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GTP Regulation of (−)-Stepholidine Binding to R_H of D_1 Dopamine Receptors in Calf Striatum

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ABSTRACT. (−)-Stepholidine (SPD) exhibits antagonist effects on normosensitive dopamine (DA) receptors, but it has an agonist action on rotation in unilaterally 6-hydroxydopamine (6-OHDA)-lesioned rats. The present work endeavors to further elucidate the mechanism of its agonist action on D_1 receptors. [^{3}H]*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine ([^{3}H]SCH-23390) and [^{3}H]spiperone were used, respectively, as radioligands in D_1 and D_2 DA receptor binding assays in calf striatal membranes. Experimental data were analyzed by a non-linear regression computer program, GraphPAD InPlot 3.15. The competition curves were fitted first by a single-site equation and then by a two-site equation. The results showed that both apomorphine (APO) and SPD competitively inhibited [^{3}H]SCH-23390 binding. Their competition curves fitted best to the two-site equation ($P < 0.05$) with a high-affinity site (R_H) and a low-affinity site (R_L) to DA receptors. The K_H and K_L values (nM) were 2.7 ± 0.45 and 378 ± 62 for APO, and 3.9 ± 2.2 and 126 ± 25 for SPD, respectively. In contrast, the competition curve of SCH-23390, a selective D_1 DA receptor antagonist, fitted best to a single-site model with a K_i value of 1.7 ± 0.5 nM. The R_H of APO or SPD could be decreased by the addition of 450 μM GTP. In the [^{3}H]spiperone binding test, the APO curve was modeled best by the two-site equation, while the SPD curve fitted best to a single-site model. In the rotational behavior test, APO induced 441 ± 20 turns/30 min in the 6-OHDA-lesioned rats, and SPD induced 310 ± 42 turns/30 min, while SCH-23390 antagonized the SPD-induced rotation but did not induce rotational behavior. These results suggest that SPD possesses agonist actions on D_1 but antagonist effects on D_2 DA receptors. BIOCHEM PHARMACOL **54**;2:227–232, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. (−)-stepholidine; apomorphine; SCH-23390; dopamine receptors; GTP; rotational behavior

SPD§, the leading compound of a series of tetrahydroprotoberberines isolated from the Chinese herb *Stephania intermedia* Lo, has been well studied and shown to be a novel antagonist of DA receptors [1, 2]. Previous results have revealed that SPD has high affinity for both D_1 and D_2 DA receptors in striatal membrane preparations, with a preference for D_1 receptors [3]. SPD antagonizes APO-induced stereotypy in rats and vomiting in dogs [4], and it reverses or antagonizes the APO-induced inhibition of the firing activity of DA neurons in the substantia nigra pars compacta [5, 6]. In biochemical determinations, SPD blocks the inhibition of tyrosine hydroxylase activity mediated via presynaptic D_2 DA receptors, increases DA biosynthesis, and facilitates DA release from nerve terminals in the striatum [7, 8]. All these results support the previous view that SPD is a DA receptor antagonist.

In the 6-OHDA-lesioned rats, however, SPD induces contralateral rotation [9–12], with characteristics similar to that of the selective D_1 receptor agonist 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1-1*H*-benzazepine (SKF 38393), including a gradually increasing response with repeated treatments and a long latent period. The rotation is primed by pretreatment with APO [11, 12] and antagonized by the D_1 receptor antagonist SCH-23390 [*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine]. From these results, it is postulated that SPD has agonist action at DA receptors.

It is well known that the D_1 DA receptors couple to AC through a G_s protein. The binding sites of DA agonists to D_1 receptors are thus regulated by GTP [13]. SCH-23390 is a selective D_1 DA receptor antagonist and completely blocks DA-stimulated AC activity. [^{3}H]SCH-23390 thus labels a homogeneous population of D_1 receptors. Experimental data indicate that antagonist competition to [^{3}H]SCH-23390 binding displays a monophasic curve. In contrast, an agonist interacts with [^{3}H]SCH-23390 binding sites in a complex manner, yielding shallow competition curves. The agonist competition consistently fits best to a two-site curve composed of an R_H and an R_L component. The ratio of agonist affinities at these sites (K_L/K_H) may

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§ *Abbreviations:* AC, adenylate cyclase; APO, apomorphine; DA, dopamine; K_i, inhibitor constant; K_H, K_i value of high affinity to receptors; K_L, K_i value of low affinity to receptors; 6-OHDA, 6-hydroxydopamine; R_H, high affinity to receptors; R_L, low affinity to receptors; and SPD, (−)-stepholidine.

