8 – ORIGINAL ARTICLE
EXPERIMENTAL NEUROLOGY

Effect of adenosine A_{2A} receptor antagonists on motor disorders induced by 6-hydroxydopamine in rat[1]

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ABSTRACT

PURPOSE: To investigate the role of adenosine A_{2A} receptors on 6-OHDA-induced motor disorder in rat.

METHODS: In order to induce experimental model of Parkinson's disease, 6-hydoxydopamine (8 µg/rat) was injected unilaterally into the SNc. After three weeks as a recovery period, 6-OHDA-induced bradykinesia and balance disturbances were assessed by using beam traversal test 10, 30 and 60 minutes after intraperitoneal injections of the drugs (caffeine, SCH58261).

RESULTS: The results showed that 6-OHDA (8 µg/rat, Intra-SNc) induced motor disorders of Parkinson's disease and increased elapsed time in the beam test ($p<0.001$). Injection of caffeine (30 mg/kg, i.p.) and SCH58261 (2 mg/kg, i.p.) attenuated elapsed time on beam ($p<0.01$ and $p<0.001$). We showed that acute administration of caffeine and SCH 58261 can improve the 6-OHDA-induced bradykinesia and motor disturbance.

CONCLUSION: Adenosine A_{2A}R antagonists improve 6-OHDA-motor deficit and this effect seems to be mediated by the inhibition of A_{2A} presynaptic receptors in substantia nigra pars compacta.

Key words: Receptor, Adenosine A2A. Oxidopamine. Parkinson Disease. Rats.

Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disease mainly caused by degeneration of dopaminergic neurons from the substantia nigra pars compacta (SNc)[1] . PD is characterized clinically by tremor, bradykinesia, rigidity and postural instability[2]. The cellular and molecular mechanisms underlying the pathogenesis of PD is unclear at present, but it has been linked increasingly to neuroinflammation and oxidative stress[3,4]. It seems that other neurotransmitter systems such as adenosinergic and serotonergic systems play important role in PD[5-9]. In the past two decades, preclinical, clinical and epidemiological studies have demonstrated that adenosine A_{2A} receptors (A_{2A} R) could be as a non dopaminergic therapeutic targets for Parkinson's disease. For instance, it has been shown that A_{2A}R antagonists enhance motor activity in animal models of PD and PD patients. Furthermore, epidemiological findings indicate inverse association between caffeine (as an A_{2A}R antagonists) consumption and PD risk[10]. In other words, coffee and tea drinking reduce the risk of developing Parkinson's disease in the worldwide population[11-14]. Moreover, according to a meta-analysis of five cohort studies and eight case control studies there is a strong epidemiological evidence that coffee drinkers have a lower risk of PD [14]. This study aimed to investigate the role of adenosine A_{2A} receptors on 6-OHDA-induced motor disorder in rat.

Caffeine, the major behavioral stimulant present in coffee, was isolated in 1820 and the correct structure of this methylxanthine was finally established in the last decade of that century[15]. It has been demonstrated that blockade of pre-synaptic A_{2A}Rs reduces transmitter release in several brain regions and in line with this, it is known that A_{2A}Rs antagonist can reduce extracellular glutamate levels induced by dihydrokainate (DHK), a non-transportable competitive inhibitor that primarily blocks the glial glutamate transporter GLT-1[16]. Thus a reduction in glutamate level in the substantia nigra via pre-synaptic blockade of A_{2A}Rs on the projections from the subthalamic nucleus may be one mechanism by which the A_{2A} antagonists protect[17]. In the present study, we investigated effect of Adenosine A_{2A} receptor antagonists on motor disorders induced by 6-hydroxydopamine in rat.

Methods

All of the procedures were carried out under the ethical guidelines of the Tabriz University of Medical Sciences and the study received approval from the Ethics Committee of the Tabriz University of Medical Sciences, according to the guide for the care and use of laboratory animals (National Institutes of Health Publication No 85–23, revised 1985).

The experimental study was carried out on male Wistar rats with weight range of 200-220g. Animals were divided into the groups contain 8 rats per group and were kept in standard condition, under a 12:12 hour light/dark schedule at an ambient temperature of 25 ± 2°C and *with free access to food and water.*

All chemicals were obtained from Sigma Chemical Co. (USA). Solutions were prepared fresh on the days of experimentation. Caffeine and SCH 58261 were dissolved in physiological saline (0.9% NaCl), and 6-Hydroxydopamine (6-OHDA) was dissolved in 0.9% saline containing 0.2% (w/v) ascorbic acid. Caffeine (10 and 30 mg/kg) and SCH 58261 (2 mg/kg) were injected intraperitoneally and 6-OHDA was infused at a flow rate of 0.2 µl/min into the substantia nigra to establish unilateral PD models (Figure-2).

