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# (-)-Stepholidine antagonizes the inhibition by $D_2$ receptor agonists on synaptosomal tyrosine hydroylase in rat corpus striatum[1]

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**AIM** To evaluate the action of (-)-stepholidine (SPD), a novel dopamine (DA) receptor antagonist, on inhibition by presynaptic $D_2$ receptor agonists on the activity of synaptosomal tyrosine hydroxylase (TH) in the striatum. **METHOD:** The TH activity was measured by HPLC-ECD. **RESULTS:** The mixed DA receptor agonist apomorphine (Apo 10.4 nmol $L^{-1}$) and selective $D_2$ receptor agonists N-0437 (0.4 pmol $L^{-1}$) and quinpirole (0.8 μmol $L^{-1}$) inhibited the activity of TH, while selective $D_1$ receptor agonist SKF 38393 (0.001-10 μmol $L^{-1}$) and SPD (1-100 μmol $L^{-1}$) failed to inhibit the TH activety. The inhibition of N-0437 and quinpirole were antagonized by $D_2$ receptor antagonist spiperone and SPD. **CONCLUSION:** The negative feedback regulation of presynaptic DA receptors is mediated via $D_2$ receptors and SPD is an antagonist on presynaptic $D_2$ DA receptors.

**KEY WORDS** berbines; (-)-stepholidine; spiperone; feedback regulation; dopaminergic agents; tyrosine hydroxylase; corpus striatum; synaptosomes

The striatal dopaminergic terminals possess dopamine (DA) autoreceptors or presynaptic DA receptors which mediate the negative feedback regulation on DA synthesis[1,2]. Tyrosine hydroxylase (TH) which is the rate-limiting enzyme in the synthesis of DA[3]. Stepholidine (SPD) reverses the negative feedback regulation of presynaptic DA receptors and increases the formation of *L*-DOPA *in vivo*[4]. In the present paper, we studied 1) the efffects of some DA receptor agonists and antagonists on the activities of rat striatal synaptosomal TH to clarify the relationship between presynaptic DA receptors and the negative feedback regulation on DA biosynthesis, and 2) the effects of SPD on the activity of synaptosomal TH from rat striatum to elucidate the mechanism of SPD in reversing the negative feedback regulation of presynaptic DA receptors on DA biosynthesis.

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## MATERIALS AND METHODS

**Reagents** SPD ($[\alpha]_D$-440° in pyridine) was isolated from *Stephania intermedia* Lo in our Institute[5]. Apomorphine (Apo), 2-(*N*-propyl-*N*-2-thienyl-ethylamino)-5-hydroxytetralin (N-0437), quinpirole hydrochloride (LY-171555), (±)-1-phenyl-2, 3, 4, 5-tetrahydro-(1*H*)-3-benzazepine-7, 8-diol (SKF 38393), spiperone (Research Biochemicals Inc, USA); *L*-DOPA, *DL*-6-methyl-5, 6, 7, 8-tetrahydropterin dihydrochloride (6-MPH), and catalase (Sigma, USA); β-mercaptoethanol (Shanghai Fourth Reagent Factory, China); *L*-tyrosine (Shanghai Institute of Biochemistry, Chinese Academy of Sciences, China); 3-hydroxybenzylhydrazin dihydrochloride (NSD-1015, Aldrich Chemie, Germany); *D*-camphor-10-sulfonic acid (CSA, BDH Chemicals Ltd, England). Other chemicals used were AR.

**Rats** Sprague-Dawley rats (237 ± *s* 19 g, ♂, Shanghai Laboratory Animal Center, Chinese Academy of Sciences, China) were used.

**Striatal synaptosomes** After decapitation of the

rat, the striatum were excised and put into ice-cold homogenizing medium (sucrose 0.32 mol $L^{-1}$, β-mercaptoethanol 2 mmol $L^{-1}$, and Tris-HCl 20 mmol $L^{-1}$, pH 7.45). Each preparation was pooled from 10 rats. Synaptosomes were prepared[6].

**TH assay** TH activity was assayed by a HPLC-ECD (high performance liquid chromatography with electrochemical detector) method. An aliquot of 50 μL of synaptosomal suspension was added into a glass tube. The assay of TH started with the addition of 50 μL of reaction medium containing acetate buffer 0.2 mol $L^{-1}$(pH 7.0), 6-MPH 0.5 mol $L^{-1}$, catalase 2500 U, *L*-tyrosine 0.4 mmol $L^{-1}$, and NSD-1015 1 mmol $L^{-1}$. Enzymatic reaction proceeded for 10 min at 37 ℃ until the addition of 100 μL of $HClO_4$ 1 mol $L^{-1}$ containing edetic acid 0.1 mmol $L^{-1}$ and 0.1 % $Na_2S_2O_5$. Reaction blanks were made using Tris-HCl 20 mmol $L^{-1}$(pH 7.0) instead of synaptosomal suspension. $HClO_4$ 1 mol $L^{-1}$ containing various concentrations of *L*-dopa was added in the blank reaction as standard. The reaction mixtures were centrifuged at 8000×*g* for 10 min. The resulting supernatants were used to elute[7] and assay the *L*-DOPA.

