The Effect of Chronic Administration of Buspirone on 6-Hydroxydopamine-Induced Catalepsy in Rats

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ABSTRACT

Purpose: Several evidences show that serotonergic neurons play a role in the regulation of movements executed by the basal ganglia. Recently we have reported that single dose of buspirone improved 6-hydroxydopamine (6-OHDA) and haloperidol-induced catalepsy. This study is aimed to investigate effect of chronic intraperitoneal (i.p.) administration of buspirone on 6-OHDA-induced catalepsy in male Wistar rats.

Methods: Catalepsy was induced by unilateral infusion of 6-OHDA (8 μg/2 μl/rat) into the central region of the SNc and was assayed by the bar-test method 5, 60, 120 and 180 min after drugs administration in 10th day. The effect of buspirone (0.5, 1 and 2 mg/kg, i.p. for 10 days) was assessed in 6-OHDA-lesioned rats. Results: The results showed that chronic injection of buspirone (0.5, 1 and 2 mg/kg, i.p. for 10 days) decreased catalepsy when compared with the control group. The best anticaletalitic effect was observed at the dose of 1 mg/kg. The catalepsy-improving effect of buspirone was reversed by 1-(2-methoxyphenyl)- 4-[4-(2-phthalimido) butyl]piperazine hydrobromide (NAN-190), 0.5 mg/kg, i.p., as a 5-HT1A receptor antagonist.

Conclusion: Our study indicates that chronic administration of buspirone improves catalepsy in a 6-OHDA-induced animal model of parkinson's disease (PD). We also suggest that buspirone may be used as an adjuvant therapy to increase effectiveness of antiparkinsonian drugs. In order to prove this hypothesis, further clinical studies should be done.

Introduction

PD is a neurodegenerative disease that leads to motor impairments such as tremor, rigidity, slowed body movements (bradykinesia), unstable posture and difficulty in walking. This disease is characterized by the loss of dopamine (DA)-producing neurons in the ventral midbrain with cell bodies in the substantia nigra pars compacta (SNc) that project to the striatum. It's motor symptoms can be treated with dopaminergic drugs, however, their effectiveness diminishes as the severity of the clinical symptoms increases due to progression of the underlying neurodegeneration. It has been suggested that non-dopaminergic regions including the serotonergic neurons of dorsal raphe nucleus (DRN) are involved in pathology of PD. 5,6 5-HT projections from the DRN innervate all components of the basal ganglia circuitry. The striatum and output regions of the basal ganglia (SNr and GPm) receive a dense serotonergic (5-HT) input. In addition to dopamine, 5-HT also modulates the actions of other neurotransmitters, including GABA and glutamate, as well as providing feedback mechanisms on 5-HT neurotransmission itself via an action in the DRN. 7,8 It is therefore plausible that serotonin plays a role in the regulation of movements executed by the basal ganglia. Among the subtypes of 5-HT receptors, 5-HT1A receptors play a particularly significant role in the regulation of motor activity. For example, 5-HT1A receptor agonists attenuate the catalepsy induced by raclopride and anti-psychotics such as haloperidol and risperidone. For this reason, there has been a substantial attention on 5-HT1A receptors and their involvement in motor control. In an experiment by Gerber and colleagues it was found that 5-HT1A receptor stimulation represented antiparkinsonian effects in 6-OHDA-lesioned rats (Gerber et al., 1988). We have shown previously that single dose of buspirone decreases haloperidol and 6-hydroxydopamine-induced catalepsy. This effect is most likely caused by the increase in 5-HT1A receptor activation resulting in an inhibition of 5-HT release. Buspirone acts as 5-HT1A receptor partial agonist and is used in chronic treatment of anxiety disorders. Since drugs may have completely different effects in
acute and chronic use, thus in this study we tried to evaluate effect of chronic administration of buspirone on catalepsy induced by 6-OHDA. Furthermore, the possible involvement of 5-HT1A receptor in mediating of observed effects was investigated.

Materials and Methods

Chemicals
All chemicals were obtained from Sigma Chemical Co. (USA) except for buspirone, which was purchased from Heumann Co. (Germany). Solutions were prepared freshly on the day of experimentation. Buspirone, and 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine hydrobromide (NAN-190) were dissolved in physiological saline (0.9% NaCl), and 6-OHDA was dissolved in 0.9% saline containing 0.2% (w/v) ascorbic acid. 6-OHDA was injected into the central region of the substantia nigra pars compacta (SNc) in a total volume of 8 μl/rat with a constant injection rate of 0.2 μl/min.