Animals were anesthetized by intraperitoneal (i.p.) injection of Ketamine (80 mg/kg) and xylasine (5 mg/kg). After anesthetization, rats were mounted in a stoelting stereotaxic frame in the flat skull position. The scalp was shaved with standard shaving machine, swabbed with iodine and a small central incision made to appear the skull (Figure-1). A 23 gauge sterile stainless steel guide cannula was implanted to inject 6-OHDA into the SNc. The coordinates for this site were based on the rat brain atlas[18]: 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-operated animals were submitted to the same procedure but received 2 µl vehicle (0.9% saline containing 0.2% (w/v) ascorbic acid). After three weeks as a recovery period, in order to adapt animals, they were located in the laboratory for 1-2 hours before beginning of behavioral studies. 6-OHDA-induced motor incoordination was measured by means of a standard beam traversal test. Balance disturbance *and bradykinesia were measured by means of a standard beam traversal test. In this method*, a wooden beam 105 cm long and 40 mm wide was elevated 80 cm above the floor. The rats were placed on the far end of the beam and trained to walk the beam toward their home cage. To quantify motor deficits, time to traverse was scored using a stopwatch. The timer was started when the rat began to move forward and ended when the first forepaw was placed in the home cage. latency to cross was scored from five repetitive trials with 15 minutes interval as a recovery period after each trial. The data were presented as the mean of five trials/per animal[19]. This test was carried out 10, 30 and 60 minute after drug administration.

Descriptive statistics and comparisons of differences between each data set were calculated using SigmaStat software. The data were expressed as mean ± SEM, and 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

6-OHDA-induced balance disturbance

Three groups of rats were subjected as normal, sham operated (receiving 2 µL vehicle of 6-OHDA) and 6-OHDA (8 µg/2 µL/rat)-lesioned group. Drugs and vehicle were injected into the SNc through the implanted guide cannula. As it has been shown, 6-OHDA induced significant ($p<0.001$) bradykinesia and balance disturbance in comparison with both normal and sham-operated rats (Figure 1).

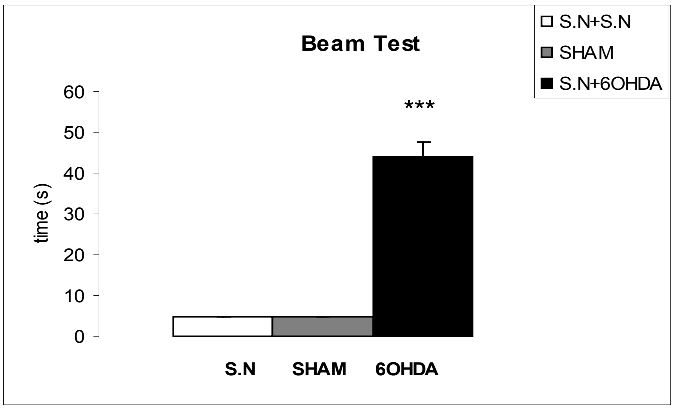

FIGURE 1 - The results of beam test in normal, sham-operated and 6-OHDA (8 µg/2µL/rat) lesioned rats. Each bar represents the mean ± SEM of elapsed time (s), n=8 per group: ***$p<0.001$ when compared with normal and sham-operated groups.

Effect of caffeine and SCH58261on normal rats

Four groups of normal rats received saline normal, one of two different doses of caffeine (10 and 30 mg/kg) and SCH58261 (2 mg/kg) intraperitoneally. There was not any significant difference in the beam test elapsed time in these groups (Figure 2).

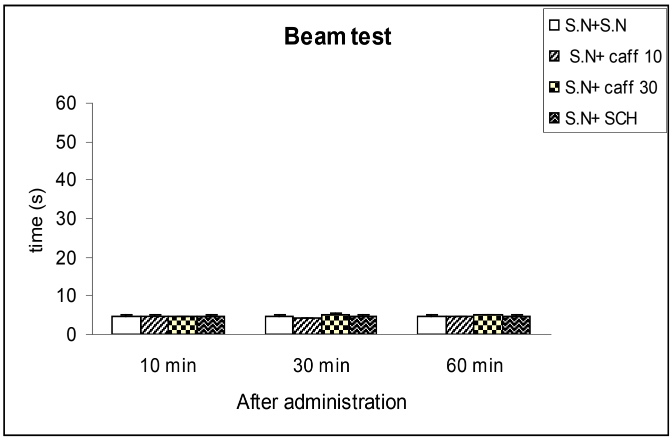

FIGURE 2 - Effect of caffeine (10 and 30 mg/kg, i.p.) and SCH58261 (2mg/kg, i.p.) on normal rats 10, 30 and 60 min after administration in beam test. Each bar represents the mean ± SEM of elapsed time (s), n=8 per group.

