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Buspirone improves the anti-cataleptic effect of levodopa in 6-hydroxydopamine-lesioned rats

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Abstract:
In Parkinson's disease (PD), prolonged exposure to L-3,4-dihydroxyphenylalanine (L-DOPA) results in motor fluctuations, such as the on-off phenomenon, and L-DOPA-induced dyskinesia. Previously, we found that activation of 5-HT_{1A} in the substantia nigra pars compacta (SNc) decreased catalepsy in parkinsonian rats. In the current investigation, we attempted to evaluate the effect of buspirone on the anti-cataleptic effect of L-DOPA in 6-hydroxydopamine (6-OHDA)-lesioned male Wistar rats. Catalepsy was induced by the unilateral infusion of 6-OHDA (8 μg/2 μl/rat) into the central region of the SNc. After a 3-week recovery period, rats received L-DOPA intraperitoneally (*ip*; 15 mg/kg) twice daily for 20 days, and the anti-cataleptic effect of L-DOPA was assessed by the bar test at days 5, 10, 15 and 20. The results showed that L-DOPA had an anti-cataleptic effect only until day 15, and its effect was abolished on day 20. On day 21, these rats were co-treated with three different doses of buspirone (0.1, 0.5 and 2.5 mg/kg, *ip*) and L-DOPA (15 mg/kg, *ip*). At a dose of 0.5 mg/kg, buspirone improved the anti-cataleptic effect of L-DOPA. Furthermore, the effect of buspirone (0.5 mg/kg, *ip*) on the anti-cataleptic effect of L-DOPA (15 mg/kg, *ip*) was reversed by 1-(2-methoxyphenyl)-4-(4-phthalimidobutyl)piperazine hydrobromide (NAN-190; 0.5 mg/kg, *ip*), a 5-HT_{1A} receptor antagonist. From these results, it may be concluded that buspirone improves the anti-cataleptic effect of L-DOPA in a 6-OHDA-induced animal model of PD through the activation of 5-HT_{1A} receptors. In this regard, further investigations should be undertaken to clarify the exact mechanism of the interaction between 5-HT_{1A} and dopaminergic neurons.

Key words:
buspirone, 5-HT_{1A} receptor, catalepsy, L-DOPA, rat

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder caused by the destruction of dopaminergic neurons projecting from the substantia nigra pars compacta (SNc) to the striatum. This degeneration results in the reduction of extracellular dopamine levels in the corpus striatum [19] and subsequently induces motor dysfunctions, such as tremor, muscle rigidity and bradykinesia [6, 15, 24].

Restoring dopamine levels in the affected area is the most effective and standard therapeutic strategy commonly used to alleviate the motor symptoms in PD [22]. L-3,4-dihydroxyphenylalanine (L-DOPA) is widely used in combination with a peripheral DOPA decarboxylase inhibitor, such as carbidopa or bensera-

zide [23]. Chronic use of L-DOPA, especially in advanced disease stages, causes patients to have motor fluctuations, such as L-DOPA-induced dyskinesia (LID) and the *wearing off* or *on-off* phenomena [14, 20, 22].

It has been demonstrated that under normal conditions, storage and release of dopamine from exogenous L-DOPA take place in the SNc dopaminergic neurons [1, 5, 13]. After dopaminergic neuron loss, hyperinnervation of serotonergic projections occurs [12], and the up-regulation of 5-HT receptors is observed, which compensates for some dopaminergic system-related defects [11, 13]. Aromatic amino acid decarboxylase (AADC) and vesicular monoamine transporter-2 (VAMT-2) in serotonergic neurons allow the conversion of L-DOPA to dopamine (DA) and the storage of DA in synaptic vesicles (along with serotonin), respectively [4, 7, 17]. The dopamine derived from L-DOPA in serotonergic neurons acts as a false neurotransmitter and co-localizes with serotonin in the same vesicles [3]. A lack of appropriate regulatory systems in the serotonergic neurons prevents them from regulating DA levels in a physiological manner [5]. Therefore, dopamine receptors are exposed to fluctuating amounts of DA [5, 23], which results in the appearance of motor fluctuations during continuous L-DOPA therapy [23]. Evidence suggests that 5-HT_{1A} receptor agonists regulate the striatal concentration of DA derived from exogenous L-DOPA [2, 24]. Recently, we have shown that buspirone, a 5-HT_{1A} activator, can improve catalepsy induced by 6-OHDA [19] and haloperidol [16]. Therefore, this study was designed to determine the impact of buspirone on the anti-cataleptic effect of L-DOPA in parkinsonian rats.