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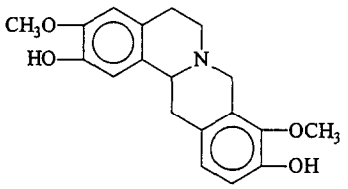
predict the intrinsic activity of the agonist [13]. After the addition of GTP, the R_H components of agonist binding sites are converted to low-affinity sites [14]. The present study was designed to compare the agonist action of SPD with APO on both D_1 and D_2 DA receptor binding assays, AC activity, and rotational behavior in unilaterally 6-OHDA-lesioned rats.

MATERIALS AND METHODS

Drugs and Reagents

6-OHDA–HCl (Sigma, U.S.A.) was dissolved in saline; (−)-SPD (Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China), m.p. 163°, $[\alpha]_D$ −440° (C = 0.002, pyridine), was dissolved in a small amount of 0.1 N H_2SO_4 and then diluted with Tris–HCl (pH ~5). SCH-23390, ketanserin (RBI, U.S.A.), and APO (Sigma) were dissolved in Tris–HCl buffer. [^{3}H]SCH-23390 (2.66 TBq/mmol) and [^{3}H]spiperone (3.7 TBq/mmol) were purchased from Amersham (U.S.A.).



Chemical structure of SPD

Receptor Binding Assay

Within 1 hr after killing the animals, the striata from male calves were dissected carefully and stored at −80° for up to 2 months. The striatum was thawed, minced, and then homogenized in a blender with 50 vol. of ice-cold 50 mM Tris–HCl (pH 7.4). The homogenate was centrifuged at 1500 *g* for 10 min at 4°. The supernatant was collected and then centrifuged at 35,000 *g* for 20 min at 4°. The pellet was rinsed once more with the same buffer, followed by recentrifugation. For the D_1 receptor binding assay, the final pellet was resuspended in assay buffer consisting of 50 mM Tris–HCl (pH 7.4), 5 mM $MgSO_4$, 0.5 nM EDTA, 40 nM ketanserin, and 0.02% ascorbic acid. For the D_2 receptor binding assay, 0.5 mM KCl, 100 mM NaCl, 5 mM $CaCl_2$, 2 mM $MgCl_2$, and 0.01% ascorbic acid were included in the reaction medium. Protein content was measured according to the method of Lowry *et al.* [15].

Incubations were initiated by adding tissue (0.4 mg protein/tube), [^{3}H]SCH-23390 for D_1 or [^{3}H]spiperone for D_2 receptors, unlabeled competitor and/or guanine nucleotides, to yield a 1 mL final assay volume, and lasted for 30 min at 37°. Non-specific binding was defined by 100 nM SCH-23390 for D_1 or by 100 nM butaclamol for D_2. Duplicate samples were filtered over glass fiber filters and then were rinsed rapidly with 10 mL of ice-cold Tris buffer. The glass fiber filters were dried at 80° and counted by LKB scintillation spectroscopy.

Curve Fitting and Statistics

The weighted non-linear curve-fitting program GraphPAD Inplot 3.15 was used for the analysis of saturation and competition experiments. All the saturation and competition studies were analyzed initially with a one-site model; the data were then analyzed with a two-site model (i.e. two distinct saturable binding sites of different affinities), and the results of this curve fitting were compared statistically with its one-site model by the F-test. The two-site model was accepted if the fit was significantly better ($P < 0.05$) than that of the one-site model. Otherwise, the one-site model was accepted when it was not significantly different from the two-site model ($P > 0.05$). The K_i values were calculated with the equation $K_i = IC_{50}/(1 + (L/K_D))$, and the experimental data are presented as means ± SEM. A direct comparison between any two groups was made by Dunnett's *t*-test. When the comparison was made between assays run in parallel on the same tissue samples, the paired Student's *t*-test was used.

Rotational Behavior Test

Sprague–Dawley rats weighing 175 ± 14 g (Shanghai Animal Center, Academia Sinica) were used. The rats were lesioned unilaterally by 6-OHDA as previously described [11]. Briefly, the rats were fixed on a stereotaxic frame, and saline solution (4 μL) containing 9.7 μg 6-OHDA was injected unilaterally into the substantia nigra at the rate of 1 μL/min. Sixty days later, the unilaterally 6-OHDA-lesioned rats manifesting contralateral rotation (towards the unlesioned side) at a speed of more than 5 turns/min in response to APO (0.2 mg/kg, i.p.) were used to evaluate the effects of drugs. The significance was evaluated by Dunnett's *t*-test.

