Intraventral Pallidum Injection of 6-OH Dopamine Induces Motor Deficit in Rat, a Novel Experimental Model for Catalepsy

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Abstract: Parkinson’s disease is a movement disorder that mainly caused by degeneration of dopaminergic neurons from the substantia nigra pars compacta. There are three current techniques for producing in vivo models of Parkinson’s disease: unilateral lesioning with 6-hydroxydopamine (6-OHDA) in rats, systemic injection of 1-methyl 4-phenyltetrahydropyridine (MPTP) in mice and monkeys, and systemic injection of rotenone in rats. Present study aimed to investigate the effect of 6-OHDA injection into the ventral pallidum, which is a group of subcortical nuclei within basal ganglia. To this end, 6-OHDA was injected into the SNc and the ventral pallidum. Subsequently, motor deficit assayed by the bar test and the rotarod test. The results demonstrated that injection of 6-OHDA into the ventral pallidum is as effective in the induction of motor deficit as injection of 6-OHDA into the SNc. In conclusion, our data suggest that injection of 6-OHDA into the ventral pallidum induces movement disorder, and it can be as a novel model of cataleptic rat and can be used in different varieties of researches related to Parkinson’s disease and motor disorders.

Key words: motor deficit, 6-OHDA, ventral pallidum, rat

INTRODUCTION

Parkinson’s disease is a progressive disorder of movement that occurs mainly in the elderly (Rang et al., 2007). The most important characteristic of PD (Parkinson’s Disease) is degeneration of the dopaminergic neurons of the substantia nigra pars compacta (SNc) (Scholtissen et al., 2006). Damage of this region result in movement disorders and the most parkinsonian symptoms are tremor, rigidity, and bradykinesia (Mink, 1996). There are three current techniques for producing in vivo models of Parkinson’s disease: unilateral lesioning with 6-hydroxydopamine (rats), systemic injection of 1-methyl 4-phenyltetrahydropyridine (MPTP) (mice and monkeys), and systemic injection of rotenone (rats) (Andreas, 2004; Blum, 2001; Sherer, 2003). 6-OHDA are used commonly to create experimental model of Parkinson’s disease, such as catalepsy, motor imbalance and slowing of movement can be modeled (Nayebi, 2010; Scholtissen et al., 2006; Wang et al., 2005). The principal advantage of this model is that it is very sensitive to dopamine agonists. 6-OHDA is one of the most common neurotoxins used to experimentally model nigral dopaminergic degeneration in vivo (Blum et al., 2001). The toxin 6-hydroxydopamine (6-OHDA) is injected on one side of the rat, while the opposite side serves as an intra-animal control. This injection produces DA neuron loss on the injected side while sparing the contralateral DA neurons (Scholtissen et al., 2006). Present study are shown that unilateral infusion of 6-OHDA into the ventral pallidum induces catalepsy in rats. This model can be used to evaluate effects of drugs on catalepsy which is a motor disorder in parkinson’s disease.

MATERIALS AND METHODS

Study was carried out on male Wistar rats weighting between 180-210 g. The rats were kept under controlled conditions (12:12 hour light/dark schedule at temperature of 25±2°C), with rat food and water freely available. The rats were housed in groups (four per cage). At least 7 days were allowed for adaptation before the rats were used in the experiments. All procedures were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals, and were approved by the Animal Ethics Committee of the Tabriz University of Medical Sciences.

Animals were anesthetized by intraperitoneal (i.p.) injection of ketamine (50mg/kg) and xylasine (5mg/kg).
After the rats were anesthetized, they were placed in a stereotaxic frame in the flat skull position and were fixed their head. The scalp was shaved, swabbed with iodine and a central incision made to expose the skull. A cannula (23 gauge stainless steel) was implanted as a guide for injection of 6-OHDA into the Ventral Pallidum and SNc. The coordinates for these sites were based on the rat brain atlas (Paxinos and Watson, 1998): Ventral Pallidum (VP) coordinates: anteroposterior (AP) 0.48 mm from bregma; mediolateral (ML) 2.2 mm from the midline and dorsoventral (DV) 7.8 from the skull. Substantia nigra Pars compacta (SNc) coordinates: anteroposterior (AP) –5.0 mm from the bregma; mediolateral (ML) –2.1 mm from the midline and dorsoventral (DV) –7.7 from the skull. Sham group animals were operated 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 ventral pallidum and Substantia nigra Pars compacta. All rats with guide cannula were sacrificed at the end of the experiments. The brains of the rats was dissected to make certain the exact implantation of the guide cannula into the VP and SNc. The brains were fixed in 10% formalin for 1 week with the injecting tube in situ. The location of the injecting tube tip was then verified in serial sections. Unilaterally injection of 6-OHDA into the both of ventral pallidum and substantia nigra compact part induced dopaminergic lesion. Infusion of 6-OHDA was carried out through implanted guide cannula at a dose of 6 μg/2 μl/rat at a rate of 0.2 μl/min. Subsequent stages of experiments were accomplished after 5 days.

Catalepsy was assessed by means of a standard bar test, which measured the time the rats maintained 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 occurs when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. The cut-off time for the test was 180 seconds. This test was accomplished 5, 60, 120 and 180 min after the beginning of the test. All observations were made between 9 a.m. and 4 p.m. by an observer who was blind to the nature of the treatments. Balance disturbances were assessed by using rotarod test 5, 60, 120 and 180 minutes after the beginning of the test. The rotarod unit consists of a rotating spindle and individual parts for each rat. The rats try to control his balance when rotating spindle rotates with speeds 18 rpm. We considered cut of time when the rats fell onto the base grid beneath the rotarod. The cut-off time for the test was 720 seconds.