Materials and Methods

Chemicals

All chemicals were purchased from Sigma Chemical Co. (USA), except for buspirone (Heumann Co., Germany), L-DOPA and carbidopa were from Ramopharmine Co., Iran. Solutions were freshly prepared on the day of experimentation by dissolving drugs in physiological saline (0.9% NaCl). The drugs were injected intraperitoneally (*ip*) and movement disorders were assessed by the bar test 5, 60, 120 and 180 min after drug administration.

Animals

The study was performed on male Wistar rats weighing 180–220 g. Animals were housed in standard polypropylene cages, four per cage, under a 12:12 light/dark program and at a temperature of 25 ± 2°C, with free access to food and water. Animals were acclimated to the testing conditions for 2 days before the behavioral investigations were conducted. Procedures were carried out under the ethical guideline of Tabriz University of Medical Sciences for the care and use of laboratory animals (National Institutes of Health Publication No. 85-23, 1985).

6-OHDA-induced SNc lesions

Animals were anesthetized with an *ip* injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). After they were deeply anesthetized, rats were mounted in a stereotaxic frame in the flat skull position. The scalp was shaved and swabbed with iodine and a small central incision was made to expose the skull. A 23-gauge sterile stainless steel guide cannula was firmly implanted into the injection site for the subsequent insertion of the injection tube into the SNc. The coordinates for this site were based on the rat brain in stereotaxic coordinates [21]: 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 *ip* 30 min before the intranigral injection of 6-OHDA to avoid the destruction of noradrenergic neurons [10]. Then, 6-OHDA (8 μg/per rat in 2 μl saline with 0.2% ascorbic acid) was infused with an infusion pump at a flow rate of 0.2 μl/min into the left substantia nigra. At the end of infusion, the injection tube was kept implanted for an additional 2 min and then was slowly retracted. Sham-operated animals were subjected to the same procedure, except 2 μl vehicle (0.9% saline containing 0.2% w/v ascorbic acid) was injected into the SNc. After a three-week recovery period, only the rats that were markedly immobilized in the bar test were subjected to further experimentation. Then, parkinsonian rats were divided randomly into equal groups and received twice daily (9 a.m., 9 p.m.) injections of L-DOPA (15 mg/kg, *ip*) for 20 days. Peripheral metabolism of L-DOPA was inhibited by concomitant administration of carbidopa (1.5 mg/kg, *ip*). Buspirone and NAN-190, a 5-HT_{1A} receptor agonist and antagonist, respectively, were injected *ip* at day 21.

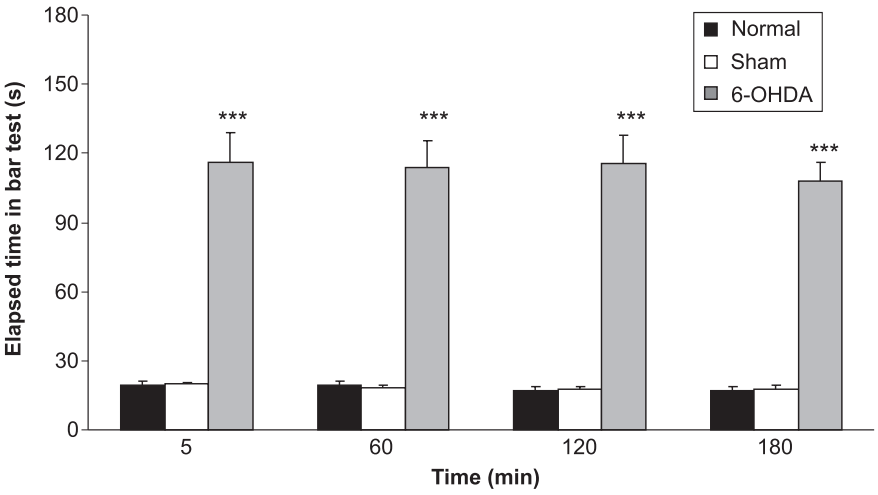
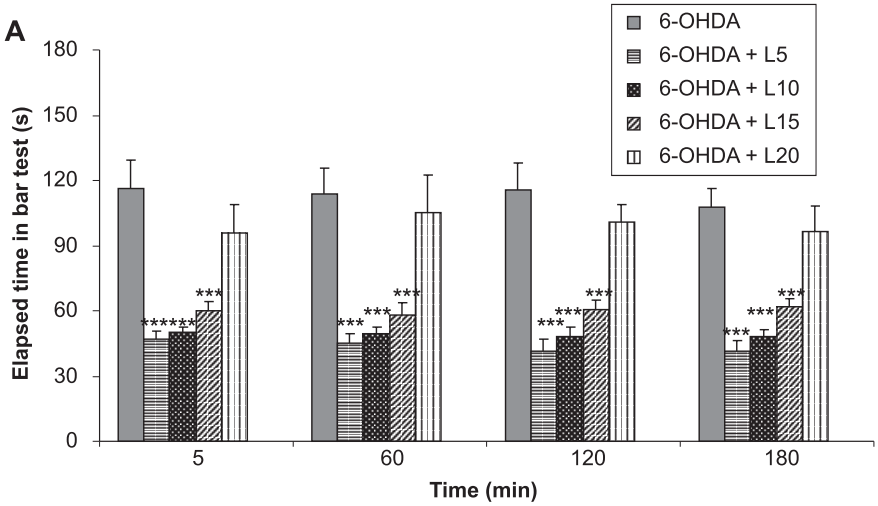


