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l-Stepholidine facilitates inhibition of mPFC DA receptors on subcortical NAc DA release[1]

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KEY WORDS stepholidine; dopamine receptors; dopamine; microdialysis; amphetamine; prefrontal cortex; nucleus accumbens; 6-hydroxydopamine

ABSTRACT

AIM: To determine whether the D_1 agonistic action of (-)-stepholidine (SPD) on the medial prefrontal cortex (mPFC) neuron is involved in the modulation of evoked subcortical dopamine (DA) release from nucleus accumbens (NAc) of rats. **METHODS**: With the microinjection of SPD into the mPFC, the ventral tegmental area (VTA)-stimulated or amphetamine (AMP)-evoked DA efflux in the NAc was detected by microdialysis + HPLC-ECD in the 6-hydroxydopamine (6-OHDA)-lesioned and intact rats. **RESULTS**: The depletion of DA in the mPFC did not modify both the basal level and the VTA-stimulated DA efflux in the NAc, but significantly facilitated the AMP (20 $\mu mol \cdot L^{-1}$)-evoked DA efflux within the NAc. It indicates that the mPFC DA system is involved in the regulation of evoked DA release in the NAc. Besides, the AMP-evoked increase of the extracellular DA release in the NAc was significantly attenuated by SPD (10, 30 $mmol \cdot L^{-1}$) microinjection into the mPFC, though this injection of SPD could not alter the response of DA release by the stimulation of the VTA. Furthermore, the inhibitory effect of SPD on the AMP-evoked DA efflux could be partially reversed by intravenous administration of D_1 antagonist Sch-23390 (1 $mg \cdot kg^{-1}$), but not by D_2 antagonist spiperone. **CONCLUSION**: SPD is capable of enhancing the function of D_1 receptors in the mPFC, by which it facilitates the inhibition of DA release in the NAc.

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INTRODUCTION

The nucleus accumbens (NAc), a dopamine (DA) enriched mesolimbic nucleus, is a crucial site involved in the pathophysiology of schizophrenia with a hyperdopaminergic state[1]. While the NAc receives a major inhibitory input from the A_{10} DA neurons originated in the ventral tegmental area (VTA), it also receives excitatory amino acid (EAA) afferents from the medial prefrontal cortex (mPFC)[2,3]. There is increasing evidence to suggest that failure of the mPFC activation in schizophrenic patients is due to hypoactivity of the meso-cortical DA innervation which, in turn, leads to hyperactivity of meso-NAc DA neurons[4,5]. Consistent with the viewpoint, previous studies utilizing 6-hydroxydopamine (6-OHDA) lesion in the area of the mPFC have clearly demonstrated that subcortical DA enriched areas, in particular the NAc, can be regulated by the meso-cortical DA pathway, which exerts an inhibitory influence on subcortical DA transmission[6,7].

Previous studies have demonstrated that (-)-stepholidine (SPD) possesses dual actions on DA receptors, ie it is an "agonist to D_1 receptor and antagonist to D_2 receptor"[8,9]. Our recent work has shown that the agonistic action of SPD on mPFC D_1 receptors markedly increased the firing activity of NAc neurons[10]. This excitatory effect might be due to the disinhibition of NAc neurons by the mPFC-NAc pathway underlying the modulation of the tonic DA release from the terminals of the meso-NAc DA neurons. It has been reported that the D_1 receptor agonist SKF-38393 tended to decrease the NAc DA response to stress by the activation of mPFC projected neuron[11]. To confirm the possibility that the activation of mPFC DA receptors by SPD is involved in modulating the response of subcortical DA release to stimuli, we used microdialysis to examine the effect of SPD in the mPFC on both VTA-stimulated and amphetamine-evoked DA ef-

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flux of the NAc in the 6-OHDA-lesioned and intact rats.

MATERIALS AND METHODS

Chemicals SPD (Shanghai Institute of Materia Medica), mp 161 ~ 162 ℃, $[\alpha]_D$-440° in pyridine, was dissolved in a small amount of H_2SO_4 0.1 mol·L^{-1}, then diluted with distilled water and adjusted with 0.1 mol·L^{-1} NaOH to pH 5.5. The other drugs used in this study were (±)-SKF-38393·HCl, Sch-23390·HCl and spiperone·HCl (Research Biochemicals Incorporated, USA); 6-OHDA·HBr, desipramine·HCl and *d*-amphetamine (AMP) were purchased from Sigma; the dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were obtained from Fluka (Buchs, Switzerland). Each drug was given as the weight of its salt.