Descriptive statistics and comparisons of differences between each data set were calculated using 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 by Tukey test.

RESULTS AND DISCUSSION

Rats were divided into five groups: normal, two sham operated (receiving 2 μl 6-OHDA vehicle into the SNc and the ventral pallidum) and two 6-OHDA (6 μg/2 μl/rat intra SNc and intra ventral pallidum)-injected. According to figure 1., the results demonstrated that 6-OHDA is able to induce significant (p < 0.001) catalepsy (both in SNc and ventral pallidum) in comparison with both normal and sham-operated rats in the bar test method.

As shown in figure 2., 6-OHDA was also able to induce significant (p < 0.001) motor disturbance (both in SNc and ventral pallidum) in comparison with both normal and sham-operated rats in the rotarod test method.

![Fig. 1: The results of bar test in control, sham-operated and 6-OHDA (6μg/2μl/rat) lesioned rats. Each bar represents mean ± SEM. n= 12 rats per group; ***P< 0.001 when compared with normal and sham-operated rats](image-url)
operated groups. (s: second)

Fig. 2: The results of rotarod test in control, sham-operated and 6-OHDA (6µg/2µl/rat) lesioned rats. Each bar represents mean ± SEM. n= 12 rats per group, ***P< 0.001 when compared with normal and sham-operated groups. (s: second)

In brief, The results demonstrated that injection of 6-OHDA into the ventral pallidum is as effective in the induction of motor deficit as injection of 6-OHDA into the SNc.

Discussion:

Parkinson’s disease is a neurological disorder characterized by damage of striatal dopaminergic neurons (smith, 2000). There are three current techniques for producing in- vivo models of Parkinson’s disease: unilateral lesioning with 6-hydroxydopamine in rats, systemic injection of 1-methyl 4-phenyltetrahydropyridine (MPTP) in mice and monkeys, and systemic injection of rotenone (rats) (Andreas, 2004; Blum et al., 2001; Sherer et al., 2003). 6OHDA rat model is the traditional model for testing Parkinson’s therapies. A selective catecholaminergic neurotoxin 6-OHDA is one of the most common neurotoxins used to experimentally model nigral dopaminergic degeneration in vivo (Blum et al., 2001).

Current study was basically designed for whether injection of 6-OHDA into ventral pallidum cause to induce catalepsy and motor disturbance. The results showed that intra-SNc injection of 6-OHDA markedly induced catalepsy and motor disturbance when assessed by the bar test and rotarod test, respectively, which are standard tests those are frequently used for evaluating catalepsy and motor imbalance induced by 6-OHDA and neuroleptic drugs in rodents (Nayebi et al., 2010b; Pires et al., 2005; Yu et al., 2008). These results were approximately predictable, but the observation of the markedly catalepsy and motor disturbance by injection of 6-OHDA into the ventral pallidum was unexpected.

The basal ganglia regulates the flow of information from the cerebral cortex to the motor neurons of the spinal cord(Laurence et al., 2008). The Ventral Pallidum is a group of subcortical nuclei within basal ganglia. The Ventral Pallidum receives dopaminergic inputs from the ventral tegmental area, and GABAergic inputs from the nucleus accumbens (Smith et al., 2000). It is a component of the mesolimbic dopamine system, a pathway thought to be the major neural correlate of addiction. Addictive drugs facilitate dopamine release in this system (Pierce et al., 2006). The pallidum consists of a large structure called the globus pallidus together with a smaller ventral extension called the ventral pallidum. The globus pallidus is divided into two functionally different parts, called GPi and GPe (Smith et al., 2000). Moreover the striatum is the principal input structure of the basal ganglia and receives excitatory glutamatergic input from many areas of cerebral cortex. Outflow from the striatum has two routes. The direct pathway, from the striatum to the substantia nigra pars reticulata (SNpr) and globus pallidus interna (GPI), uses GABA as a neurotransmitter. The indirect pathway, from the striatum through the globus pallidus externa (GPe) and the subthalamic nucleus (STN) to the SNpr and GPe, uses the excitatory transmitter glutamate and inhibitory transmitter GABA. The substantia nigra pars compacta (SNpc) sends dopaminergic projections to the striatal neurons. These projections reach both the direct and indirect pathways, and regulates the activity of these two pathways. The SNpr and GPe are the output structures of the basal ganglia and provide feedback to the cerebral cortex through the ventroanterior and ventrolateral nuclei of the thalamus (VA/VL) (Laurence et al., 2008). Stimulation of the direct pathway at the level of the striatum lead to increase the excitatory outflow from the thalamus to the cortex; stimulating the indirect pathway at the level of the striatum result in attenuate the excitatory outflow from the thalamus
to the cerebral cortex (Laurence et al., 2008). Differential effect of dopamine on the direct and indirect pathways follow by the symptoms observed in Parkinson’s disease as a result of loss of dopamine neurons. These effects related to unique basal ganglia function. The net effect of the reduced dopaminergic input in PD is to increase markedly the inhibitory outflow from the SNpr and globus pallidus interna (GPI) to the thalamus and reduce excitation of the motor cortex (Laurence et al., 2008).

In conclusion, our data suggest that injection of 6-OHDA into the ventral pallidum induces cataleptic rats, inasmuch as it receives dopaminergic and GABAergic inputs, therefore it interferes in movement activities, and consequently 6-OHDA affect the motor neurons of the ventral pallidum. Hence, it can be as a novel model of cataleptic rat and can be used in different varieties of researches related to Parkinson’s disease and motor disorders.

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REFERENCES