Fig. 1. The bar test results of control, sham-operated and 6-OHDA (8 µg/2 µl/rat)- lesioned rats. Each bar represents the mean ± SEM of elapsed time (s); n = 8 rats for each group; *** $p < 0.001$ when compared with normal and sham-operated groups



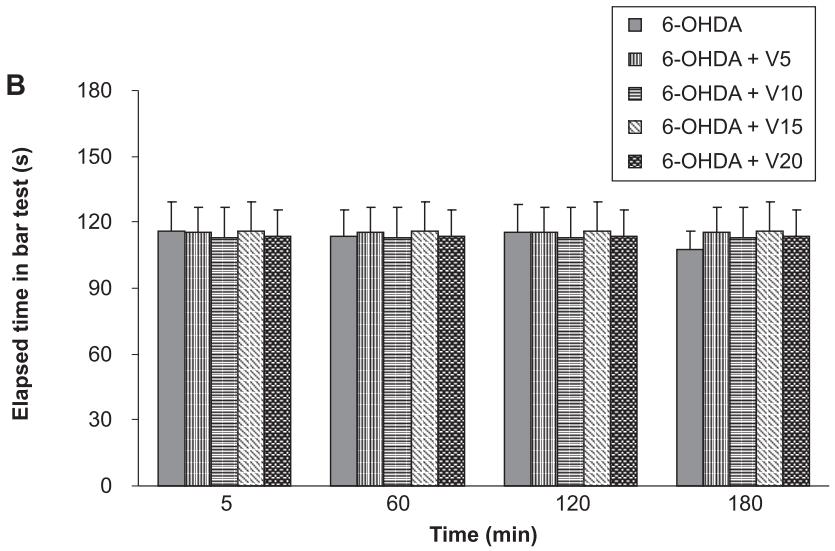


Fig. 2. The bar test results of 6-OHDA (8 µg/2 µl/rat)-lesioned rats treated with L-DOPA (15 mg/kg) (**A**) and vehicle (**B**) twice daily on days 5, 10, 15 and 20. Each bar represents the mean ± SEM of elapsed time (s); n = 8 rats for each group; *** $p < 0.001$ when compared with 6-OHDA-lesioned rats.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

Catalepsy test

Catalepsy was measured using a standard bar test. In this method, forepaws of rats were placed over a 9-cm-high standard wooden bar, and the duration of retention of rats in this imposed posture was considered as the bar test elapsed time. The end point of catalepsy was considered to occur when both front paws were removed from the bar or when the animal moved its head in an exploratory manner. The cut-off time of the test was 720 s. All observations were made between 9 a.m. and 4 p.m. by an observer who was blind to the nature of treatments.

Histology

All rats with guide cannulae were sacrificed at the end of the experiments. Brain dissections were performed in all animals to confirm the exact implantation of guide cannulae into the SNc. Brains with the injecting tube *in situ* were fixed in 10% formalin for 1 week. The location of the injecting tube tip was then verified in serial sections. Only the results from bar tests in animals with the tip of the injecting tube within the SNc area were used for statistical analysis.

Statistical analysis

Statistical analysis of each data set was performed by SigmaStat software. The data were expressed as the mean ± SEM and were analyzed by one-way ANOVA in each experiment. Statistical significance was accepted at the level of $p < 0.05$. In the case of significant variation ($p < 0.05$), the values were compared using *post-hoc* Tukey's test.

Results

The effect of intra-SNc-injected 6-OHDA

The elapsed time of catalepsy was assessed in three groups of rats: normal, sham-operated and 6-OHDA (8 µg/2 µl/rat)-lesioned groups. As shown in Figure 1, 6-OHDA (8 µg/2 µl/rat) induced cataleptic immobilization as compared with normal and sham operated groups ($p < 0.001$).

