BIBLID: ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 Jul; 13 (4): 289-292

Effects of *l*-stepholidine on tyrosine hydroxylase activity in rat corpus striatum

GUO Xin, CHEN Li-Juan, Michel BUDA[1], JIN Guo-Zhang
(*Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China; [1]INSERM U 171-CNRS URA 1195, Lyon, France*)

ABSTRACT *l*-Stepholidine (SPD) 2.5 and haloperidol (Hal) 1.0 mg · kg^{-1} ip increased rat striatal *L*-3,4-dihydroxyphenylalanine (DOPA) and 3,4-dihydroxyphenylacetic acid (DOPAC) accumulation induced by NSD 1015 (50 mg · kg^{-1}, ip), a decarboxylase inhibitor. SPD (2.5 mg · kg^{-1}, ip) did not alter but apomorphine (2.0 mg · kg^{-1}, ip) decreased the dopamine (DA) content elevated by γ-butyrolactone (GBL, 750 mg · kg^{-1}, ip) in the rat striatum. Ip injection of either SPD 5.0 or Hal 2.5 mg · kg^{-1} after NSD 1015 50 mg · kg^{-1} or NSD 1015 plus GBL 750 mg · kg^{-1} also augmented tyrosine hydroxylase (TH) activity in the rat striatum. These results suggest that SPD produces an antagonistic effect on presynaptic DA receptors.

KEY WORDS *l*-stepholidine; tyrosine hydroxylase; corpus striatum; haloperidol; apomorphine

l-Stepholidine (SPD), an alkaloid isolated from *Stephania intermedia* Lo, is a novel dopamine (DA) antagonist proved in many biochemical, electrophysiological, and pharmacological studies. In the previous study, SPD increased the rat striatal *L*-3,4-dihydroxyphenylalanine (DOPA) level and reversed apomorphine-induced decrease of DOPA accumulation[1]. But in rotational behavior test, SPD showed an agonistic effect on DA receptors in 6-hydroxydopamine-lesioned rats[2]. To assess further the properties of SPD acting on DA receptors, we studied the effects of SPD on the feedback regulation of tyrosine hydroxylase (TH) activity in the rat striatum.

Received 1992 Mar 2 Accepted 1992 Apr 28

MATERIALS AND METHODS

Drugs and chemicals SPD was isolated and purified by this Institute. The alkaloid was dissolved in distilled water with the aid of 10% H_3PO_4 and then neutralized with NaOH to pH 5.0. Other chemicals were haloperidol (Hal, Haipu Pharmaceutic Factory, Shanghai, China), apomorphine hydrochloride (Shenyang First Pharmaceutic Factory, China), γ-butyrolactone (GBL, synthesized by this Institute), 3-hydroxybenzylhydrazine dihydrochloride (NSD 1015, Aldrich Chemie, Germany), catalase (65 000 U · mg^{-1}, Boehringer Mannheim GmbH, Germany), *D,L*-6-methyl-5,6,7,8-tetrahydropterin dihydrochloride (6-MPH$_4$, Sigma, USA), 3,4-dihydroxyphenylacetic acid (DOPAC, Fluka AG, Switzerland), DOPA (Sigma, USA), *L*-tyrosine (Shanghai Institute of Biochemistry, China), DA hydrochloride (Fluka AG, Switzerland), β-mercaptoethanol (Shanghai Fourth Reagent Factory, China), *D*-camphor-10-sulphonic acid (CSA, BDH Chemicals Ltd, England) and Sephadex G25 (Pharmacia, Sweden). Tris(hydroxymethyl)-aminomethane (Tris), alumina (Al_2O_3), EDTA, sodium metabisulfite ($Na_2S_2O_5$), methanol, perchloric acid ($HClO_4$), and chloroacetic acid were of AR grade and obtained from commercial sources.

Rats and medications Sprague-Dawley rats (♂, 247 ± *s* 21 g, Shanghai Laboratory Animal Center, China) were used. The drugs were ip injected. Doses referred to their salts. GBL and NSD 1015 were injected 5 and 10 min, respectively, after SPD, Hal or apomorphine. Rats were killed 30 min after NSD 1015.