6-OHDA lesion of mPFC Sprague-Dawley rats (♂, 180 – 200 g, Grade Ⅱ Shanghai Experimental Animal Center, Certificate № 005) were pretreated with the norepinephrine uptake blocker, desipramine (25 mg·kg^{-1}, ip) and anesthetized with pentobarbital (40 mg·kg^{-1}, ip) 30 min later. A glass pipette (tip OD = 100 – 150 μm) was positioned in the mPFC using the following coordinates: AP + 3.2 mm, ML ± 0.7 mm from bregma and DV – 3.2 mm from dura[12]. The pipette was left in position for 4 min and then 2 μg 6-OHDA in 2 μL of vehicle (0.9 % NaCl containing 0.03 % ascorbic acid) was infused into each hemisphere over a 4-min period with pressure ejection. The pipette was left in position for additional 4 min to allow for dispersal of the toxin. After surgery, rats were caged for recovery for 13 – 15 days.

Guide cannula placement and Brain microdialysis After the lesioned and naïve rats were anesthesized with chloralhydrate (400 mg·kg^{-1}, ip), both guide cannula (22 gauge) and concentric-style microdialysis probe (the active dialysing area: (0.12 × 2) mm, provided by Yale University) were separately implanted into the mPFC (AP + 3.2 mm, ML 0.8 mm, DV – 3.2 mm) and the NAc (AP + 1.7 mm, ML 1.4 mm, DV – 7.5 mm); and then fixed to the skull by dental cement and screws. The microdialysis probe was connected to an infusion pump (B Braun Melsungen AG, Germany) via a length of polyethylene tubing. The rats were perfused at a rate of 1.5 μL·min^{-1} with artificial cerebrospinal fluid (pH 7.4) containing: NaCl 145, KCl 2.7, $MgCl_2$ 1.0, $CaCl_2$ 1.2, glucose 5.4 and Vit C 0.125 (mmol·L^{-1}). After 2-h continuous perfusion with the fluid, two basal dialysate samples were collected (15 min sampling intervals), and then followed by 10 min perfusion of *d*-amphetamine (AMP, 20 μmol·L^{-1}) and an additional 30-min sampling. Dialysate samples were immediately injected into a liquid chromatography (CC-5, Bioanalytical Systems, West Lafayette, IN, USA) with electrochemical detection (LC-44, BAS) (HPLC-ECD).

For intracranial injection, the drug solutions or vehicle (all at pH 5.5) were injected at a constant rate of 0.5 μL over 1 min via a stainless steel internal cannula, which was connected to a syringe infusion pump. SPD and D_1 antagonist Sch-23390 or D_2 antagonist spiperone (dissolved in NN-Dimethyl formamide solution) were made fresh on the day of the experiment.

Electrical stimulation of VTA Stimulation was accomplished with a bipolar tungsten electrode (gifted by Prof LI Chao-Yi, Shanghai Institute of Physiology), with 200 μm tips separated by approximately 100 μm. Square pulses (300 μA, 0.5 ms) were generated by a electronic stimulator (SEN-7103, Japan) via an isolator to the VTA (AP – 5 mm, ML ± 0.8 mm, DV – 7.8 ~ – 8.2 mm). The main stimulation parameters included: train × 10, interval × 50 ms, cycles × 60, main interval × 1 min for total 10 min of the episodes.

HPLC conditions The effluxes of dopamine and its metabolites were measured by HPLC-ECD. A phase-Ⅱ ODS column (3 m, 100 × 3.2 mm ID, BAS) was used for all separations with flow rate maintained at 0.4 mL·min^{-1} (PM-80, BAS). The potential of the electrode was set at + 750 mV, and the column temperature was controlled at 35 ℃ (LC-22C). Every litre of mobile phase consisted of NaH_2PO_4 14.5 mmol·L^{-1}, sodium citrate 30 mmol·L^{-1}, Na_2EDTA 27 μmol·L^{-1}, diethylamine·HCl 10 mmol·L^{-1} and sodium octyl sulfate 2.2 mmol·L^{-1}, 80 mL acetonitrile and 10 mL tetrahydrofuran (pH adjusted to 3.4 with H_3PO_4).