The effect of chronic administration of L-DOPA

The effect of L-DOPA (15 mg/kg, *ip*) and its vehicle was investigated in 6-OHDA-lesioned rats for 20 days. In these groups, catalepsy was assessed on days 5, 10, 15 and 20. As shown in Figure 2A, L-DOPA significantly decreased the bar test elapsed time on days 5, 10, 15 ($p < 0.001$), whereas its anti-cataleptic effect was abolished on day 20. There was not any significant difference in the bar test elapsed time in vehicle-treated rats (Fig. 2B).

The effect of buspirone on the anti-cataleptic effect of L-DOPA

In the groups of animals treated with L-DOPA (15 mg/kg/day for 21 days, *ip*), buspirone also was *ip* injected at doses of 0.1, 0.5 and 2.5 mg/kg on day 21. The results showed that buspirone improved the anti-cataleptic effect of L-DOPA at the dose of 0.5 mg/kg ($p < 0.01$ and 0.001) (Fig. 3).

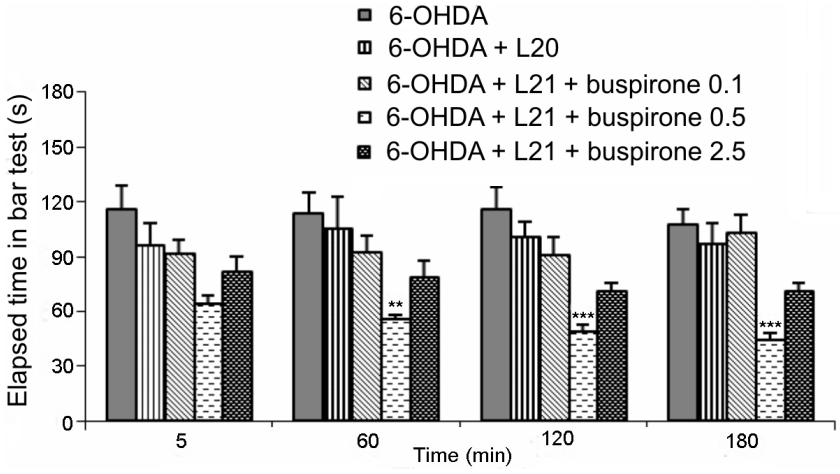


Fig. 3. The bar test results from the co-administration of buspirone (0.1, 0.5 and 2.5 mg/kg) with L-DOPA (15 mg/kg) on day 21 in 6-OHDA-lesioned rats and 6-OHDA-lesioned rats treated with L-DOPA (15 mg/kg) on day 20. Each bar represents the mean ± SEM of elapsed time (s); n = 8 rats for each group; ** $p < 0.01$ and *** $p < 0.001$ when compared with 6-OHDA-lesioned rats

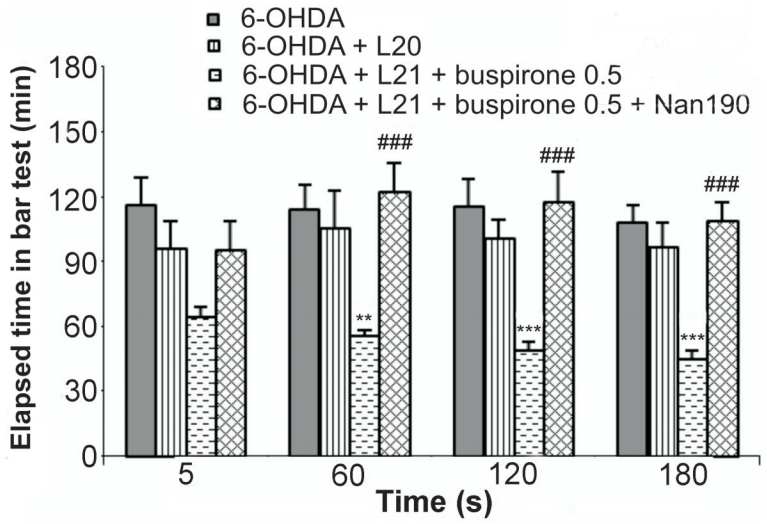


Fig. 4. The bar test results from the co-administration of NAN-190 (0.5 mg/kg) with L-DOPA (15 mg/kg) and buspirone (0.5 mg/kg) on day 21 in 6-OHDA lesioned rats treated with L-DOPA (15 mg/kg) on day 21 and in 6-OHDA (8 μg/2 μl/rat)-lesioned rats. Each bar represents the mean ± SEM of elapsed time (s); n = 8 rats for each group; ** $p < 0.01$ and *** $p < 0.001$ when compared with 6-OHDA-lesioned rats; ### $p < 0.001$ when compared with 6-OHDA-lesioned rats co-treated with L-DOPA (15 mg/kg) and buspirone (0.5 mg/kg) on day 21

