Steroidal anti inflammatory drug betamethasone significantly alters level of striatal dopamine in a rat model of Parkinson's disease

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Abstract

Many scientific efforts have been well done to investigate the effects of anti inflammatory agents on the degenerative brain diseases such as Parkinson's (PD) or Alzheimer's disease and their affiliated sings. Previously we showed the effectiveness of steroids on rigidity of PD and in the study for further mechanistic investigation of that observation the microdialysis technique was employed to determine the striatal dopamine changes in parkinsonian rats after administration of betamethasone (0.12, 0.24 mg/kg) respectively. Our findings showed us the significant increase in the striatal dopaminergic neurotransmission ($P<0.05$) after administration of betamethasone comparing to the controls. These observations suggest a new mechanism for betamethasone on striatum dopaminergic neurotransmission leading us to gather further evidence about effectiveness of betamethasone in PD.

Keywords: Parkinson's disease, betamethasone, glutamate, microdialysis technique

Introduction

Parkinson's disease (PD) is a degenerative neurodopaminergic disease in the nigrostriatal pathway of humans/ animals, and the resultant losses of nerve terminals accompanied by dopamine deficiency in this pathway are responsible for most of the movement disorders. [1, 2] Increasing evidence suggests that an inflammatory reaction and pathological processes seen in many neurodegenerative disorders, including PD. [2] In cell culture and animal models, inflammation contributes to the neuronal damage and anti inflammatory agents such as dexamethasone has been shown to provide some neuroprotection or treatment of PD affiliated disorders such as rigidity by aspirin and betamethasone in animal model of PD. [3] In agreement the significant changes in the striatal neurotransmissions after the Cyclooxygenase-2 (COX-2) as an important component of the inflammation[4] but not COX-1 selective inhibition were observed. These researches showed the significant increase in dopaminergic or decrease in gamma amino butyric acid (GABA)ergic-glutamatergic neurotransmissions after only selective COX-2 inhibition, coincidently the PD affiliated disorders significantly improved. [5-6] Notified researches intended to explore the important role for inflammation especially COX-2 in PD affiliated disorders and Striatum neurotransmissions. The study of striatal dopaminergic interactions has special importance due to the physiological and pathophysiological processes of these systems, such as Parkinson's or Alzheimer's disease. [1, 2]

The present work studies, assay of neurotransmitter dopamine concentration in the striatum of parkinsonian rats following administration of steroidal anti inflammatory drug betamethasone.

Materials and Methods

Animals

Male albino Wistar rats were distributed into four groups that each group contained ten rats (200-250g). The animals were housed in stainless steel cages, handled daily, and provided food and water ad libitum. A 12h light/12-h dark cycle was maintained, and the animals were tested during the light cycle. These animal experiments were carried out in accordance with recommendations from the declaration of Helsinki and the internationally accepted principles in the use of experimental animals.

Surgery

Each rat was anesthetized separately by injection of 75 mg/kg ketamine combined with 8 mg/kg Xylazin intraperitoneally (i.p.). Then we prepared the rats for surgery and placed them in the stereotaxic instrument. The left SNc [A/P -4.8 mm; M/L -1.6 mm and D/V +8.2 mm for left SNc according to the atlas [7]] was destroyed using electrical lesion, 1 mA for 8 Sec [3] thus creating parkinsonian rats. Then the skull was 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 according to the atlas. [7] 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. Surgery was performed using sterile instruments and aseptic conditions. Rats were allowed to recover from the surgery for 7–10 days.

