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(−)-Stepholidine: a dopamine receptor antagonist shows agonistic effect on rotational behavior in 6-hydroxydopamine-lesioned rats[1]

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ABSTRACT (−)-Stepholidine ((−)-SPD), a well demonstrated dopamine (DA) receptor antagonist in normal rats, could markedly induce contralateral rotational behavior in 6-hydroxydopamine (6-OHDA)-lesioned rats. This peculiar behavioral action of (−)-SPD, proposed to be an agonistic action on DA receptors, was further studied in this paper. The rotational behavior challenged by (−)-SPD (4 mg · kg^{-1}, ip) or SKF-38393 (selective D_1 agonist) had a gradually progressive process with a long latent period and a plateau response after 56-67 postlesion days. On the contrary, the action of apomorphine (APO) or D_2 selective agonist N-0347, had a short latent period and a plateau response after d 21 of postlesion. In the latent period of rotation, (−)-SPD (4 mg · kg^{-1}) was primed by pretreatment of APO (0.2 mg · kg^{-1}, ip). In the period of steady contralateral rotation, a dose-dependent action of (−)-SPD was found in a range of 0.5-8 mg · kg^{-1} and a linear correlation ($r = 0.77$, $P < 0.01$) between the rotation induced by (−)-SPD (4 mg · kg^{-1}) and by APO (0.2 mg · kg^{-1}) was also shown. These results suggest that the agonistic effect of (−)-SPD on rotation behavior in 6-OHDA-lesioned rats resembles that of D_1 agonist SKF-38393. Taken together, the dual actions of (−)-SPD, antagonistic on normosensitive DA receptors and agonistic on supersensitive DA receptors, were proposed.

KEY WORDS stepholidine; berbines; rotation; dopaminergic agents

It is well-known that rotational behavior can be induced by DA agonists in the rats with 6-hydroxydopamine (6-OHDA) lesions of unilateral substantia nigra pars compacta (SNC)[1]. Both D_1 and D_2 selective agonists can induce remarkable contralateral rotation (away from the lesioned side) in the lesioned rats and they have a synergistic effect. The synergism has been suggested to be due to stimulation of D_1 and D_2 subtypes located in the substantia nigra zona reticulata (SNR) and the corpus striatum respectively[2-4]. The rotational behaviour induced by D_1 and D_2 agonists can be separately attenuated by pretreatment with D_1 and D_2 antagonist[5-7].

Recently, several experiments showed that DA receptors appear to loss their selectivity when they become supersensitive due to deterioration of DA neurons[8,9]. This evidence indicates that the functional state of DA receptors is changed during supersensitivity.

(−)-Stepholidine ((−)-SPD), isolated from Chinese herb *Stephania intermedia* Lo, belongs to the tetrahydroprotoberberine analogs (THPBs). Since 1980, it has been well established that (−)-SPD is a novel DA receptor antagonist testified by a vast number of studies on postsynaptic and presynaptic DA receptors[10-12]. But, (−)-SPD could induce contralateral rotation in 6-OHDA-lesioned rats and had a synergistic effect on the action of DA agonist APO, while it antagonized the amphetamine-induced rotational behavior[10,13]. This is a new phenomenon quite different from that of other DA receptor antagonists. And these findings indicate that the actions of (−)-SPD depend on functional state of DA receptors. In this paper, further observations were performed on the rotational behavior

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in 6-OHDA-lesioned rats in order to elucidate the characteristics of the agonistic action of (−)-SPD on DA receptors.

MATERIALS AND METHODS

Drugs 6-OHDA-HCl (Sigma), SKF-38393-HCl (RBI) and N-0437 (Nelson) were dissolved in saline. (−)-SPD (Shanghai Institute of Materia Medica) and SCH-23390 (RBI) were dissolved in 0.2% H_3PO_4 adjusted by 0.2 mol · L^{-1} NaOH (pH 4-5). Spiperone (RBI) was dissolved in dimethylformamide (DMF). Apomorphine (APO 5 mg · ml^{-1}) was purchased from Shengyang No. 1 Pharmaceutical Co.

Rotational model Male Sprague-Dawley rats weighing 170 ± *s* 11 g (Shanghai Animal Center, Academia Sinica) were used. Anesthetized with pentobarbitol (40 mg · kg^{-1}, ip), the rats were fixed on a stereotaxic frame and the saline solution (4 μl) containing 9.7 μg 6-OHDA-HCl (equivalent 8 μg free base) and 1 μg ascorbic acid was injected unilaterally into the substantia nigra at the rate of 1 μl · min^{-1}, according to the brain atlas of Paxino and Watson (1985). A small glass pipette with 0.2 mm tip diameter pulled from the glass pipette was joined with a syringe (5 μl) through a connecting tube (diameter 0.2 mm). When the injection was finished, the glass pipette was withdrawn after remaining *in situ* for 4 min.