Histology At the end of the experiments, all the animals were deeply anesthetized with sodium pentobarbital (70 mg·kg^{-1}, ip) and transcardially perfused with saline followed by 10 % formalin. Brains were removed and sliced in 40 μm sections for verification of probe, electrode or cannulae placements.

Statistical analysis Data were expressed as $x \pm s$. The percent changes of DA peak area from baseline (pg·20 μL^{-1}) were calculated by ChromGraph (BAS) analysis software. Statistical analyses were per-

formed using an analysis of variance and student's t-test to identify significant differences between experimental groups.

RESULTS

Effect of mPFC DA depletion on basal DA level in the NAc After 2 weeks of the mPFC 6-OHDA lesion, the basal concentration of either extracellular DA or HVA in the NAc was not significantly changed in comparison with that of intact rats (Tab 1). The average concentrations of these samples of NAc microdialysate were represented as a baseline prior to drug administration or pre-stimulation level in rats.

Tab 1. Effect of mPFC 6-OHDA lesion on basal extracellular levels of both DA and HVA in the NAc microdialysate of rats $\bar{x} \pm s$, $^aP < 0.05$ *vs* intact group).

Groups	n	Changes of DA and HVA peak area in the NAc	
		DA (pg·20 μL^{-1})	HVA (pg·20 μL^{-1})
Intact	8	86 ± 7 (7.4 ± 0.3)	147 ± 10 (12.8 ± 0.4)
Lesioned	34	98 ± 6 (8.5 ± 0.2)^a	165 ± 14 (14.3 ± 0.6)
Baseline	42	92 ± 7	156 ± 12

Effects of 6-OHDA lesions of the mPFC on both VTA-stimulated and AMP-evoked DA release in the NAc After 10 min electrical-stimulation of VTA, the DA level of NAc microdialysate was higher than that of the pre-stimulus for both the 6-OHDA lesioned and intact rats ($F = 7.82$, $F = 3.95$; $P < 0.01$ *vs* baseline), but there was no significant difference between the two groups (Fig 1). However, compared with the changes in DA release in VTA-stimulated groups, the increase of DA efflux evoked by intraaccumbens perfusion of amphetamine (AMP, 20 μmol·L^{-1}) for 10 min in all the tested rats was more potent, and in particular, extremely significant in the mPFC 6-OHDA lesioned rats ($F = 56.71$, $F = 113.73$; $P < 0.01$ *vs* baseline, $P < 0.05$, $P < 0.01$ *vs* VTA-stimulated group) (Fig 2).

Effects of SPD microinjected into the mPFC on either VTA-stimulated or AMP-evoked DA release in the NAc Microinjection of SPD (30 mmol·L^{-1}, μL) into the mPFC did not alter the DA efflux of the NAc induced by electrical stimulation of VTA (Fig 1). However in the mPFC 6-OHDA-lesioned rats, the same treatment by SPD (10, 30 mmol·L^{-1}) significantly attenuated the effects of AMP on extracellular DA in the NAc ($F = 11.42$, $F = 3.74$; $P < 0.01$ *vs* baseline).

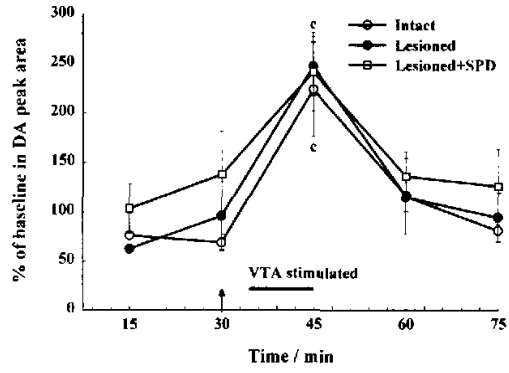

Fig 1. Effect of electrical stimulation (300 μA, 10 Hz, 10 min) at the VTA on extracellular dopamine level in NAc microdialysate of rats with or without mPFC 6-OHDA-lesion. Arrows indicate microinjection of *l*-SPD (30 mmol·L^{-1}) into the mPFC. Bar represents the duration of VTA stimulated. $^cP < 0.01$ *vs* baseline.