**HPLC-ECD** The HPLC system consisted of Model 510 pump (Waters), a Model U6K Universal Injector (Waters), and 2 reversed-phase Linchrosob C8 columns (50 mm×4 mm, 200 mm×4 mm, 5 μm). The column elute was measured with a Waters 460 Electrochemical Detector. The detector potential was set at 0.7 V. The mobile phase was a degassed mixture of $ClCH_2COOH$ 0.16 mol $L^{-1}$, NaOH 100 mmol $L^{-1}$, edetic acid 0.1 mmol $L^{-1}$, CSA 25 mmol $L^{-1}$, and methanol 10 % (pH 2.8). The flow rate was 1 mL $min^{-1}$.

**Protein assay** Protein of synaptosomes was determined colorimetrically[8]. Bovine serum albumin was used as standard.

**Statistical analysis** The means $\bar{x} \pm s$ of the groups were compated with *t*-test.

## RESULTS

**Inhiition of $D_2$ receptor agonists on synaptosomal TH** Striatal synaptosomes were preincubated at 37 ℃ for 5 min with Apo, SKF 38393, N-0437, and quinpirole prior to the addition of reaction medium. Apo, N-0437 (Fig 1), and quinpirole (Tab 1) inhibited the activities of synaptosomal TH in a concentration-dependent manner with $IC_{50}$ values of 10.4 nmol $L^{-1}$, 0.4 pmol $L^{-1}$, and 0.8 μmol $L^{-1}$, respectively.

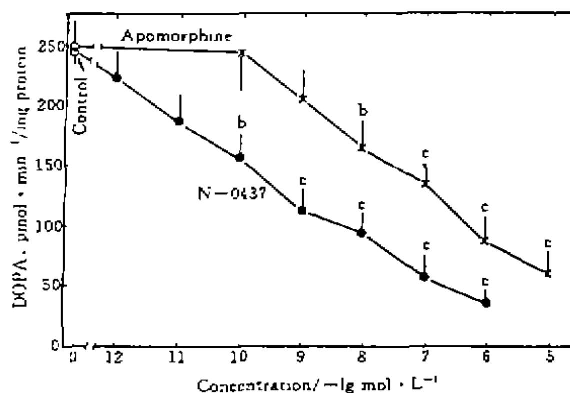


**Fig 1. Effects of Apo and N-0437 on activity of synaptosomal tyrosine hydroxylase (TH) from rat striatum. $n=4$ homogenates (each was pooled from 10 rats and assayed in triplicate). $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* control.**

**Tab 1. Activity of synaptosomal tyrosine hydroxylase (DOPA/%) after quinpirole hydrochloride with/without SPD (100 μmol $L^{-1}$). $n=4$ homogenates (each was pooled from 10 rats and assayed in triplicate). $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* control. $^eP<0.05$, $^fP<0.01$ *vs* corresponding quinpirole.**

| Quinpirole/ μmol $L^{-1}$ | DOPA/% No SPD | SPD |
|---|---|---|
| 0 | 98±4 | 98±4 |
| 0.05 | 94±5 | 98±8 |
| 0.1 | 87±6 | 97±16 |
| 0.5 | 77±1.5[b] | 92±5 |
| 1 | 70±3[c] | 88±11 |
| 5 | 47±4[c] | 76±14[be] |
| 10 | 31±6[c] | 58±15[cf] |

The maximal inhibition of Apo, N-0437, and quinpirole on TH activity were 76 %, 84 %, and 70 %, respectively decrease of basal enzyme activity. SKF 38393 (0.001−10 μmol $L^{-1}$) did not inhibit the activity of synaptosomal TH from rat striatum (The con-

trol TH activity was DOPA 0.21 ± 0.03 nmol $min^{-1}$/mg protein).

SPD (1—100 μmol $L^{-1}$) did not affect the activities of synaptosomal TH from rat striatum, *vs* the control activity (DOPA 0.24 ± 0.02 nmol $min^{-1}$/mg protein).

