Iranian Journal of Pharmaceutical Research (2008), 7 (3): 229-232
Received: November 2007
Accepted: May 2008

Short communication

Betamethasone Can Significantly Decrease Level of Striatal Glutamate in Animal Model of Parkinson's Disease

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Abstract

In the present study, the effect of steroidal anti-inflammatory drug betamethasone (0.12, 0.24 mg/kg, i.p. acutely) on striatal glutamate level in Parkinsonian rats was studied using the microdialysis technique. Our results showed significant differences ($p<0.05$) in the level of striatal glutamate between treated and non-treated damaged rats.

Keywords*:* Microdialysis; Betamethasone; Glutamate; Parkinson's disease; Rat Model.

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder, which involves the the loss of dopaminergic neurons of the substantia nigra pars compacta (SNc). Its prevalence and incidence rates increase with age, and more than 2% of the population aged over 65 years and ~5–20/100,000 individuals per year are affected by the disease. The diagnosis of PD is based on medical history and a neurological examination, and can be difficult to be proven accurately. This neurodegeneration leads to a decrease in dopamine content in both SN and striatum, which has been ascertained by several neuroimaging studies. The reduction of ^{18} F-fluoro-L- Dopa and dopamine presynaptic transporter radioligand ^{18} F-CIT/FCCIT in the striatum has been demonstrated using positronemission tomography (PET) and single photon emission computed tomography (SPECT) scanning (1). Increasing evidence suggests that an inflammatory reaction and pathological processes could be seen in many neurodegenerative disorders, including PD (2, 3). Furthermore, dexamethasone as on steroidal anti-inflammatory agent, has shown to provide some neuroprotection (4) or rigidity recovery (5) in PD animal models. The role of glutamate, the major excitatory neurotransmitter in the brain, is regularly mentioned as a possible mechanism of cell death, although controversial in PD (6). Animal models studies have shown the effect of glutamate on degeneration of DA neurons and the beneficial effects of N-methyl D-aspartate (NMDA) receptor antagonists (7). In addition, defective glutamate transport in astrocytes exposing the neuron to excess synaptic glutamate may be involved (8). Thus glutamate transporters have a high potential as drug targets (9), even though evidence of a role on the SN neurons in PD patients still has to be found to support the theory.

The study of striatal glutaminergic interaction has a special importance due to the physiological and pathophysiological process of these systems, such as Parkinson's or Huntington's diseases (8). It has also been shown that an excessive release of excitatory amino acids such as glutamate may

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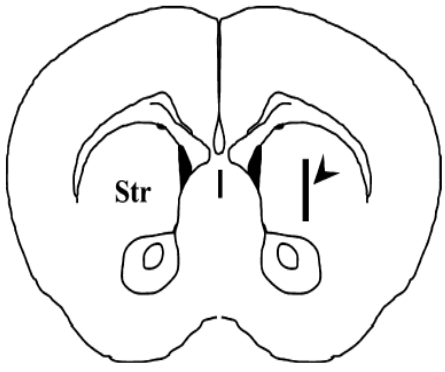

Figure 1. Microdialysis probe placement within the rat striatum (Str).
[With permission and Courtesy of Professor Yi Fan]

be involved in the pathogenesis of PD and also some reports have suggested an inerease in the release of glutamate exhibited by prostaglandins in neuronal damage (10).

In vivo assay of glutamate as an affecting neurotransmitter in the striatal pathway in SNc-lesioned rats after administration of betamethasone has been investigated in this study.

Experimental

Adult male albino Wistar rats (200-250 g) were subjects of this study. The animals were housed in groups of six in stainless steel cages, handled daily, and provided food and water ad libitum. A 12 h light/12-h dark cycle was maintained, and the animals were tested during the light cycle. These animal studies were carried out in accordance to the recommendations from the declaration of Helsinki and the internationally accepted principles in the use of experimental animals. In this study the subjected animals were defined as normal or parkinsonian groups, each group being divided into four sub-groups [each sub-group contained 6 rats] who received betamethasone or the vehicle.

Each rat was anesthetized separately by injection of 75 mg/kg ketamine combined with 8 mg/kg xylazin intraperitoneally. Then, the rats were prepared for surgery and placed within the stereotaxic appartus. The left SNc with the following coordinates was used: A/P -4.8 mm; M/L -1.6 mm and D/V +8.2, according to the atlas of Paxinos and Watson (11). Next, the stainless steel electrode was placed in the left SNc and destroyed it by the Electrical Lesion Maker (Siemens Company, Germany), using an electrical current (1 mA, 8 sec). This procedure resulted in the creation of parkinsonian rats (5, 12). the skull was then exposed and a hole was drilled through it in the area overlying the right striatum, using the following coordinates with respect to the bregma: A/P+1 mm; M/L+3 mm, D/V+6 mm, based on the atlas. A guide-cannula lowered into the brain for inserting the microdialysis probe which delivered a modified Ringer solution through the probe, was fixed to the cranium and the incision was closed. The typical position of probe placing in the striatum has been depicted in Figure 1. Surgery was performed using sterile instruments and under aseptic conditions. Rats were allowed to recover from the surgery over a period of 7-10 days. Microdialysis experiments were performed in normal and SNc-lesioned animals. On the day of experiment, a microdialysis probe was inserted into the cannula, and the inputs of the probes were connected to a microperfusion pump CMA/102 infusion pump, which delivered a modified Ringer solution (146 mM NaCl, 1.21 mM $CaCl_2$, 2.6 mM KCl, 1.01 mM $MgCl_2$ and 0.04 mM ascorbic acid) through the probe at a flow rate of 2 µl/min. Ringer solution was then infused for 3–3.5 h, before the collection of baseline samples, in order to obtain a stable basal extracellular level of glutamate. The microdialysate samples (20 µl) were collected every 20 min. When a stable outflow was shown by four consecutive samples of neurotransmitters, rats were given betamethasone (0.12, 0.24 mg/kg) and the vehicle, i.p. Also control rats received saline injection (1 ml/kg) i.p. Glutamate was analyzed by reverse-phase HPLC using a fluorimetric detector (13).