The rats were screened starting on d 10 after lesion at an interval of 3-4 d until steady rotational response to systemic DA agonist and / or (−)-SPD was obtained. The rats were gently placed in a bowl (diameter 24 cm) and the rotation was measured in each turn of 360°. Only the rats showing contralateral rotation at a speed of more than 5 turns / min in response to apomorphine (APO 0.2 mg · kg^{-1}, ip) or (−)-SPD (4 mg · kg^{-1}, ip) were used.

Drug administration and statistics The lesioned rats were divided into several groups (4-6 rats in each). Each rat was tested repeatedly at an interval of 3-7 d. The total turns were measured for the first 30 min test period. The data were expressed as $\bar{x} \pm s$. The significance was evaluated by Dunnett's test.

Histological examination At the end of experiments, some rats were perfused under pentobarbital with 10% formalin 50 ml in phosphate buffer (pH 7.4). The brain was then removed, fixed, freeze-sectioned (50 μm thick) and stained with cresyl violet. The deterioration of DA neurons due to 6-OHDA lesion in the substantia nigra zona compacta (SNC) was observed.

RESULTS

Differential development of rotational behavior challenged by various compounds in 6-OHDA-lesioned rats In the 6-OHDA-lesioned and drug-naive rats, DA agonists (APO, N-0437), and (−)-SPD exhibited differential developmental process of contralateral rotation. The remarkable and steady rotation was challenged by APO 0.2 mg · kg^{-1} (ip) on d 21-26 after lesion (Fig 1, upper panel), and this plateau response was kept for 2 months. The action of N-0437, a selective D_2 agonist, had the same developmental pattern as that of APO (Fig 1, upper panel). However, as for (−)-SPD (4 mg · kg^{-1}, ip), there was rare contralateral rotation observed in drug-naive rats until 47 d later, and then the plateau response of contralateral rotation was found on d 63. Thus, the process of development of rotational behavior challenged by (−)-SPD showed a progressive pattern, consisting of a latent period and a plateau response period. This gradually developing pattern of rotation to (−)-SPD was similar to that of SKF-38393 (2 mg · kg^{-1}, ip), of which the plateaus response appeared on d 54, but obviously

different from those of APO and N–0437 (Fig 1, upper panel).

"Priming" of rotational behavior challenged by (–)–SPD in 6–OHDA–lesioned rats Although the rotational behavior provoked by (–)–SPD required a long latent period, the pretreatment of APO primed the (–)–SPD–induced rotation. If the rats had received APO (0.2 mg · kg^{-1}) during 14–26 d after lesion, 3 d later these rats could rotate contralaterally in response to (–)–SPD (4 mg · kg^{-1}) and the steady rotational plateau response maintained more than a month (Fig 1, lower panel). Therefore, the effect of (–)–SPD in the APO–treated rats did not require such a long latent period as that in drug–naive rats.

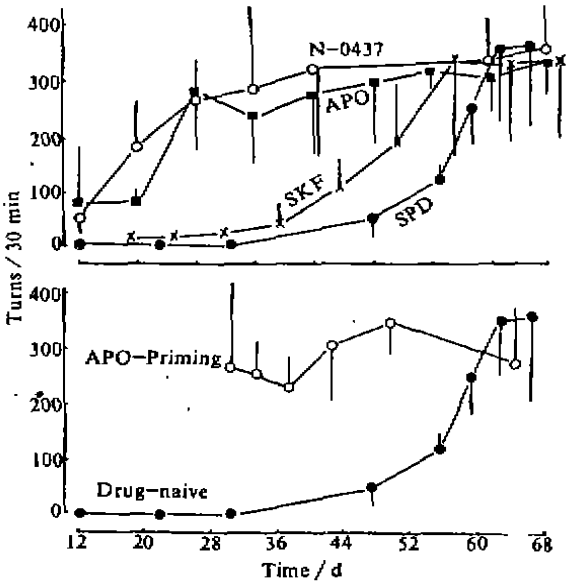

Fig 1. Rotational behavior. Upper panel: induced by dopamine agonists in 6–OHDA–lesioned rats. $\bar{x} \pm s$. **APO (0.2 mg · kg^{-1}), $n=6$; N–0437 (0.2 mg · kg^{-1}), $n=3$; SKF–38393 (2 mg · kg^{-1}), $n=6$; (–)–SPD (4 mg · kg^{-1}), $n=4$. Lower panel: induced by (–)–SPD primed by APO in 6–OHDA–lesioned rats. Drug–naive group, rats received (–)–SPD 4 mg · kg^{-1} (ip); priming group, rats received APO (0.2 mg · kg^{-1}, ip) on d 26 after 6–OHDA.**

Correlation between effects of (–)–SPD and APO After the primed or naive rats showed steady rotation to (–)–SPD (4 mg · kg^{-1}), the effect of (–)–SPD was found to be in a dose–dependent way in a range of 0.5–8 mg · kg^{-1} (Fig 2). Moreover, the responsibility to (–)–SPD (4 mg · kg^{-1}) was proportional to that to APO (0.2 mg · kg^{-1}) ($r=0.77$, $P<0.01$) in rats showing the steady response to APO as well as (–)–SPD (Fig 3), when each rat was tested with (–)–SPD and APO (or reversed order) at 1 wk interval.