Preparation of DOPA, DOPAC, and DA samples Rats were decapitated and the striata were dissected on ice plates. The tissues (~ 80 mg) were homogenized 1 : 10 (wt : vol) in $HClO_4$ 0.1 mol · L^{-1} containing EDTA 0.1 mmol · L^{-1} and $Na_2S_2O_5$ 0.1% at 4 ℃. Following centrifugation (15 min, 10 000 × g, 4℃), an aliquot of 100 μl supernatants

mixed with 100 μl Tris–HCl 1 mol · L^{-1} (pH 8.9, containing EDTA 0.1 mmol · L^{-1} and $Na_2S_2O_5$ 0.1%) was purified on an alumina column which was prepared as follows: approximately 15 mg of acid-washed and heat-activated alumina was added in a microtube followed by washing with Tris–HCl 0.1 mol · L^{-1} 200 μl (pH 8.6, containing EDTA 0.1 mmol · L^{-1} and $Na_2S_2O_5$ 0.1%, buffer A) for twice. The mixed solution (200 μl) was shaken for 3 min, and the supernatant was discarded. After washed with buffer A and distilled water separately, the catechols were eluted with 100 μl $HClO_4$ 0.1 mol · L^{-1}. Following centrifugation (1000 × *g*, 5 min, 4 ℃), 50 μl of the supernatants in the alumina microtube were injected into the chromatography

HPLC–ECD The HPLC system consisted of a Model 510 pump (Waters), a Model U6K Universal Injector (Waters), and 2 reversephase Lichrosob C_{18} columns (50 × 4 mm, 200 × 4 mm, 5 μm). The column elute was monitored with a Waters 460 Electrochemical Detector (ECD). The detector potential was set at 0.7 V. Mobile phase ($ClCH_2COOH$ 0.16 mol · L^{-1}, NaOH 0.1 mol · L^{-1}, EDTA 0.1 mmol · L^{-1}, CSA 25 mmol · L^{-1}, methanol 10%, pH 2.8) flow rate was 1 ml · min^{-1}.

Preparation of soluble TH After given drugs, rat striata were dissected and homogenized at 4 ℃ in Tris–HCl 20 mmol · L^{-1} 0.5 ml (pH 7.0, containing β-mercaptoethanol 2 mmol · L^{-1}, buffer B). The homogenates were centrifuged for 30 min at 40 000 × *g* and 4 ℃. The supernatant was passed through a Sephadex G25 column (10 × 1 cm). Elution was achieved with buffer B. Approximately 0.4 ml of the elute fraction which contained the highest concentration of protein were collected.

TH assay A procedure for TH assay originally described by Nagatsu[3] was used with modifications as follows. An aliquot of 50 μl Sephadex G25 elutes was added in a glass tube. The assay of TH started with the addition of 50 μl solution containing acetate buffer 0.2 mol · L^{-1} (pH 7.0), 6-MPH_4 0.5 mmol · L^{-1}, catalase 2500 u, and *L*-tyrosine 0.4 mmol · L^{-1}. The reaction was proceeded for 10 min at 37 ℃ until the addtion of $HClO_4$ 1 mol · L^{-1} 50 μl containing EDTA 0.1 mmol · L^{-1} and $Na_2S_2O_5$ 0.1%. Reaction blanks were obtained using buffer B instead of the eluted enzyme solution. $HClO_4$ 1 mol · L^{-1} 50 μl added in the blank reaction solution contained DOPA 20, 40, and 80 ng as the standard. The final reaction solution was purified on an alumina column and then injected into the HPLC.

Protein measurement and statistics Protein concentration was measured with colorimetric method[4] with bovine serum albumin as the standard. Statistical analyses were made with *t* test.

RESULTS

Effects of SPD and Hal on accumulation of DOPA and DOPAC In rats ip treated with NSD 1015 at 30 min prior to decapitation, DOPA accumulation in the striatum was detectable. When rats were treated with SPD or Hal 10 min before NSD 1015, the concentrations of DOPA and DOPAC in striata were increased as compared with NSD 1015 alone (Tab 1).

Tab 1. Effects of SPD and Hal on striatal DOPA and DOPAC accumulation (ng / mg tissue). SPD 2.5, Hal 1.0, and NSD 1015 50 mg · kg^{-1} were ip injected 40, 40, and 30 min before decapitation, respectively. $n = 6$, $\bar{x} \pm s$, $^{**}P < 0.05$ *vs* NSD 1015.

Drugs	DOPA	DOPAC
NSD 1015	1.2 ± 0.7	0.30 ± 0.19
NSD 1015 + SPD	5.0 ± 1.6**	0.94 ± 0.25**
NSD 1015 + Hal	8.2 ± 1.7**	1.28 ± 0.25**

Effects of SPD and apomorphine on striatal DA content In rats ip treated with GBL at 35 min prior to decapitation, DA content increased as compared with the control. SPD 2.5 mg · kg^{-1} ip injected 5 min before GBL did not change the GBL-induced increase on DA level. But apomorphine (2.0 mg · kg^{-1}, ip) attenuated the GBL-induced DA increase (Tab 2).