Results and Discussion

The average concentration of four stable samples before drug or vehicle [glycerin/ acetone] administration was considered as

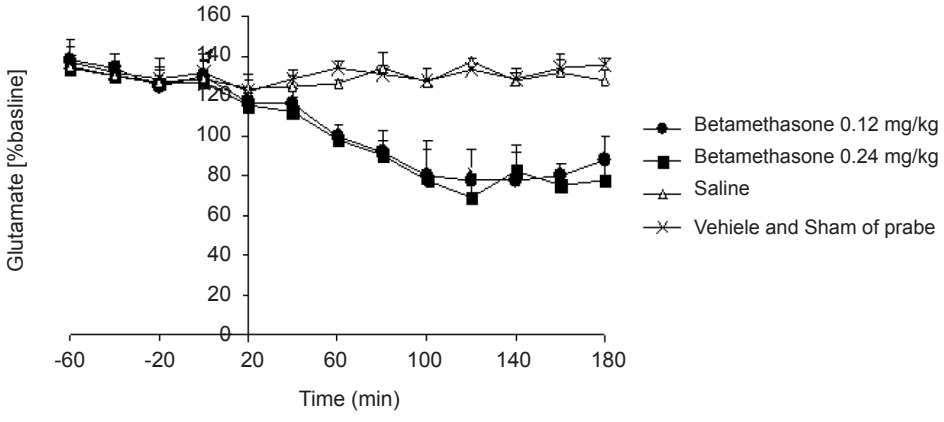

Figure 2. Effects of Betamethasone (0.12, 0.24 mg/kg) on glutamatergic neurotransmission. All the doses were found to significantly ($P<0.05$) diminish the striatal glutamatergic neurotransmission, especially during the 40-180 min period after betamethasone administration.

the basal glutamate level in SNc lesioned rats. Statistical evaluation of the results was performed by means of one-way analysis of variance (ANOVA) and Student-Newman-Keuls multiple range test, considering $P<0.05$ as the significant level ofdifference.

The striatal extraneuronal (i.e. microdialysate) level of glutamate in SNc-lesioned and normal rats of all the examined groups before drug-vehicle injection (baseline) were 2.91±0.38 pg/20 µl and 2.2±0.23 pg/20 µl, respectively. Betamethasone (0.12, 0.24 mg/kg) was observed to modify glutamate release in the striatum of parkinsonian rats, during the observation period, as depicted in Figure 2. Additionally the changes observed seen to be significant ($P<0.05$) for levels of glutamate on or after 60 min, up to 180 min. It should be noted that no significant changes in the striatal level of glutamate of normal rats were observed after administration of betamethasone. Moreover, glutamatergic neurotransmission was diminished by 17.82% (a range of 40-180) after administration of betamethasone compared with the normal rats, and 32.33% (a range of 40-180) after administration of betamethasone, in comparison to the parkinsonian rats.

The results indicated that changes in the dose of betamethasone have not affected the striatal glutamate level except after 120 and 180 min. These finding suggest another possible additional mechanism of betamethasone action on stiatal neurotransmission, wich is the decreasing levels of striatal glutamate. It seems that inflammation and its mediators such as prostaglandins (PGs) have an important role in neurotransmitter release, as noted in a previous report (14), suggesting that inflammation causes increased levels of acetylcholine in the brain via production of PGE2 and increases in expression of cholinergic markers, such as choline acetyl transfrase and vesicular acetylcholine transporter protein. It was also noted that prostaglandins have modulatory effects on adrenergic, noradrenergic and glutaminergic transmission, specially PGE2, and prostaglandin synthesis inhibitors induced increases in the blood pressure via increases in the release of the catecholamine: For instance, the use of large doses of glucocorticoids in human may cause insomnia, euphoria and an increased the intracranial pressure (14). Further studies are necessary in order to clarify the role of anti inflammatory agents such as betamethasone in the other striatal neurotransmissions.

Acknowledgments

The authors would like to sincerely thank and express their deep gratitude to Prof. Yi Fan [Laboratory of Neuropharmacology, Department

of Pharmacology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, China] for his kindness and invaluable collaborations, also permitting the use Figure 1 of his article [Neuroscience Letters, 422 (2007) 175–180].

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