**Antagonism of SPD on inhibition of $D_2$ receptor agonists on synaptosomal TH** Striatal synaptosomes were preincubated at 37 ℃ for 5 min with either SPD 100 μmol $L^{-1}$ or spiperone 10 μmol $L^{-1}$ before the addition of reaction medium and N-03437 or quinpirole. Spiperone antagonized the inhibition of N-0437 on the activity of synaptosomal TH (Fig 2). Similarly, SPD also antagonized the inhibitions of N-0437 and quinpirole on TH activity uplift (Fig 2, Tab 1).

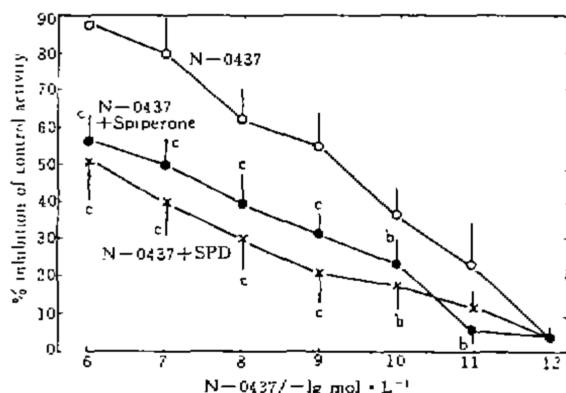


**Fig 2. Inhibition of N-0437 with spiperone 10 μmol $L^{-1}$ or SPD 100 μmol $L^{-1}$ on synaptosomal TH. $n=4$ homogenates (each was pooled from 10 rats and assayed in triplicate). $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* corresponding N-0437. Control activity (as 100 %) of N-0437, N-0437 plus spiperone, and N-0437 plus SPD were 2.37 ± 0.61, 2.72 ± 0.71, and 2.15 ± 0.51 DOPA μmol min/mg protein respectively.**

## DISCUSSION

The synaptosome isolated from striatum has been shown to be an effective model system for study of presynaptic DA receptor feedback[5]. In the present study using this model system, it was found that both mixed DA receptor agonist Apo and selective $D_2$ receptor agonists (N-0437 and quinpirole inhibited the activities of synaptosomal TH from rat striatum, while selective $D_1$ receptor agonist failed to affect the TH activity. The inhibitions of Apo and $D_2$ receptor agonists on TH avtivities were effectively antagonized by selective $D_2$ receptor antagonist spiperone. These results indicated that the negative feedback regulation of presynaptic DA receptors on DA biosynthesis was mediated by $D_2$ receptors rather than by $D_1$ receptors. In combination with the previous findings that DA inhibited the activity of synaptosomal adenylate cyclase (AC) from rat striatum through the activation of $D_2$ receptors[10], it is presumed that presynaptic $D_2$ receptor-mediated inhibition of TH activity may result from the attenuation of cAMP-dependent phosphorylation so that the formation of *L*-DOPA is decreased.

It was also observed that SPD antagonized inhibition induced by both N-0437 and quinpirole on synaptosomal TH activity resulting from the activation of $D_2$ receptors. The results were consistent with both facts that SPD blocks DA autoreceptors[11], and SPD reverses the negative feedback regulation of presynaptic DA receptors on DA biosynthesis in rat striatum[4,12]. Based upon the results that SPD reverses the $D_2$ receptor-mediated inhibition of synaptosomal AC activity[10], it is deduced reasonably that the reversal induced by SPD on the negative feedback regulation of presynaptic DA biosynthesis is due to the block of $D_2$ receptors and the release from the negative coupling of $D_2$ receptors with AC activity.

SPD is a novel DA receptor antagonist with $D_1$ agonistic action[13,14]. However, in the present study, SPD only showed antago-

nistic action without agonistic or partial agonistic effects on presynaptic $D_2$ receptors.

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## 左旋千金藤立定拮抗 $D_2$ 受体激动剂对大鼠突触体酪氨酸羟化酶活性的负反馈调节

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**目的**：研究左旋千金藤立定（SPD）对大鼠纹状体突触体酪氨酸羟化酶（TH）活性的影响. **方法**：利用高效液相-电化学法（HPLC-ECD）检测 *l*-DOPA 的含量变化. **结果**：选择性 $D_2$ 受体激动剂 N-0437和喹吡罗均能抑制大鼠纹状体 TH 的活性，$D_1$ 受体激动剂 SKF 38393和 SPD 都不能抑制 TH 的活性；$D_2$ 受体阻滞剂螺哌隆和 SPD 均能拮抗 $D_2$ 受体激动剂对 TH 的抑制作用. **结论**：$D_2$ 受体介导突触前 DA 受体的负反馈调控. SPD 对 $D_2$ 受体无激动作用，而是 $D_2$ 受体的阻滞剂.

**关键词** 小檗因类；左旋千金藤立定；螺哌隆；反馈调节；多巴胺受体激动剂；酪氨酸羟化酶；纹状体；突触体