Effects of caffeine and SCH58261 on 6OHDA-lesioned rats

Four groups of 6-OHDA-lesioned rats respectively received saline, caffeine (10 and 30 mg/kg) and SCH58261 (2 mg/kg) intraperitoneally. As shown in Figure 3, beam test demonstrated a statistically significant decrease in the elapsed time after caffeine (30 mg/kg) and SCH58261(2 mg/kg) treatment in comparison with 6-OHDA-lesioned rats ($p<0.01$ and $p<0.001$).

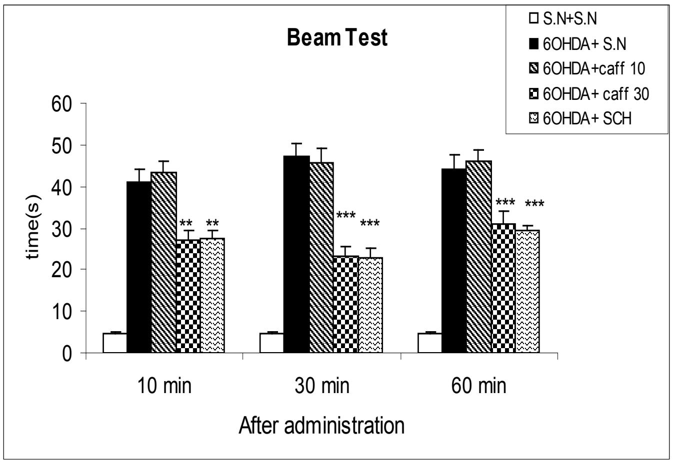

FIGURE 3 - Effect of caffeine (10 and 30 mg/kg, i.p.) and SCH58261 (2mg/kg, i.p.) on 6OHDA-lesioned rats 10, 30 and 60 min after administration in beam test. Each bar represents the mean ± SEM of elapsed time (s), n=8 per group. ** $p<0.01$ and *** $p<0.001$ when compared with 6-OHDA lesioned rats.

Discussion

This study aimed to investigate the role of adenosine A_{2A} receptors on 6-OHDA-induced motor disorder in rat. The rat model of 6-OHDA-induced PD is frequently used to investigate PD[20]. We showed that acute administration of caffeine and SCH 58261 can improve the 6-OHDA-induced bradykinesia and motor disturbance as assessed by the beam traversal test.

The potential therapeutic treatment of Parkinson's diseases by A_{2A}-adenosine receptor antagonists has been the subject of recent comprehensive reviews[21,23]. Convergent evidence suggests that caffeine consumption in beverages reduces risk of Parkinson's disease[21] and also caffeine in rodent models has protective effects[24].

The abovementioned results and also genetic and pharmacologic studies showing an antiparkinsonian action of selective adenosine A_{2A} receptor antagonists in different animal models of Parkinson's disease[25-27], suggest that A2A receptor antagonists seem to be very promising compounds in the treatment of Parkinson's disease. The accepted mechanisms that underlie the neuroprotective properties of A2A receptor antagonists include neuronal, vascular and microglial elements. It has been shown that the stimulation of A2A receptors leading to enhancement of glutamate release and excitotoxicity and A_{2A} R inactivation reduces neurotoxicity[10]. It is known that blockade of pre-synaptic A_{2A}Rs reduces transmitter release in several brain regions. Thus a reduction in glutamate release in the substantia nigra via pre-synaptic blockade of A_{2A}Rs on the projections from the subthalamic nucleus may be one mechanism by which the A_{2A} antagonists protect. These studies provide a neurobiological basis for the inverse relationship between increased caffeine consumption and reduced risk of developing PD[10]. Thus inactivation of pre-synaptic A_{2A}Rs on the projections from the subthalamic nucleus result in diminution of glutamate release in the substantia nigra, and this may be one mechanism to justify the neuroprotective role of A_{2A} R antagonists. We have demonstrated that the pharmacological inactivation of A_{2A} R inhibits 6-OHDA-induced motor deficit. This confirms the results of another study showing that A_{2A}R blockade attenuates striatal dopamine loss[28] and increases motor activity in animal models[29].

Conclusion

Adenosine A_{2A} R antagonists improve 6-OHDA-motor disturbances and this effect seems to be mediated by the inhibition of A_{2A} presynaptic receptors in SNc.

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