5-HT<sub>1A</sub> receptor activation improves anti-cataleptic effects of levodopa in 6-hydroxydopamine-lesioned rats

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Received 15 March 2011; Revised 15 Aug 2011; Accepted 17 Aug 2011

ABSTRACT

Background and the purpose of the study: In Parkinson's disease (PD) prolong use of L-DOPA causes some motor disorders such as wearing-off and L-DOPA induced dyskinesia (LID). In this investigation the effect of 8-OHDAPT, as a 5-HT<sub>1A</sub> agonist on anti-cataleptic effect of L-DOPA in 6-hydroxydopamine (6-OHDA) lesioned male Wistar rats was investigated.

Methods: Catalepsy was induced by unilateral injection of 6-OHDA (8 µg/2µl/rat) into the central region of the SNc. After 3 weeks as a recovery period, animals received intraperitoneally (i.p.) L-DOPA (15 mg/kg) twice daily for 20 days, and anti-cataleptic effect of L-DOPA was assessed by bar-test at days of 5, 10, 15 and 20.

Results and major conclusion: The results showed that L-DOPA had anti-cataleptic effect only until the day of 15, and its effect was decreased on the day of 20. On the day of 21, rats were co-injected with three different doses of 8-OHDAPT (0.1, 0.5 and 2.5 mg/kg, i.p.) and L-DOPA (15 mg/kg) twice daily for 20 days, and anti-cataleptic effect of L-DOPA was assessed by bar-test at days of 5, 10, 15 and 20.

8-Hydroxy-2-(di-n-propylamino) tetralin (8-OHDAPT) improved anti-cataleptic effect of L-DOPA at the dose of 0.5 mg/kg. Moreover the effect of 8-OHDAPT on anti-cataleptic effect of L-DOPA (15 mg/kg, ip) was abolished by 1-(2-methoxyphenyl)-4-[4-(2-phthalamido) butyl] piperazine hydrobromide (NAN-190; 0.5 mg/kg, i.p.) as a 5-HT<sub>1A</sub> receptor antagonist. According to the obtained results, it may be concluded that activation of 5-HT<sub>1A</sub> receptors by 8-OHDAPT may improve anti-cataleptic effect of L-DOPA in a 6-OHDA-induced rat model of PD. Further studies are required to clarify the exact mechanism of interaction between 5-HT<sub>1A</sub> and dopaminergic neurons.

Keywords: 8-OHDAPT; 5-HT<sub>1A</sub> receptor, Catalepsy, L-DOPA.

INTRODUCTION

L-Dihydroxyphenylalanine (L-DOPA) is the most effective drug which is commonly used in PD (1). However prolong use of L-DOPA may cause some motor problems such as wearing-off and L-DOPA induced dyskinesia (LID) (2). Although the main mechanisms underlying these complications are unclear but they are attributed to the maladaptive plastic changes in the brain such as changes in the synthesis, release, inactivation and vigorous axonal sprouting of dopamine (3).

There are some evidences that pronounce axial role of serotonergic system in the PD. Accordingly, in the 6-hydroxy dopamine lesioned rats, increase of serotine levels (3) and hyperinnervation of serotonergic neurons take place within the striatum and these neurons compensate some roles of the lost dopaminergic neurons (4). In addition to these, serotonergic neurons express aromatic L-amino acid decarboxylase and vesicular monoamine transporter 2, which convert L-DOPA to the dopamine (3). It has been shown that L-DOPA derived dopamine is co-stored with serotonin into same vesicles and acts as a false transmitter (5).

Dopaminergic neurons are able to keep dopamine levels at the physiologic levels (6). This regulatory mechanism is mediated by D<sub>2</sub> auto-receptors and dopamine transporters (7). However, serotonergic neurons have not these regulatory components and they are not able to exert enough control on the release of dopamine to the striatum (2). Therefore release of dopamine from these neurons has an activity-dependent-fashion (6). Under this condition fluctuation in dopamine levels result in intermittent activation of dopamine receptors and motor fluctuation (2). It has been shown that 5-HT<sub>1A</sub> receptors are present on dorsal raphe neurons with efferents to the striatum and on cortical neurons that send glutamatergic projections to the basal ganglia.
ganglia (8). Stimulation of 5-HT1A receptors in these regions leads to dopamine release (9) via inhibition of adenyl cyclase and opening of potassium channels (10). Recently it was shown that 5-HT1A agonists exert anti-cataleptic effect in animal model of PD (11, 12) and also regulatory effect of 5-HT1A receptors on L-DOPA derived dopamine levels has been reported (13). However there is no report about 5-HT1A effects on anti-cataleptic effect of L-DOPA. In this study effect of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DAPFT), as a 5-HT1A receptor agonist on anti-cataleptic effect of L-DOPA in 6-hydroxydopamine-lesioned rats was investigated.