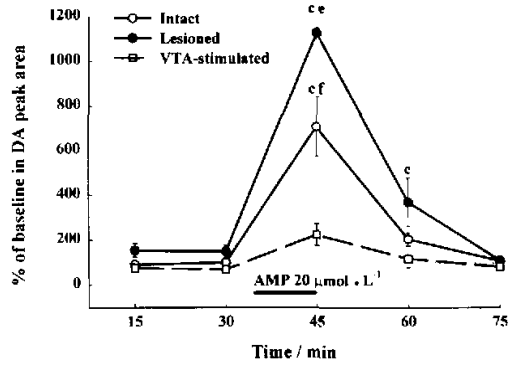

Fig 2. Effect of amphetamine (AMP, 20 μmol·L^{-1}) perfusion on dopamine efflux in NAc microdialysate of rats with or without mPFC 6-OHDA-lesion. Bar represents the duration of AMP-perfusion. $^cP < 0.01$ *vs* baseline. $^eP < 0.05$, $^fP < 0.01$ *vs* VTA-stimulated group.

The inhibitory effect of SPD (30 mmol·L^{-1}) on AMP-evoked DA efflux from NAc was very significant as compared to the vehicle ($t = 4.20$, $P < 0.01$ *vs* vehicle) (Fig 3).

Roles of DA antagonists on the inhibition of SPD for AMP-evoked DA release in the NAc In order to ascertain actions of DA receptor subtypes underlying the modulation of SPD on the inhibition of DA release in the NAc, either selective D_1 or D_2 antagonists

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were injected via the tail vein just as SPD was microinjected into the mPFC. The inhibitory effect of SPD (30 mmol·L^{-1}) on the AMP-evoked DA efflux of NAc could be partially reversed by D_1 antagonist Sch-23390 (1 mg·kg^{-1}, iv), but not by D_2 antagonist spiperone (1 mg·kg^{-1}), ($F = 22.09$, $P < 0.01$ *vs* baseline) (Fig 4).

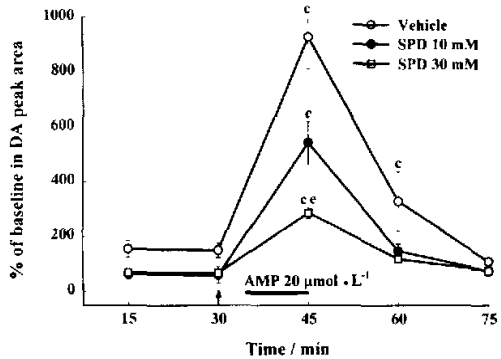

Fig 3. Effect of microinjected SPD (10, 30 mmol·L^{-1}) into the mPFC on AMP-induced dopamine efflux in NAc microdialysate of rats with the mPFC 6-OHDA-lesion. Arrow indicates the drug given in the mPFC. Bar represents the duration of AMP-perfusion. $^cP < 0.01$ *vs* baseline; $^eP < 0.05$ *vs* vehicle.

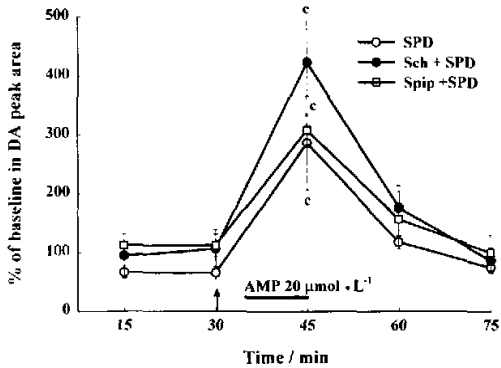

Fig 4. Effect of D_1 antagonist Sch-23390 (1 mg·kg^{-1}, iv) and D_2 antagonist spiperone (1 mg·kg^{-1}, iv) on the AMP-induced dopamine efflux in NAc microdialysate of rats with the mPFC 6-OHDA-lesion, which were pretreated with SPD (30 mmol·L^{-1}) microinjected into the mPFC. Arrow indicates the injection of DA antagonists from the tail vein. Bar represents the duration of AMP-perfusion in the NAc. $^cP < 0.01$ *vs* baseline.

