Effects of glutamate and MK-801 on the metabolism of dopamine in the striatum of normal and parkinsonian rats

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Abstract: The direct effects of glutamate and dizocilpine maleate (MK-801, non-competitive N-Methyl-D-aspartate glutamate receptor antagonist) on the metabolism of dopamine were investigated in the striatum of normal and parkinsonian rats. L-dopa, L-glutamic acid and MK-801 were administered in the striatum locally by microdialysis. 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were simultaneously sampled by microdialysis. The concentrations of DOPAC and HVA were assayed by high performance liquid chromatography with electrochemical detection (HPLC-ECD). L-dopa increased the concentrations of DOPAC and HVA in the striatum of normal rats but not parkinsonian rats. MK-801 increased the concentrations of DOPAC and HVA in the striatum of normal rats but not parkinsonian rats. MK-801 prevented the L-glutamic acid-induced decrease of DOPAC and HVA in the striatum of normal rats. Our results indicate that glutamate modulates the metabolism of dopamine (DA) through NMDA receptors and that the improvement of PD by MK-801 is not through improving the metabolism of DA.

Key words: Parkinson's disease; microdialysis; rat; glutamate; dizocilpine maleate; 3,4-dihydroxyphenylacetic acid; homovanillic acid

谷氨酸和 MK-801 对正常和帕金森模型大鼠纹状体内多巴胺代谢的影响

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摘 要: 采用微透析和高效液相色谱-电化学 (HPLC-ECD)技术研究了谷氨酸和 MK-801 对正常和帕金森模型大鼠纹状体内多巴胺代谢的影响。用微透析技术在大鼠纹状体内分别定位给以左旋多巴、L-谷氨酸和/或 MK-801, 同时收集透析液, 用 HPLC-ECD 方法测定透析液中多巴胺代谢产物的浓度。微透析和 HPLC-ECD分析结果表明: 纹状体内定位给以左旋多巴, 正常大鼠和帕金森模型纹状体内多巴胺代谢产物的浓度均升高; 纹状体内定位给以 L-谷氨酸, 可使正常大鼠纹状体内多巴胺代谢产物的浓度降低, 但对帕金森大鼠模型纹状体内多巴胺代谢产物浓度的降低不显著; 纹状体内定位给以 MK-801, 正常大鼠纹状体内多巴胺代谢产物的浓度升高; 但对帕金森大鼠模型纹状体内多巴胺代谢产物浓度的升高不显著; 纹状体内同时定位给以 MK-801 和 L-谷氨酸, 可以有效防止 L-谷氨酸所致正常大鼠纹状体内多巴胺代谢产物浓度的降低。结果提示, 谷氨酸可以通过 NMDA 受体调节多巴胺的代谢。尽管非竞争性 NMDA 拮抗剂 MK-801 可以有效防止 L-谷氨酸所致正常大鼠纹状体内多巴胺代谢产物浓度的降低, 但却不能有效地改善帕金森大鼠模型纹状体内多巴胺的代谢水平。因此在正常及帕金森病情况下, 谷氨酸 - 多巴胺相互作用机制和 MK-801 改善帕金森病的机制还有待进一步研究。

关键词: 帕金森病; 微透析; 大鼠; 谷氨酸; MK-801; 3,4-二羟基苯乙酸; 高香草酸

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Parkinson's disease (PD) is characterized by the progressive loss of the dopaminergic neurons in the substantia nigra and a severe decrease in dopamine (DA) in the striatum[1]. Dopaminomimetic therapy is the most commonly...
available treatment for PD. The PD patients initially respond well to drugs such as levodopa or DA agonists that improve dopaminergic transmission. Later, however, the response to pharmacological agents of this type becomes increasingly less satisfactory, mainly due to the appearance of treatment-refractory signs (gait and balance disorders, freezing, dementia, and affective changes) and various adverse events, especially motor response complications. Most commonly, these later responses include various types of fluctuations and dyskinesias. This prompts the search for nondopaminergic therapies for PD in animal models and clinical patients to overcome these limitations.

It is well established that the striatum receives excitatory inputs from both the cortex and the subthalamic nuclei, and that glutamate is the transmitter released from all these excitatory fibres. Furthermore, there is evidence that the decreased dopaminergic innervation of the striatum in Parkinson’s disease is associated with over-activity of the above-mentioned excitatory systems. This thought contributes to the clinical manifestations of the disorder, a contention supported by the positive clinical effects obtained with the use of compounds capable of attenuating glutamatergic transmission. There is evidence that all three types of glutamate receptors are present on midbrain dopaminergic neurons. NMDA and DA receptors are co-expressed in close proximity along the distal dendrites of medium spiny neurons. NMDA glutamate receptors are a class of excitatory amino acid receptors, which have several important functions in the motor circuits of the basal ganglia, and are viewed as important targets for the development of new drugs to prevent or treat PD. NMDA receptors present in the striatum are crucial for dopamine-glutamate interactions.