**MATERIAL AND METHODS**

**Chemicals**
All chemicals except L-DOPA and Carbidopa which were obtained from Ramopharmin, Iran were purchased from Sigma Chemical Co. (USA). L-DOPA solutions were made freshly on the days of experimentation by dissolving in 0.9% NaCl. The drugs other than 6-hydroxydopamine (6-OHDA) which was injected into left substantia nigra were injected intraperitoneally (i.p.). Movement disorder was assessed by bar test on 5, 60, 120 and 180 min after drugs administration

**Animals**
Seventy two male wistar rats weighting 180-200g were housed in standard polypropylene cages, four per cage, under 12:12 light/dark schedule and at 25±2°C with free access to food and water. All experiments were performed according to the ethical guideline of Tabriz University of Medical Sciences for the care and use of laboratory animals.

6-OHDA-induced lesion of SNc Rats were anaesthetized deeply by intraperitoneal (i.p.) injection of Ketamine (50 mg/kg) and xylazine (5 mg/kg). Then their heads were fixed in a stereotactic apparatus frame at flat skull position. The scalp was shaved and scrubbed with iodine and a small central incision made to appear skull. A 23 gauge sterile stainless steel cannula as a guide cannula, was firstly implanted to the injection site for subsequent insertion of the injection tube into the SNc. The coordinates for this position were determined according to the rat brain in stereotaxic coordinates (14): which are anteroposterior from bregma (AP)=−5 mm, mediolateral from the midline (ML)=−2.2 mm and dorsoventral from the skull (DV)=−8.8 mm. The guide cannula was then secured to the cranium with dental cement. Desipramine (25 mg/kg) was injected intraperitoneally 30 min before intranigral injection of 6-OHDA to avoid destruction of noradrenergic neurons (15). Then 6-OHDA (8 μg/per rat in 2 μl of saline containing 0.2 % w/v of ascorbic acid) was infused by infusion pump at the flow rate of 0.2 μl/min into the left substantia nigra. At the end of injection, the tube was kept for an additional 2 min and then slowly withdrawn. All these procedure were exploited for control animals except that they were injected 2 μl vehicle of 6-OHDA (0.9% saline containing 0.2% (w/v) ascorbic acid). After three weeks as a recovery period, only rats that showed marked catalepsy in bar test were subjected to further experimentation. Then parkisonian rats were divided randomly into groups of 8 and treated with L-DOPA (15 mg/kg, i.p.) twice daily (9 AM and 9 PM) for 20 days. Peripheral metabolism of L-DOPA was inhibited by concomitant administration of carbidopa (1.5 mg/kg, i.p.). Other drugs such as 8-OH-DPAT and NAN-190 were injected once on the day of 21.

**Catalepsy test**
Catalepsy was measured by means of a standard bar test. In this method, forepaws of rats were placed over a 9 cm high standard wooden bar and duration of retention of rats in this imposed posture was considered as a bar test elapsed time (16). The endpoint of catalepsy was considered to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. A cut-off time of 720 seconds was applied. All observations were carried out by a person who was unaware from entity of treatment.

**Statistical analyses**
Statistical analysis of each data set was calculated by use of SigmaStat software. Data were expressed as the mean±SEM, and analyzed by one-way ANOVA in each experiment. In the case of significant variation (p<0.05), the values were compared by Tukey test.

**RESULTS**

**Effect of intra-SNc injected 6-OHDA on bar test**
Catalepsy elapsed time was measured in three groups of rats that were as normal, control and 6-OHDA (8 mg/2 μl/rat)-lesioned groups. As is has been shown in figure 1, 6-OHDA (8 mg/2μl/rat) compared with normal and control group was able to induce (p<0.001) marked catalepsy.

**Effect of chronic administration of L-DOPA on bar test**
The impact of L-DOPA (15 mg/kg, i.p.) and its vehicle on 6-OHDA-induced catalepsy was assessed for 20 days. In these groups catalepsy was tested on days of 5, 10, 15 and 20. There was significant reduction (p<0.0001) on bar test elapsed time in L-DOPA treated rats on days of 5, 10, 15, whereas its anti-cataleptic effect was abolished on the day of 20 (Fig. 2A). No alteration was observed on bar test.
Figure 1. The results of bar test in normal, control and 6-OHDA (8 μg/2μl/rat) lesioned rats. Each bar shows the mean±SEM of elapsed time (s), n=8 rats for each group; *** p<0.001 when compared with normal and control groups.