RESULTS

APO, SPD and SCH-23390 Competition for [^{3}H]SCH-23390 Binding

The total binding of [^{3}H]SCH-23390 to D_1 receptor sites in calf striatum homogenates showed a saturable curve, while non-specific binding increased linearly over the entire concentration range, 0.13 to 16 nM. The Scatchard plots of saturation data indicated a K_D of 1.99 ± 0.12 nM and a B_{max} of 510 ± 13.2 fmol/mg protein (N = 3). The Hill coefficient estimated from the saturation binding experimental data was 1.014, indicating a homogeneous population of binding sites. No detectable effect of GTP on the affinity of D_1 receptors for [^{3}H]SCH-23390 was observed (B_{max} = 510 ± 17.3 fmol/mg protein).

The competition curves of SCH-23390 for [^{3}H]SCH-23390 binding in calf striatal homogenates fitted best to a single homogeneous population of binding sites with a K_i value of 1.7 ± 0.29 nM (N = 3, Fig. 1). In contrast to SCH-23390, APO and SPD were best modeled by a heterogeneous, two-site fit (Figs. 2 and 3). The percentages

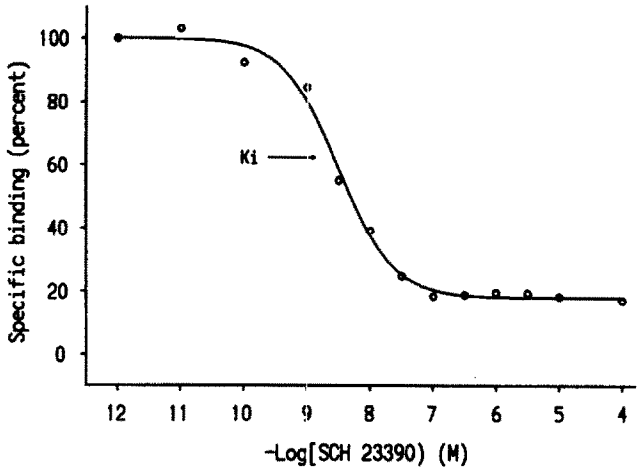


FIG. 1. Competition curve of SCH-23390 for [^{3}H]SCH-23390 binding in calf striatal membranes. The curve was analyzed by GraphPAD Inplot 3.15 and fitted best to a single-site model. The K_i of SCH-23390 for [^{3}H]SCH-23390 binding was 1.7 ± 0.29 nM (N = 3).

of high affinity sites for APO and SPD were 38.2 ± 4.9 and 59.1 ± 3.6, respectively. The K_i values for APO at high (K_H) and low (K_L) affinity sites were 2.7 ± 0.45 and 378 ± 62 nM, respectively (N = 4), (Table 1). The K_H and the K_L for SPD were 3.9 ± 1.1 and 126 ± 25 nM (N = 4). The ratios of K_L/K_H for APO and SPD were 140 and 32.3, respectively. The addition of 0.45 mM GTP shifted the competition curves of APO and SPD to the right and converted to a single-site fit for competition with [^{3}H]SCH-23390 binding, with K_i values of 155 ± 39 and 85.7 ± 13.3 nM, respectively.