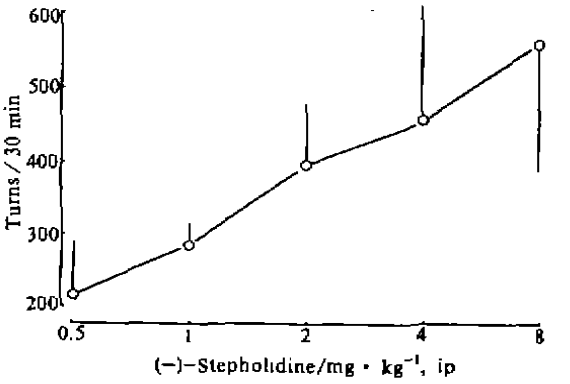

Fig 2. Contralateral rotation induced by (–)–SPD in 6–OHDA–lesioned rats. $n=4$. $\bar{x} \pm s$.

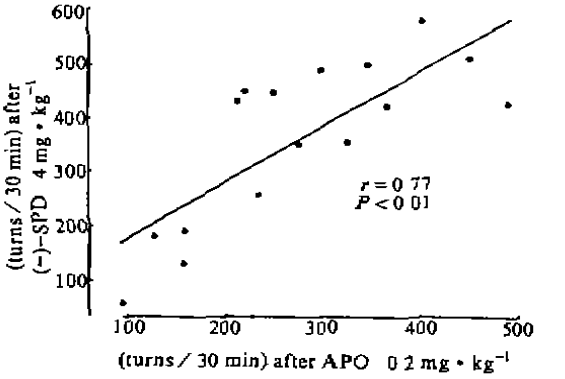

Fig 3. Linear correlation between the rotation by (–)–SPD ip and that by APO ip in the same 6–OHDA–lesioned rats.

DISCUSSION

(−)−SPD acts as a DA agonist in 6−OHDA−lesioned rats The present work verified a fact first found in 1984 that (−)−SPD induced remarkable contralateral rotation in 6−OHDA−lesioned rats[13]. Moreover, this rotational behavior of (−)−SPD was dose−dependent and proportional to the DA agonist APO. The evidence against the conclusion derived from other neuropharmacological studies in unlesioned animals were listed in Tab 1. According to the widely accepted concept that DA agonists could induce contralateral rotation in 6−OHDA−lesioned rats and DA antagonists block this effect, (−)−SPD would be considered to be a DA agonist in such case (Fig 4). Further studies have shown that selective D_1 antagonist SCH−23390 could preferably attenuate the (−)−SPD−induced contralateral rotation, but selective D_2 antagonists could not. Hence, (−)−SPD is considered to act as a D_1 agonist in 6−OHDA−lesioned rats (Huang *et al*, to be published).

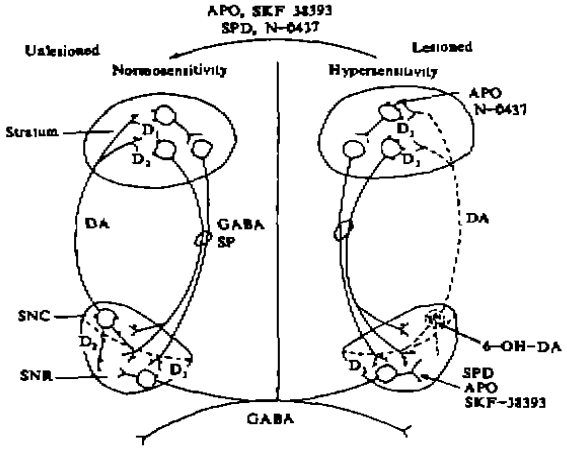

Fig 4. Proposed mechanism underlying the action of (−)−SPD and DA agonists in rotational behavior of rats lesioned with 6−OHDA. APO, SKF−38393, pergolide, N−0347, and (−)−SPD induced the contralateral rotation in rats via stimulation of supersensitive DA receptors in lesioned side.