Figure 2. The results of bar test in 6-OHDA (8 μg/2 μl/rat) lesioned rats injected with L-DOPA (15 mg/kg) (Fig. 2A) and L-DOPA's vehicle (Fig. 2B) twice daily on the days of 5, 10, 15 and 20. Each bar represents the mean±SEM of elapsed time (s), n=8 rats for each group; *** p<0.001 when compared with 6-OHDA lesioned rats. (L=L-DOPA on days of 5, 10, 15, 20); (V=Vehicle on days of 5, 10, 15, 20)
elapsed time in vehicle-treated rats (Fig. 2B).

**Effect of 8-OH-DAPT on anti-cataleptic effect of L-DOPA**

In three groups of rats that received L-DOPA (15 mg/kg ip), 8-OH-DAPT was also injected intraperitoneally at the dose of 0.1, 0.5 and 2.5 mg/kg on the day of 21. Results showed that 8-OH-DAPT enhanced (p<0.05 and 0.01) anti-cataleptic effects of L-DOPA at the dose of 0.25 and 0.5 mg/kg (Fig. 3).

**Effects of co-administration of NAN-190 and 8-OH-DAPT on anti-cataleptic effect of L-DOPA**

On the day of 21, the effect of co-treatment of NAN-190 (0.5 mg/kg, i.p.) and 8-OH-DAPT (0.5 mg/kg, i.p.) in L-DOPA (15 mg/kg, i.p.) injected rats was investigated. As it has been shown in figure 4, catalepsy improving effect of 8-OH-DAPT was abolished (p<0.05 and p<0.01) in the presence of NAN-190.

**DISCUSSION**

Results of this study showed that acute administration of 8-OH-DAPT, as an agonist of 5-HT$_{1A}$ increased anti-cataleptic effect of L-DOPA in a dose-dependent manner. This is in accordance with results of our previous studies concerning an anti-cataleptic effect for 5-HT$_{1A}$ agonists in 6-OHDA (11) and haloperidol (12) induced PD.

It has been reported that in 6-OHDA lesioned rats, serotonergic neurons are responsible for not-physiologically release of dopamine in the striatum (6). This pulsatile release of dopamine may cause intermittent dopamine receptor stimulation and subsequent motor problems like wearing off and LID (2). It has been shown that serotonin depletion by fenfloramine and 5, 7-dihydroxytryptamine (5, 7-DHT) induce anti-dyskinetic effect in parkinsonian animals (6, 17). Also transplantation of serotonin neurons-rich graft to the striatum in 6-OHDA lesioned rats has worsened dyskinetic condition (18). According to the other report stimulation of 5-HT$_{7}$ receptors found on dorsal raphe neurons reduce activities of serotonergic neurons (19, 20). Therefore, it may be postulated that inhibition of serotonergic system can provide benefits in treatment of the induced motor disorders during long-term L-DOPA therapy.

According to results of this study, 8-OH-DAPT increased markedly anti-cataleptic effect of L-DOPA which is in accordance to results of our previous study describing an anti-cataleptic effect for 8-OH-DAPT in haloperidol treated animals (13). Additionally in other studies anti-dyskinetic effect of 8-OH-DAPT has been observed (6, 21).

In addition to agonistic effect of 8-OH-DAPT on 5-HT$_{1A}$, it has low affinity for 5-HT$_{7}$ receptors and α$_2$-adrenoceptors (22). Thus, receptors other than 5-HT$_{1A}$ are thought to be involved in anti-cataleptic improving effect of L-DOPA. In order to rule out possible involvement of 5-HT, and α$_2$ receptors, the effect of 8-OH-DAPT on catalepsy improving effect of L-DOPA in the presence of NAN-190 (5HT$_{1A}$ antagonist) was investigated. Results showed that NAN-190 could abolish catalepsy improving effect of 8-OH-DAPT in L-DOPA treated rats. Therefore it may be speculated that anti-cataleptic improving effect 8-OH-DAPT on L-DOPA treated rats is mediated through the 5-HT$_{1A}$ receptors and the role of 5-HT7 and α$_2$ receptors in this effect may be neglected. These findings are consistent with results of another study reporting an anti-cataleptic effect for 5-HT$_{1A}$ agonists (23).

In conclusion it is suggested that 8-OH-DAPT may improve anti-cataleptic effect of L-DOPA in 6-OHDA-lesioned rats by stimulation of 5-HT$_{1A}$ receptors. It seems that 5-HT$_{1A}$ receptor agonists can be used as an
effective adjuvant therapy for the treatment of motor fluctuations during long term L-DOPA therapy in PD. Further investigations are required to determine exact mechanisms underlying interaction between serotonergic and dopaminergic systems.

**ACKNOWLEDGEMENT**

We wish to thank the Director of Drug Applied Research Center and Research Vice-Chancellor, Tabriz University of Medical Sciences for supporting this study.

**REFERENCES**