Effects of SPD and Hal on TH activity SPD, Hal, or GBL ip injected produced an increase of tyrosine hydroxylation as compared with NSD 1015 alone. When rats were ip treated with either SPD 5.0 or Hal 2.5 mg · kg^{-1} before GBL, the TH activity was also increased as compared with NSD 1015 alone,

Tab 2. Effects of SPD and apomorphine on striatal DA content. SPD 2.5, apomorphine 2.0, and GBL 750 mg · kg^{-1} were ip injected 40, 40, and 35 min before decapitation, respectively. $n=6$, $\bar{x} \pm s$, $^{*}P>0.05$, $^{**}P<0.05$ *vs* GBL.

Drugs	DA (ng / mg tissue)
Control	11.1 ± 1.0**
GBL	14.8 ± 1.6
GBL + SPD	15.0 ± 1.0*
GBL + apomorphine	11.5 ± 1.9**

but had no significant differences as compared with NSD 1015 plus GBL (Tab 3).

Tab 3. Effects of SPD and Hal on striatal TH activity. GBL, SPD, and Hal were given 5, 10, and 10 min before NSD 1015, respectively. Rats were killed 30 min after NSD 1015. Numbers in parentheses referred to doses in mg · kg^{-1}, ip. $\bar{x} \pm s$, $^{**}P<0.05$ *vs* NSD 1015; $^{+}P>0.05$ *vs* NSD 1015 + GBL.

NSD 1015 (50) +	Rats	TH activity, pmol / (mg protein · min)
−	6	130 ± 50
SPD (5.0)	6	340 ± 200**
Hal (2.5)	6	460 ± 300**
GBL (750)	10	400 ± 210**
GBL (750) + SPD (5.0)	10	470 ± 330**+
GBL (750) + Hal (2.5)	10	450 ± 150**+

DISCUSSION

The measurement of DOPA accumulation induced by NSD 1015, an inhibitor of aromatic *L*-amino acid decarboxylase, in the rat striatum is a reflection of the TH activity. DA antagonist Hal increases the DOPA and DOPAC formation when injected systematically[5,6]. The present results showed that SPD exhibited the same effect as Hal, which indicated that SPD augmented TH activity when ip injected. GBL which blocks impulse flow in DA neurons increases DA synthesis[7]. Animals treated with GBL are more sensitive to detect the action of DA agonists than antagonists, and it is widely used as an effective model to test whether or not a compound is an agonist on DA receptors[8,9]. In the present studies, apomorphine attenuated striatal DA content in GBL treated rats, but SPD was ineffective. These results suggest at least that SPD has no agonistic action on presynaptic DA receptors.

TH activity is an index of the feedback regulation of DA synthesis through DA autoreceptors[10,11]. Thus, in present work, TH activity was also measured. The results demonstrated that systematic injection of either SPD or Hal prior to NSD 1015 enhanced the tyrosine hydroxylation assayed *in vitro*. But pretreatment of SPD 5.0 or Hal 2.5 mg · kg^{-1} failed to increase the activity of TH compared with NSD 1015 plus GBL treated animals. However, at the same doses, both compounds increased DOPA formation in NSD 1015 plus GBL treated rats[1]. This difference suggests that the determination of DOPA formation *in vivo* is more sensitive to reflect the blockade of presynaptic DA receptor than the determination of TH activity *in vitro*. In conclusion, SPD systematically injected produces an increase of TH activity, which suggests a blockade of DA autoreceptors.

REFERENCES

1 Chen LJ, Guo X, Wang QM, Jin GZ. Feed-back regulation of presynaptic D_2 receptors was blocked by (−)-stepholidine and (−)-tetrahydropalmatine. *Acta Pharmacol Sin*. In press.

2 Huang KX, Sun BC, Jin GZ. (−)-Stepholidine: a dopamine receptor antagonist shows agonistic effect on rotational behavior in 6-hydroxydopamine-lesioned rats. *Acta Pharmacol Sin* 1992, 13 : 17-22

3 Nagatsu T, Levitt M, Udenfriend S. A rapid and simple radioassay for tyrosine hydroxylase activity. *Anal Biochem* 1964; 9 : 122-6.