The interaction between glutamate (Glu) and DA in vivo in the striatum of normal rats have been studied. In these studies, the pharmacological compounds (Glu-receptor agonists or inhibitor of Glu uptake) were perfused into the striatum of normal rats. It would be of interest to investigate the interactions of Glu itself and DA in normal and parkinsonian rats and further study the mechanisms of antiparkinsonian targeting of glutamatergic system.

Our present study was aimed to clarify the effects of L-glutamic acid and dizocilpine maleate (MK-801) on the metabolism of dopamine in the striatum of normal and parkinsonian rats. Also, the mechanisms of glutamate-dopamine interaction and the mechanisms of MK-801 for prevention or treatment of PD were investigated.

1 MATERIALS AND METHODS

1.1 Animals and reagents
Female Sprague-Dawley rats (320 ± 60) g for acute experiment, (200 ± 10) g for chronic experiment, Grade II, Certificate No SCUK11-00-0012 were housed at 20–22°C on a 12h light/12h dark schedule, with free access to food and water. DOPAC (3,4-dihydroxyphenylacetic acid), HVA (homovanillic acid), 6-OHDA (6-hydroxydopamine), L-dopa (3,4-dihydroxy-L-phenylalanine), L-glutamic acid and MK-801 were purchased from Sigma (USA). All other reagents were obtained from standard commercial sources.

1.2 Unilateral 6-OHDA lesions
Unilateral 6-OHDA lesions and rotational behavior assessment were performed as described previously. In brief, adult female Sprague Dawley rats (190–220 g) were injected unilaterally with 6-OHDA (24 mg/8 ml) of saline containing 0.1% ascorbic acid. Two stereotactic injections of 6-OHDA were made into the left mesostriatal dopaminergic pathway at the following coordinates (in mm, with reference to bregma and dura): (1) 4 µl of 6-OHDA at anteroposterior (AP), −4.0; lateral (L), −0.8; ventral (V), 8.0 [tooth bar (TB), +3.4]; (2) 4 µl of 6-OHDA at AP, −4.4; L, −1.2; V, 7.8 (TB, −2.4). One month later, the rats were tested with a subcutaneous injection of 0.1 mg/kg apomorphine, and the contralateral turns were recorded with automatic rotometers for 40 min. Only those rats consistently making at least 240 contralateral turns were used for our studies.

1.3 Microdialysis
The dialysis probes utilized in the present experiment were of the concentric type (0.5 mm diameter, CMA/12) with a molecular weight cut-off of 20 kD. All the dialysis membrane of the microdialysis probes was 4 mm or 2 mm. The probes were implanted in the striatum (ventral rostrum of caudate-putamen) of the rats under anesthesia (400 mg/kg chloral hydrate) at the following coordinates: +0.0 mm anterior, ±3.0 mm lateral to bregma (lesion side for parkinsonian rats), and −6.0 mm ventral from dura, with the incisor bar set at −2.4 mm below the intraaural line. For each individual experiment, the probe was constantly perfused with artificial cerebrospinal fluid (ACSF) (NaCl 145, CaCl2 1.2, KCl 2.8, MgCl2 1.2, D-glucose 5.4, ascorbic acid 0.25, pH 7.2–7.4, filtered through 0.22 µm filter) or 2 µmol/L L-dopa in ACSF, or 1 µmol/L L-glutamic acid in ACSF, or 5 µmol/L MK-801 in ACSF, or 5 µmol/L MK-801 and 1 µmol/L L-glutamic acid in ACSF at a rate of 1 µl/min. Samples were continuously collected into 500
µl Eppendorf microtubes containing 3 µl of 1.0 mol/L perchloric acid solution (containing 5 mmol/L Na₂EDTA, 0.1% L-cysteine) on ice and 2 samples were collected after stabilizing for 90 min under anesthesia. At the end of experiments, the rats were anesthetized with chloral hydrate and perfused intracardially with 0.9% saline followed by 10% formalin. The brain was removed and placement of the microdialysis probe was verified with a cryostat microtome and viewing lens.