Progressive development of rotational behavior The (−)−SPD−induced rotational behavior in 6−OHDA−lesioned rats was characterized by a long progressive latent period similar as D_1 agonist SKF−38393. In this period, (−)−SPD could not induce noticeable contralateral rotation, suggesting that some mechanisms may underlie the (−)−SPD−induced rotation due to DA deterioration. However, the (−)−SPD−induced rotation could be primed by pretreatment of APO. The mechanism of priming would be possible to be the prior stimulation of DA receptors. Recently, it was reported that the prior D_2 receptor stimulation primed SKF−38393−induced rotation[14]. It is thought that D_2 receptor stimulation could produce a "primer" of D_1 agonist induced rotation[14], but the mechanism of "priming" remains obscure. Nevertheless, the data resulting from receptor binding assay supported the D_1 receptors upregulated progressively after DA deterioration. D_1 receptors did not become supersensitive 10−15 d after 6−OHDA−lesion[15], they required longer time for upregulation. On the other hand, it was shown that the maximal D_2 receptor population appeared d 21 and lasted 100 d[2]. This evidence coincided with the fact that the steady rotation (plateau) induced by APO and N−0437 was on d 21, different from that by (−)−SPD and SKF−38393. Therefore, these works suggested a differential regulation between D_1 and D_2 subtypes after DA neuron deterioration.

Dual actions of (−)−SPD on DA receptors The present study provided a reliable evidence that (−)−SPD can act as a DA agonist on supersensitive DA receptors. Summarizing all previous works on (−)−SPD (Tab 1), it would be presumed that the pharmacological actions of (−)−SPD might be convertible from DA antagonistic to

agonistic, depending on the DA receptor sensitivity. No matter what the acute experiments of biochemical, neuropharmacological or electrophysiological study were performed, the antagonistic effect of (−)−SPD was observed under the normosensitive state of DA receptors (Tab 1). However, under 6−OHDA−lesioned condition, when the nigro−striatal DA receptors become supersensitive[2−3], (−)−SPD exhibits a DA agonistic action. The suggestion that the supersensitive DA receptors would lose their selectivity[7,9] really provides a possibility for convertible effects of (−)−SPD. One possibility is that DA receptor supersensitivity was underlain by the change of some molecular structures of receptor (or conformation) in membrane, and thus the DA receptor could recognize and interact with some compounds, which have no intrinsic activity under normosensitive DA receptors. Further findings (Huang *et al* to be published) would support the abovementioned speculation and suggest that (−)−SPD has differential effects on D_1 and D_2 receptor subtype when they became upregulated. Under supersensitive state (−)−SPD would be converted to an agonist of D_1 receptor subtype, while it

Tab 1. Overview of dual actions of (−)−SPD related to DA receptor sensitivity

Experiment	Sensitivity of DA receptors	Effect of (−)−SPD	Reference
Biochemical			
DA autoreceptor−mediated feedback regulation in striatum	normal	antagonistic	(11,17)
DA−induced formation of cAMP	normal	antagonistic	(12)
Neuropharmacololgical			
stereotypy induced by DA agonist	normal	antagonistic	(16)
spontaneous activity of mice induced by DA agonists	normal	antagonistic	(16)
amphetamine−induced ipsilateral rotation in 6−OHDA−lesioned rats	normal	antagonistic	(10,13)
apomorphine−induced contralateral rotation in 6−OHDA−lesioned rats	supersensitive	agonistic	(10,13)
contralateral rotation in 6−OHDA−lesioned rat	supersensitive	agonistic	(10,13,20)
apomorphine−induced ipsilateral rotation in kainic acid plus 6−OHDA−lesioned rats	normal	antagonistic	(19)
Electrophysiological			
DA autoreceptor−regulated firing activity of nigral DA neurons	normal	antagonistic	(19)
Electrochemical			
in vivo DA release and DOPAC level in striatum	normal	antagonistic	(11,17,18)

Note: (16): Zhang *et al*. *Acta Pharmacol Sin* 1986; 7: 522. (17): Jin *et al*. *Chin J Physiol Sci* 1991; 7: in press. (18): Huang *et al*. *Acta Pharmacol Sin* 1991; **12**: 32 (19): Huang & Jin. *Sci Sin* 1991; to be published; (20): This paper

would retain the antagonistic action to the D_2 receptor subtype. This may be the mechanism underlying dual actions of (−)-SPD.

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左旋千金藤立定：多巴胺受体拮抗剂对 6-羟基多巴胺损毁大鼠的旋转行为呈现激动作用

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提要 (−)-SPD 是新型 DA 受体拮抗剂，然而对 6-OHDA 损毁后大鼠的旋转行为呈激动作用、并有量-效关系、与 APO 作用有相关性 ($r=0.77$、$P<0.01$)；它的激动作用在大鼠损毁 6-7 周后才能观察到、与 SKF-38393(专一 D_1 激动剂)相似、而 N-0347(专一 D_2 激动剂)和 APO 仅需 3 周潜伏期. 它提示(−)-SPD 的激动作用与 D_1 受体亚型的超敏发展时程有关系.

关键词 左旋千金藤立定；小檗因类；旋转；多巴胺能药物