4 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193 : 265-75

5 Bannon MJ, Reinhard JF Jr, Bunney EB, Roth RH. Unique response to antipsychotic drugs is due to absence of terminal autoreceptors in mesocortical dopamine

neurones *Nature* 1982; **296** : 444–6.
6 Bannon MJ, Wolf ME, Roth RH. Pharmacology of dopamine neurons innervating the prefrontal, cingulate and piriform cortices. *Eur J Pharmacol* 1983; **92** : 119–25
7 Walters JR, Roth RH. Dopaminergic neurons : an in vivo system for measuring drug interactions with presynaptic receptors. *Naunyn Schmiedebergs Arch Pharmacol* 1976; **296** 5–14.
8 Coward DM, Dixon AK, Urwyler S, White TG, Enz A, Karobath M, *et al.* Partial dopamine–agonistic and atypical neuroleptic properties of the amino–ergolines SDZ 208–911 and SDZ 208–912. *J Pharmacol Exp Ther* 1990; **252** · 279–85
9 Roth RH, Galloway MP, Tam SY, Ono N, Wolf ME. Dopamine autoreceptors, studies on their distribution and mode of action. In: Woodruff GN, Poat JA, Roberts PJ, editors. *Dopaminergic systems and their regulation.* London: Macmillan, 1986 : 45–61.
10 Goldstein M. Regulatory mechanisms of dopamine biosynthesis at the tyrosine hydroxylase step *Ann NY Acad Sci* 1984; **430** : 1–5.
11 Levitt M, Spector S, Sjoerdsma A, Udenfriend S Elucidation of the rate–limiting step in norepinephrine biosynthesis in the perfused guinea–pig heart. *J Pharmacol Exp Ther* 1965; **148** . 1–8.

279-292

左旋千金藤立定对大鼠纹状体酪氨酸羟化酶活性的影响

R963

郭 新、陈丽娟、Michel BUDA[1]、金国章
(中国科学院上海药物研究所，上海 200031，中国；[1]*INSERM U 171–CNRS URA 1195, Lyon, France*)

提要 本文研究 SPD 对大鼠纹状体 DOPA、DA 和 DOPAC 含量及酪氨酸羟化酶活性的影响. ip SPD 2.5 和氟哌啶醇 1.0 mg · kg^{-1} 增强 NSD 1015 引起的大鼠纹状体 DOPA 和 DOPAC 累积 SPD 不改变 GBL 增加大鼠纹状体 DA 含量的作用，但阿扑吗啡显著抑制 GBL 的作用. ip SPD 5 或氟哌啶醇 2.5 mg · kg^{-1} 显著增强大鼠纹状体酪氨酸羟化酶的活性. 结果提示 SPD 对突触前 DA 受体表现阻滞作用.

关键词 左旋千金藤立定；酪氨酸羟化酶；纹状体；氟哌啶醇；阿扑吗啡

SPD

BIBLID: ISSN 0253–9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 Jul; 13 (4) · 292–297

Comparison of effects of tetrahydropalmatine enantiomers on firing activity of dopamine neurons in substantia nigra pars compacta[1]

SUN Bao–Cun, HUANG Kai–Xing, JIN Guo–Zhang[2]
(Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China)

ABSTRACT Extracellular single unit recording techniques were used to elucidate the effects of enantiomers of tetrahydropalmatine (THP) on the firing activity of dopamine (DA) neurons in substantia nigra pars compacta (SNC). (−)–THP rapidly reversed the apomorphine (Apo)–induced inhibition of the SNC DA cell firing activity (ED_{50} = 0.77, 0.52–1.14, mg · kg^{-1}), while much larger doses of (+)–THP were required to reverse the Apo–induced inhibition (ED_{50} = 23, 15.2–34.7, mg · kg^{-1}) and the maximal reversal caused by (+)–THP was 79 ± 9% of the basal firing rate. In paralyzed rats, (−)–THP (0.5–16 mg · kg^{-1}) significantly increased the spontaneous firing rate of SNC DA neurons dose–dependently, while (+)–THP did not until the dose reached 16 mg · kg^{-1}. Pretreatment with (−)–THP 4 mg · kg^{-1} attenuated Apo–induced inhibition of SNC DA cell firing rate, while (+)–THP 32 mg · kg^{-1} revealed a similar potency to block the Apo–induced inhibition. In addition, (+)–THP did not potentiate the effect caused by *d*–amphetamine (Amp) as some behavioral experiments have shown, but large dose of (+)–THP (32 mg · kg^{-1}) blocked the Amp–induced inhibition of SNC DA cell firing activity as (−)–THP (4 mg · kg^{-1}) did. These results sug–

Received 1991 Jun 28 Accepted 1992 Apr 14
[1] Project supported by the National Natural Science Foundation of China, № 3870905.
[2] To whom correspondence should be addressed.