The effect of NAN-190 co-treatment with buspirone on the anti-cataleptic effect of L-DOPA

On day 21, the effect of NAN-190 (0.5 mg/kg, *ip*) co-injected with buspirone (0.5 mg/kg, *ip*) was investigated in rats treated with L-DOPA (15 mg/kg, *ip*). As shown in Figure 4, the catalepsy-improving effect of buspirone was reversed ($p < 0.001$) in the presence of NAN-190.

Discussion

The rat model of 6-OHDA-induced PD is frequently used to investigate PD- and L-DOPA-related movement disorders [11, 23]. We showed that acute administration of buspirone can improve the anti-cataleptic effect of L-DOPA in 6-OHDA-lesioned rats as assessed by the bar test method. This is a standard test frequently used for evaluating catalepsy induced by 6-OHDA [19] and neuroleptic drugs [16] in rodents. In this study, intra-SNc injection of 6-OHDA caused marked catalepsy. Continuous administration of L-DOPA in parkinsonian rats alleviated catalepsy on days 5, 10 and 15. The anti-cataleptic effect of L-DOPA was abolished on day 20, which may explain the behavioral sensitization or motor fluctuations due to chronic administration of L-DOPA [23]. It seems that neuronal alterations in serotonergic and dopaminergic neurons in parkinsonian patients are responsible for motor fluctuations; e.g., wearing off and LID [23].

Furthermore, it has been demonstrated that the excessive release of DA from serotonergic neurons and the lack of feedback control in these neurons may result in L-DOPA-induced motor complications [6]. According to the results obtained here, co-administration of buspirone and L-DOPA reduced catalepsy time and improved L-DOPA effectiveness in the alleviation of catalepsy in 6-OHDA-lesioned rats on day 21. This observation confirms other findings that indicate an anti-cataleptic effect for buspirone in 6-OHDA-lesioned [19] or haloperidol-treated [16] animals. Moreover, other investigations have shown that buspirone alleviates L-DOPA-related motor complications in parkinsonian rats [11, 23]; however, its effect on the anti-cataleptic action of L-DOPA has not been reported.

Studies show that the serotonergic system plays an important role in the regulation of normal motor functions. This effect is exerted through 5-HT_{1A} receptors within the basal ganglia [8]. 5-HT_{1A} receptors that modify their own activity are also found in dorsal raphe neurons [8, 11]. In advanced Parkinson's disease, L-DOPA may be converted to dopamine in serotonergic neurons [11]. These neurons do not have enough control over the release of dopamine. Activation of 5-HT_{1A} receptor agonists can modulate the release of DA from these neurons to the striatum [8, 9], which results in prolonged DA effects in parkinsonian animals [11]. It has been reported that the anti-dyskinetic effect of 5-HT_{1A} receptors may be mediated through the activation of these receptors in the striatum [9]. It has been shown that the transplantation of serotonin neuron-rich grafts to the striatum of 6-OHDA-lesioned rats markedly increase the magnitude and du-

ration of dyskinesia [3]. Accordingly, it may be postulated that the dampening of serotonergic neuronal activity through the activation of 5-HT_{1A} receptors can be useful in motor disorders related to the long-term use of L-DOPA [18]. Apart from affecting 5-HT_{1A} receptors, buspirone also has D_2-and α_2-adrenoceptor blocking effects [18, 19]. Thus, it is possible that the effects of buspirone on the anti-cataleptic effect of L-DOPA may be due to its action on these receptors. In this study, it was shown that NAN-190, a 5-HT_{1A} receptor antagonist, reverses the catalepsy-improving effect of buspirone in L-DOPA-treated rats. Therefore, it is possible that the effects of NAN-190 to improve the anti-cataleptic effect of L-DOPA may be due to its action on 5-HT_{1A} receptors and, as a result, involvement of the α_2 and D_2 receptors may be neglected [18].

In conclusion, we suggest that buspirone improves the ability of L-DOPA to alleviate catalepsy in 6-OHDA-lesioned rats. This effect is mediated by the stimulation of striatal 5-HT_{1A} receptors. In regards to the anti-parkinsonism effect of buspirone, it seems that buspirone can be used as an adjuvant therapy to reduce L-DOPA-induced motor complications. Further investigations are needed to reveal the exact mechanism of the interactions between serotonergic and dopaminergic neurons.

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