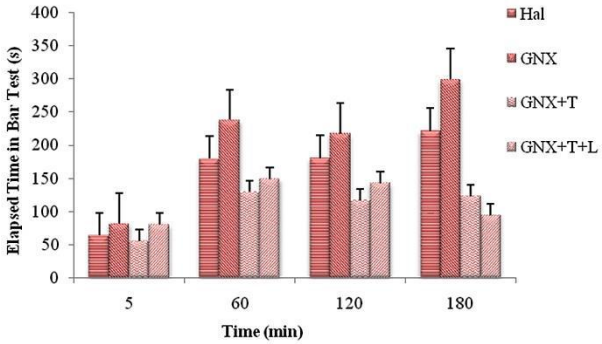

Figure 4. Effect of Letrozole and Testosterone on haloperidol-induced catalepsy in castrated animals.Each bar represents the mean±SEM of elapsed time (s), n=8 rats for each group. Hal= Haloperidol, GNX= Gonadectomy, T= Testosterone, L= Letrozole.

Discussion

Parkinson's disease is the progressive neurodegeneration of nigrostriatum substantia nigra, pars compacta. One of the important motor symptoms of PD is catalepsy, which can be induced in animal models by some neurotoxins such as MPTP and 6-OHDA or neuroleptic drugs like haloperidol.[1,12,13] In the present study, administration of haloperidol 1 mg/kg i.p., as a D_2 antagonist, was able to induce ambient catalepsy.

Incidence and prevalence of PD are both 1.5–2 times higher in men than in women.[14,15] Sex steroids such as estrogen and testosterone have been indicated to significantly regulate the incidence and severity of particular extrapyramidal movement disorders involving the nigrostriatum.[5] It is found in female rats that action of estrogen in intrinsic striatal GABAergic neurons and on dopamine terminals causes rapid and direct enhancement of dopamine release. Also, greater

dopamine neuron terminal density in female caudate nucleus represents gender differences in dopaminergic function.[16] Not only animal studies, but also human investigations prove the dopaminergic system differences of the two genders. It has been shown that both healthy and parkinsonian women have higher striatal dopamine transport binding than healthy and parkinsonian men, respectively.[2,15] Also testosterone deficiency is 20-25% common in males over 65 years and this deficiency enhances up to 35% in male parkinsonian patients who are over 65 years old. However in a case report a notable improvement in parkinsonian symptoms of a male subject have been represented after the administration of intramuscular testosterone, it is still unclear that to what extent testosterone deficiency is led to symptomatic PD and whether testosterone replacement can actually improve motor symptoms directly or not.[5]

The effect of testosterone on cataleptic symptoms is still controversial in animal studies. While a study on rats shows positive effect of acute testosterone administration on dopaminergic tonus,[10] another study represents that acute testosterone therapy increases the catalepsy in haloperidol-induced cataleptic rats.[3] Bourque et al (2009) stated that testosterone can be biotransformed into 17β-estradiol by an aromatization process in the brain while dihydrotestosterone cannot be converted into 17β-estradiol. In MPTP treated mice, a pre and post-treatment schedule with testosterone (100 µg/day for 10 days or a 21 day release via a 0.1 mg testosterone pellet for 17days) or dihydrotestosterone (2 or 100 µg/day for 10 days) did not protect against MPTP induced dopamine depletion in intact or castrated male mice. Also, testosterone and dihydrotestosterone do not prevent the decreased dopamine induced by MPTP. These results suggest that at the doses used, testosterone is not transformed into 17β-estradiol in the brain in sufficient concentrations to reach protective levels. Moreover, according to this investigation androgenic activity, evaluated with dihydrotestosterone, is also not able to protect striatal dopamine against MPTP toxicity.[17]

Our study's results of castrated animals represented the negative effect of castration on catalepsy, and following this result, the positive effect of testosterone administration on improvement of castrated animals was significant. However, in some time intervals after induction of catalepsy, testosterone was not able to reverse catalepsy, which can be as a result of insufficient dose of testosterone that can be increased in future studies.

Castration procedure not only eliminates testosterone, but also can decrease estrogen level, because of elimination of estrogen production through testosterone aromatization pathway. In order to clarify mechanism of action of testosterone, the androgen receptor blocker, flutamide, and the aromatase inhibitor, letrozole, were used.

The highest used dose of flutamide, was able to induce catalepsy the same as control groups of animals, which reflects the intrinsic direct effect of testosterone on reducing catalepsy. However higher doses of flutamide may represent higher catalepsy. On the other hand, lower used doses, represented improvement in catalepsy, which can be as a result of HPA inhibition via low doses of androgen receptor blockade, that compensatory causes ease of testosterone synthesis. Through increasing doses of testosterone receptor blocker, extent antagonization in peripheral and central regions occurs despite administration of testosterone. Although, we suggest measurement of testosterone level in flutamide-treated animals in future investigations.

Usage of letrozole was due to representing indirect effect of testosterone through aromatization to estrogen. Results of castrated group treated by letrozole and testosterone represented that letrozole had no effect on testosterone's impression on catalepsy improvement which supports testosterone's effect through androgenic receptors.

Conclusion

Male Wistar rats with testosterone deficiency experienced increased catalepsy following haloperidol injection and subchronic testosterone replacement could decline this catalepsy. Results of this study suggest that the effect of testosterone on catalepsy improvement is through androgenic receptor in compare to estrogenic pathway. However other pathways such as non genomic effects of testosterone were not objected in this study which can be considered in future investigations.

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Conflict of Interest

The authors report no conflicts of interest.

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