Animals
The experiments were carried out on male Wistar rats weighing 260-300 g. Animals were housed in standard polypropylene cages, four per cage, under a 12:12 h light/dark schedule at an ambient temperature of 25 ± 2°C and were allowed food and water ad libitum. Animals were acclimated to the testing conditions for 2 days before the behavioral experiment was conducted. All of the procedures were carried out under the ethical guidelines of the Tabriz University of Medical Sciences.

6-OHDA-induced SNc lesion
Animals were anesthetized with an ip injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). After they were deeply anesthetized (loss of corneal and toe pad reflexes), rats were mounted in a stereotaxic frame in the flat skull position. The scalp was shaved, swabbed with povidone iodine 10% and a central incision made to expose the skull. 6-OHDA was injected thorough a guide cannula (23 gauge stainless steel) implanted in the SNc. The coordinates for this site were based on the rat brain atlas [30]: anteroposterior (AP): −5.0 mm from the bregma; mediolateral (ML): −2.1 mm from the midline and dorsoventral (DV): −7.7 from the skull. Desipramine (25 mg/kg, ip) was injected 30 min before the intranigral injection of 6-OHDA to avoid the destruction of noradrenergic neurons. Then, 6-OHDA (8 μg/per rat in 2 μl saline with 0.2% ascorbic acid) was infused with an infusion pump at a constant flow rate of 0.2 μl/min into the left SNc. 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 submitted to the same procedure but 2 μl vehicle (0.9% saline containing 0.2% (w/v) ascorbic acid) of 6-OHDA was infused into the SNc.

Catalepsy test
Catalepsy was measured by 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. This test was carried out 5, 60, 120 and 180 min after drug administration. All observations were made between 9 a.m. and 4 p.m. 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 once daily (9 a.m.) injections of buspirone, a 5-HT1A receptor partial agonist (0.5,1 and 2 mg/kg, ip) for 10 days. NAN-190, as a 5-HT1A receptor antagonist, was injected (0.5 mg/kg, ip) concomitantly with the effective dose of buspirone.

Statistical analysis
Statistical analysis of each data set was calculated by use of SPSS software (version 16.0). Data were expressed as the mean±SEM, and were 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
Catalepsy induced by 6-OHDA
Rats were divided into three groups: normal, sham operated (receiving 2 μl vehicle) and 6-OHDA (8 μg/2μl/rat)-injected rats. Drugs and vehicle were injected into the SNc through the implanted guide cannula. As shown in Figure 1, 6-OHDA was able to induce significant (p < 0.05 and 0.01) catalepsy in comparison with both normal and sham-operated rats (Figure 1).

Figure 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.(Cont.=control)
Effect of buspirone on 6-OHDA induced catalepsy

Four groups of 6-OHDA-lesioned rats received saline or one of three different doses of buspirone (0.5, 1 and 2mg/kg, ip), respectively for 10 days. The results indicated that buspirone attenuated the severity of 6-OHDA induced catalepsy (p < 0.05) (Figure 2).

![Figure 2](image)

**Figure 2.** The bar test results of 6-OHDA (8 µg/2 µl/rat)-lesioned rats treated with Buspirone (0.5,1 and 2mg/kg,ip for 10 days). Each bar represents the mean ± SEM of catalepsy time (s); n = 8 rats for each group; * p < 0.001 when compared with 6-OHDA-lesioned rats. (Bus=Buspirone)

Effect of NAN-190 co-injection with buspirone on 6-OHDA-induced catalepsy

Three groups of 6-OHDA-lesioned animals respectively received saline, buspirone (1 mg/kg, ip) or buspirone (1 mg/kg, ip) with NAN-190 (0.5µmg/rat, ip). The results showed that the catalepsy-ameliorating effect of buspirone was abolished (p < 0.05) by NAN-190 (Figure 3).