DISCUSSION

The present study showed that disruption of the dopaminergic innervation of the mPFC by 6-OHDA did not modify both the basal level and the VTA-stimulated DA outflow in the NAc, but significantly facilitated the amphetamine-evoked DA efflux within the NAc. This evidence implies that the mPFC DA system is involved in the modulation of evoked DA release in the NAc, which is consistent with other recent reports suggesting that DA depletion of the mPFC enhances the effects of stress or amphetamine on extracellular DA in the NAc[4,6]. These findings lead to the hypothesis that the mPFC DA system exerts an inhibitory influence on subcortical DA transmission. Once there is DA loss in the mPFC, it might lower the threshold for stress- or amphetamine-induced activation of the mesoaccumbens DA system[13]. Our current data significantly revealed that the AMP-evoked DA efflux of the NAc in mPFC 6-OHDA-lesioned rats was more potent than that in the intact rats. While, the 6-OHDA lesion of the mPFC did not alter the DA release induced by stimulation of the VTA in comparison with the intact group, which may be due to the compensatory mechanisms of the meso-cortico-limbic DA system. Since the 6-OHDA lesion in the mPFC causes retrograde degeneration of DA axons and cell bodies, the loss of DA cell bodies in the VTA may decrease somatodendritic DA release and thereby alter the activity or sensitivity of remaining DA cells projecting to the NAc to stimuli[14].

Under the conditions of DA depletion in the mPFC, the inhibitory modulation of mPFC DA system on the VTA-NAc DA system was attenuated. Thus, the increase in the evoked DA release by AMP infusion into the NAc followed. However, the local administration of SPD in the mPFC significantly antagonized the effect of AMP on extracellular DA in the NAc, which could be partially reversed by D_1 antagonist Sch-23390 suggesting that SPD facilitates the inhibitory action of mPFC neurons on the activity of the VTA-NAc DA system. The D_1 receptors in the mPFC may be involved in the modulation of subcortical DA release activity. In addition, the evidence that microinjection of SPD in the mPFC had no effect on the VTA-stimulated DA efflux of the NAc, suggests that the effect of SPD in the mPFC on the DA release of NAc should be more possibly linked to the neural pathways of the mPFC-NAc, rather than the mPFC-VTA projection system. Based on our recent results showing that SPD possesses D_1 agonistic action on the firing activity of the mPFC neurons[10], it can be deduced that SPD inhibits the activity of mPFC neurons projecting to the NAc via the mPFC D_1 receptors, which in turn depress

the subcortical DA release activity. This would be more benificial for the treatment of schizophrenia.

In conclusion, SPD is capable of enhancing the function of D_1 receptors in the mPFC, which facilitate the inhibition of DA release from the NAc by the modulation of mPFC neurons.

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左旋千金藤立定增强 mPFC DA 受体对皮层下 NAc DA 释放的抑制作用[1]

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关键词 千金藤立定；多巴胺受体；多巴胺；微透析；苯丙胺；前额皮层；伏隔核；6-羟基多巴胺

目的：研究左旋千金藤立定（SPD）激动内侧前额皮层（mPFC）D_1 受体对皮层下伏隔核 DA 诱发释放的影响. **方法**：6-羟基多巴胺损伤大鼠 mPFC 两周后，同侧皮层内微量注射 SPD，微透析检测电刺激中脑腹侧被盖区（VTA）或苯丙胺（AMP）诱导的 NAc DA 释放. **结果**：mPFC DA 耗竭未改变 NAc DA 的基础水平和电刺激 VTA 诱发的 DA 释放，却明显易化 AMP 灌流诱发的 NAc DA 释放，表明 mPFC DA 系统参与调节 NAc DA 的诱发释放. mPFC 内微量注射 SPD 未能改变电刺激 VTA 诱发的 DA 释放，但显著减弱 AMP 对 NAc DA 的诱发释放；该作用可被 D_1 拮抗剂 Sch-23390 部分翻转，而 D_2 拮抗剂 spiperone 无作用. **结论**：SPD 强化 mPFC D_1 受体对皮层下伏隔核 DA 释放的抑制性调节作用.

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