1.4 HPLC
The high performance liquid chromatograph (HPLC) consisted of an online degassor (CMA, Sweden), a PM-80 pump and LC-4C electrochemical detector (BAS, USA) with a glassy carbon oxidative flow cell. The HPLC-ECD separation was a reverse phase ion-pair system. A reversed phase HPLC column-Phase II™ (ODS, 3.2 mm×10 mm, φ3 µm, BAS, USA) was used. Freshly prepared mobile phase buffer [0.05 mol/L citric acid, 0.05 mol/L sodium acetate, 1.0 mmol/L 1-octanesulfonic acid sodium salt monohydrate, 5 mmol/L triethylamine, 0.2 mmol/L Na₂-EDTA, 2% (v/v) methol, pH 3.6] was filtered through 0.22 µm filter. Chromatography was performed at 25ºC with a flow rate of 0.4 ml/min which produced a back pressure of about 1700 PSI. DA and its metabolites were subsequently determined electrochemically when the eluent passed into a glass carbon oxidative flow cell. The electrode potential was held at +0.7 V versus Ag/AgCl and a 10 nA range with a 0.1 Hz filter. A typical injection volume was 20 µl. A range of calibration standards were made up in each batch of samples. The amounts of DA, DOPAC and HVA in each sample were quantitated by comparing the peak area relative to the standards with electrochemical detection.

1.5 Statistics
The results were expressed as mean±SD and assayed by t-test (paired comparisons).

2 RESULTS
HPLC-ECD is suitable for the measurement of monoamines (Fig.1A, B). Components and their retention time in minutes are as follows: L-dopa 2.720; Noradrenaline 4.015; Adrenaline 5.688; 3,4-dihydroxyphenylacetic acid 10.027; Dopamine 13.297; 5-hydroxyindoleacetic acid 19.869; Homovanillic acid 29.414; 5-hydroxytryptamine 41.148.

The placement of the microdialysis probe is in the ventral rostrum of caudate-putamen (Fig.2).

The effects of L-dopa, L-glutamic acid and MK-801 on extracellular concentrations of DOPAC and HVA were shown from Fig. 4 to Fig. 8. L-dopa (2 µmol/L, dissolved in ACSF), L-glutamic acid (1 mmol/L, dissolved in ACSF), and MK-801 (5 µmol/L, dissolved in ACSF) were locally perfused into the striatum of normal and parkinsonian rats by microdialysis. The dialysate concentrations of DOPAC and HVA in the striatum of Parkinsonian rats were decreased over 98% (n=30, P<0.001) (Fig. 3). L-dopa increased the dialysate concentrations of DOPAC (n=22, P<0.05) and HVA (n=22, P<0.01) in the striatum of normal rats. L-dopa increased the dialysate concentrations of DOPAC (n=8,
Fig. 3. Concentration (nmol/L) of DOPAC and HVA in the striatum of normal \((n = 42)\) and parkinsonian rats \((n = 30)\). ***\(P<0.001\) (6-OHDA vs normal).

Fig. 4. Effects of L-dopa \((n = 22)\), L-glutamic acid \((n = 11)\) and MK-801 \((n = 10)\) on DOPAC in the striatum of normal rats. Pre-, before administration of drugs; Post-, after administration of drugs. *\(P<0.05\) (post-vs pre-), ***\(P<0.001\) (post-vs pre-).

Fig. 5. Effects of L-dopa \((n = 22)\), L-glutamic acid \((n = 11)\) and MK-801 \((n = 10)\) on HVA in the striatum of normal rats. Pre-, before administration of drugs. Post-, after administration of drugs. *\(P<0.05\) (post-vs pre-); **\(P<0.01\) (post-vs pre-).

Fig. 6. Effects of MK-801 \((n = 10)\), MK-801+L-glutamic acid \((n = 10)\) on DOPAC and HVA in the striatum of normal rats. *\(P<0.05\) (MK-801 vs ACSF); ***\(P<0.001\) (MK-801+Glu vs ACSF).

Fig. 7. Effects of L-dopa \((n = 8)\), L-glutamic acid \((n = 10)\) and MK-801 \((n = 9)\) on DOPAC in the striatum of parkinsonian rats. Pre-, before administration of drugs; Post-, after administration of drugs. ***\(P<0.001\) (Post-vs Pre-).