![Figure 3](image)

**Figure 3.** The bar test results from the co-administration of NAN-190 (0.5 mg/kg, ip) with buspirone (1mg/kg, ip for 10 days) in 6-OHDA-lesioned rats. Each bar represents the mean ± SEM of catalepsy time (s); n = 8 rats for each group,a, p < 0.001 when compared with 6-OHDA-lesioned rats; b, p < 0.001 when compared with 6-OHDA-lesioned rats co-treated with NAN-190(0.5µmg/rat,ip) and buspirone (1 mg/kg,ip). Cont.=control, Bus.=buspirone

Discussion

In our previous study the potential antiparkinsonian effects of 5-HT₁A agonists in 6-OHDA-lesioned rats were investigated in single dose administrations. Since in clinic drugs are used chronically, therefore in this study we studied the potential antiparkinsonian effects of chronic administration of buspirone in 6-OHDA-lesioned rats. Our results showed intra-SNC injection of 6-OHDA induced catalepsy when assessed by the bar test. This is a standard test frequently used for evaluating catalepsy induced by 6-OHDA and neuroleptic drugs in rodents. According to the results, buspirone, a partial agonist of 5-HT₁A receptors, improved catalepsy in 6-OHDA-lesioned rats. This confirms our previous study reporting a promising role for 5-HT₁A agonists in decreasing the motor disorders associated with PD.

5-Hydroxytryptamine 1A (5-HT₁A) receptors are widely distributed throughout the basal ganglia. They are located on dorsal raphe neurons with efferents to the striatum and on cortical neurons that send glutamatergic projections to the basal ganglia. Stimulation of 5-HT₁A receptors in these regions leads to dopamine release via the inhibition of adenyl cyclase and the opening of potassium channels. It seems that 5-HT₁A receptor agonists can reduce serotonergic synthesis and release by activation of presynaptic, somatodendritic autoreceptors on raphe serotonergic cell bodies. A potential role of 5-HT₁A agonists in the enhancement of motor activity was suggested because stimulation of somatodendritic 5-HT₁A receptors by a selective agonist such as 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) inhibited synthesis of 5-HT to decrease its release from the nerve endings.

The mechanism by which buspirone exerts an antiparkinsonian effect in 6-OHDA-lesioned rats was studied by using 5-HT₁A receptor antagonists. In addition to 5-HT₁A receptors, buspirone has D₂ and α₂-adrenoceptor blocking effects. Thus, it seems that D₂ and α₂ receptors may be involved in the observed effects of buspirone. In this study the catalepsy-improving effect of buspirone was reversed by NAN-190, a 5-HT₁A receptor antagonist. This confirms the involvement of 5-HT₁A receptors in the observed effect of buspirone. According to the obtained results, we suggest that chronic administration of buspirone alleviates catalepsy in 6-OHDA-lesioned rats by acting on 5-HT₁A receptors in the SNC, and may it be used as a potential adjuvant drug together with routinely used antiparkinsonian drugs.

We investigated antiparkinsonian effect of different chronic doses of buspirone. The results showed that antiparkinsonian effect of buspirone at dose of 1 mg/kg was more than doses of 0.5 and 2. On the other hand, the antiparkinsonian effect of buspirone was attenuated by increasing the dose. Studies show that buspirone exerts dose-dependent opposite effects on the function of the nigrostriatal dopaminergic neurons so that at high doses, it acts as an antagonist on D₂ receptors. Furthermore, the affinity of buspirone on 5-HT₁A receprots is higher than D₂ receptors. Thus the possible involvement of D₂ receptors should not be neglected at it's high doses.

In conclusion our data suggest that chronic administration of buspirone improves catalepsy in 6-OHDA-lesioned rats. Inaddition we suggest that it may be used as an adjuvant therapy for improving effects of
antiparkinson drugs. However, further clinical investigations should be carried out to prove it.

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**Conflict of Interest**

There is no conflict of interest in this study.

**References**


17. Harrington MA, Oksenberg D, Peroutka SJ. 5-Hydroxytryptamine 1A receptors are linked to a Gi/adenylate cyclase complex in rat hippocampus. Eur J Pharmacol 1988; 154:95–98.


20. Kazuo Matsubaara, , Keiko Shimizub, Manabu Sunoa, Kento Ogawah, Toshio Awayaa, Takehiro Yamadaa, Toshihiro Nooda, Machiko Satomia, Kichi Ohtakia, Kaoru Chibna, Yoshikazu Tasakia, Hiroshi Shionob. Tandospirone, a 5-HT1A agonist, ameliorates movement disorder via non-dopaminergic systems in rats with unilateral 6-
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