Microdialysis procedure

Betamethasone (0.12, 0.24 mg/kg) was administered i.p to parkinsonian rats and the microdialysate samples (20 µl) were collected every 20 min for 180 min after injection. Also control rats received saline injection (1 ml/kg) i.p. Dopamine was analyzed by reverse-phase HPLC with electrochemical detection. All samples were injected onto a reversed phase 5 µ C18 250 mm × 4.6 mm column, which was protected by Krudkatcher disposable pre-column filters and SecurityGuard cartridges. The mobile phase that was used on the ECD system was composed of a mixture of 0.11M di-sodium hydrogen orthophosphate/51 µM EDTA (pH 5.5, 1.1 M OPA) and HPLC grade methanol (32:61). The mobile phase was filtered through a Millipore 0.44 µm HV Durapore membrane filters and vacuum degassed prior to use. Dopamine concentrations were eluted isocratically over a 20 min runtime at a flow rate of 0.65 ml/min after a 20 µl injection. The column was maintained at a temperature of 30°C and samples/standards were kept at 4°C in the cooled autoinjector prior to analysis unless otherwise stated.

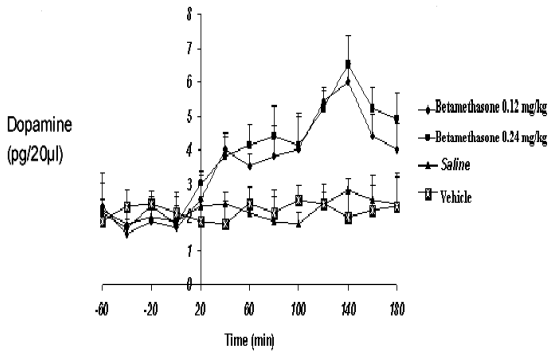

Figure 1: Effects of Betamethasone (0.12, 0.24 mg/kg) on dopaminergic neurotransmission. All doses were observed to be enhanced significantly P<0.05 the striatal dopaminergic neurotransmission especially during within 40-180 min after betamethasone administration.

Results

The results showed the significant increase in amount of dopamine P<0.05 after administering the betamethasone (0.12, 0.24 mg/kg). The results are shown as the mean ± S.E.M. relative to the basal levels. The average concentration of three stable samples before drugs or vehicle [glycerin-acetone] administration was considered as the basal levels. These basal levels were taken as 100% in order to compare the different response of neurotransmitter after drug administration. Statistical evaluation of the results was performed by means of one-way analysis of variance (ANOVA) and Student–Newman–Keuls multiple range test, considering the following significant differences: $p < 0.05$. The striatal extraneuronal (i.e. microdialysate) levels of dopamine in SNc-lesioned rats of all examined groups before drug-vehicle injection (baseline) were 2.3±0.62 pg/20µl, respectively. betamethasone (0.12, 0.24 mg/kg) were observed to modify dopamine release in the striatum during within the observation period, as depicted in Figure 1. Statistically, the aforementioned changes were shown to be significant (p<0.05) for levels of dopamine on or after 20 min throughout until 180 min (with the exception of betamethasone 0.12mg/kg affected levels of dopamine which were significant only on or after 40min). Moreover Dopaminergic neurotransmission was enhanced 33.82% [average of 40-180] after administration of betamethasone 0.12 mg/kg and 42.33% [average of 40-180] after administration of betamethasone 0.24 mg/kg comparing to the control groups.

Discussion

The study indicated the possibility of betamethasone to increase the release of dopamine in the striatal pathway, subsequently motivating the use of this agent to address improvements in brain degeneration such as PD, movement disorders effects as investigated previously [4, 8] The present study did not directly study improvements in movement disorders or the production of neuroprotective effects (our next area of investigation); at the same time, the present results hint at one possible mechanisms via which these effects can occur when steroidal anti inflammatory agents are used: increasing levels of striatal dopamine. It seems that inflammation and its mediators such as prostaglandins (PGs) have an important role in neurotransmitter release as noted in previous report,[8-11] 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 example using large doses of glucocorticoids in humans may cause insomnia, euphoria and increase the intracranial pressure [8-11].

Possible inflammatory components related to the PD and their exactly mode of actions should be elucidated by another future studies. Also it is necessary to clarify the role of steroidal anti inflammatory agents such as betamethasone on other striatal neurotransmissions.

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