Fig. 8. Effects of L-dopa \((n = 8)\), L-glutamic acid \((n = 10)\) and MK-801 \((n = 9)\) on HVA in the striatum of parkinsonian rats. Pre-, before administration of drugs; Post-, after administration of drugs. **\(P<0.01\) (Post-vs Pre-).
\( P<0.001 \) and HVA \((n=8, P<0.01)\) in the striatum of parkinsonian rats. L-glutamic acid decreased the dialysate concentrations of DOPAC \((n=11, P<0.001)\) and HVA \((n=11, P<0.05)\) in the striatum of normal rats but not parkinsonian rats \((n=10, P>0.05)\). MK-801 increased the dialysate concentrations of DOPAC and HVA in the striatum of normal rats \((n=10, P>0.05)\) but not parkinsonian rats \((n=9, P>0.05)\). MK-801 could prevent the decreases of DOPAC and HVA caused by L-glutamic acid (Fig. 5).

3 DISCUSSION

In the studies of other authors, pharmacological compounds (Glu-receptor agonists or inhibitor of Glu uptake)\(^{15,16}\) were used to study the interaction of Glu and DA in the striatum of normal rats. However, there are few studies investigating the interaction of Glu and DA and its changes in the striatum of parkinsonian rats. Recently, herbicide paraquat (involved in the etiology of PD), 6-OHDA (used in parkinsonian rats), and L-DOPA (the current mainstay drug in PD treatment) have been reported to increase glutamate\(^{20,22}\) in the striatum. So we thought that the Glu-DA interactions or its changes might be important in the pathogenesis of PD and its treatment. L-glutamic acid mimics the action of Glu better than those pharmacological compounds under normal and some pathophysiological conditions. We therefore used L-glutamic acid to study the interaction of Glu and DA in the striatum of normal and parkinsonian rats.

In the present study the microdialysis and HPLC-ECD were used to measure the extracellular concentrations of DOPAC and HVA in the striatum of normal and parkinsonian rats under anesthesia. ACSF, L-dopa (2 µmol/L, dissolved in ACSF), L-glutamic acid (1mmol/L, dissolved in ACSF), and MK-801 (5 µmol/L, dissolved in ACSF) were perfused in the striatum (ventral rostrum of caudate-putamen). This approach allowed us to determine the effects of L-dopa, L-glutamic acid and MK-801 on the metabolism of dopamine.

In our study, local administration of L-dopa increased the concentrations of DOPAC and HVA (the major metabolites of DA, which closely linked with the concentration of DA) in the striatum of normal and parkinsonian rats. However the increased concentrations of DOPAC and HVA in the striatum of parkinsonian rats were still lower than normal concentrations. The increased concentrations of DOPAC and HVA could not efficiently improve the motor function of the PD rats. For the parkinsonian rats, local administration of L-dopa was not fully metabolized to DA due to the lower activity of AADC\(^{19}\), which catalyzes the product of DA from L-dopa.

In our results, intrastratial infusion of L-glutamic acid \((1 \text{mmol/L, which is in excess of normal extracellular levels} - 1 \text{to} 3 \mu\text{mol/L})\) decreased the concentrations of DOPAC and HVA in the striatum of normal rats but not parkinsonian rats. Local administration of MK-801 \((5 \mu\text{mol/L})\) in the striatum of normal rats could effectively prevent the decreases of DOPAC and HVA from the excessive L-glutamic acid infusion. The results indicate that glutamate modulates the metabolism of DA through NMDA receptors under normal condition. Local administration of MK-801 in the striatum of normal rats increased the concentrations of DOPAC and HVA but not parkinsonian rats. The results suggested that MK-801 enhanced the metabolism of DA in normal rats, but could not improve the concentrations of DA and its metabolism of parkinsonian rats. The mechanisms of Glu-DA interaction in normal rats and parkinsonian rats may be different. The prevention or treatment by MK-801 PD is possibly not through improving the metabolism of dopamine. The mechanism needs to be further investigated. Whether MK-801 exerts its effect through the over-activity of glutamate in the striatum or through its neuroprotective effect is to be further studied.

The data presented in our study suggest that glutamate modulates the metabolism of DA through NMDA receptors in the striatum of normal rats but not parkinsonian rats. The mechanism of MK-801 for the treatment of PD is not through improving the metabolism of DA. Clearly, the need for improved palliative treatments for PD continues is to be a crucial goal for pharmacological discovery. The precise mechanisms of antiparkinsonian targeting of glutamatergic system need to be further investigated. Also, considerable additional investigative efforts are required to clarify the Glu-DA interactions and the interactions among other neurotransmitters in the striatum of patients with PD.

REFERENCES

4. Parain K, Hapdey C, Rousselet E, Marchand V, Dumery B, Hirsch EC. Cigarette smoke and nicotine protect dopaminergic...

5 Chen JF. The adenosine A(2A) receptor as an attractive target for Parkinson's disease treatment. Drug News Perspect 2003; 16(9): 597-604.