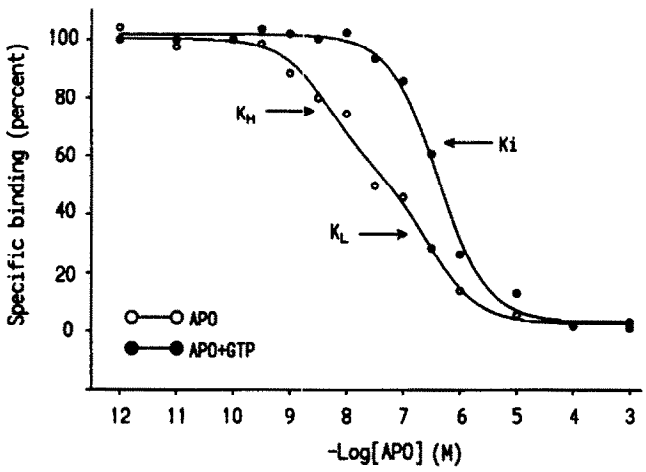


FIG. 2. Competition curve of APO for specific [^{3}H]SCH-23390 binding in calf striatal membranes, calculated from 4 separate experiments. The curves were analyzed by GraphPAD InPlot 3.15. The concentration of [^{3}H]SCH-23390 was 0.6 to 0.7 nM. The APO curves in the absence of GTP (N = 4) fitted best to a two-site model with a K_H value of 2.7 ± 0.45 nM and a K_L value of 378 ± 62 nM, while the APO curves (N = 4) in the presence of 450 μM GTP fitted best to a single-site model, with K_i = 155 ± 39 nM.

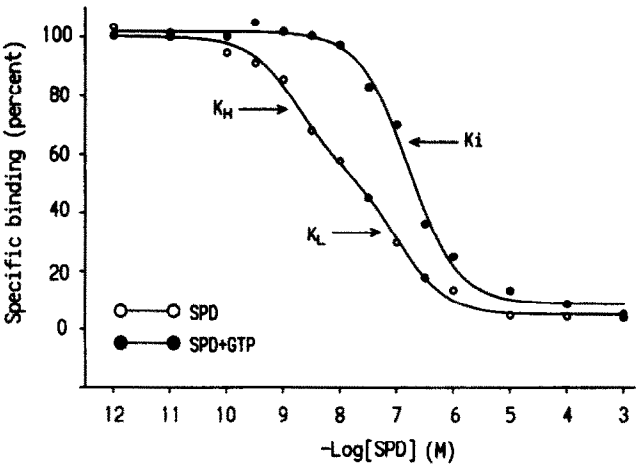


FIG. 3. Competition curve of SPD for specific [^{3}H]SCH-23390 binding in calf striatal membranes, calculated from 4 separate experiments. The concentration of [^{3}H]SCH-23390 was 0.5 to 0.7 nM. The data were analyzed by GraphPAD Inplot 3.15 with non-specific binding defined by 100 nM SCH-23390. The competition curves of SPD (N = 4) in the absence of GTP fitted best to a two-site model ($P < 0.05$), with the K_H and K_L values being 3.9 ± 1.13 and 126 ± 25 nM. In the presence of 450 μM GTP (N = 4), the competition curves of SPD fitted best to a single-site model ($P > 0.05$) with a K_i value of 85.7 ± 13.3 nM.

APO and SPD Competition for [^{3}H]Spiperone Binding

Specific binding of [^{3}H]spiperone was saturable and of high affinity to D_2 DA receptors in calf striatum homogenates. The B_{max} and K_D values calculated from the experimental data were 464 fmol/mg protein and 2.2 nM, respectively. The competitive ability of APO and SPD with D_2 specific [^{3}H]spiperone binding sites to the calf striatum membrane was examined. Both APO and SPD significantly inhibited [^{3}H]spiperone binding to D_2 receptors in a concentration-dependent manner. The competitive curve of SPD to [^{3}H]spiperone was modeled and fitted best to a single homogeneous site (Fig. 4) with a K_i value of 81.9 ± 14.4 nM. However, the curve of APO fitted best to a two-component equation with a high-affinity site (K_H = 2.9 ± 0.69 nM, N = 3) and a low-affinity site (K_L = 721.8 ± 141.5 nM, N = 3) (Fig. 5). The percentages of high affinity and low affinity to D_2 receptors for APO binding were 53 and 47, respectively. After the addition of GTP (450 nM), the APO binding curve was shifted significantly to the right and showed a K_i value of 227.9 nM.

Rotational Behavior

Contralateral rotation was induced by SPD as well as APO in a dose-dependent manner (a range of 100 μmol/kg to 10 nmol/kg), although the activity of SPD was weaker than that of APO (Fig. 6). Furthermore, at an equimolar dose (10 μmol/kg body weight, i.p.), the rotation induced by APO (N = 17) was significantly stronger (441 ± 20 turns in 30 min) than that by SPD (310 ± 42 turns in 30 min, N = 8, $P < 0.05$). In contrast, no rotational behavior was

TABLE 1. Computer-modeled parameters for APO, SPD, and SCH-23390 inhibition of [^{3}H]SCH-23390 binding in calf striatal membranes

	Without GTP					With GTP
	K_H (nM)	R_H (%)	K_L (nM)	R_L (%)	K_L/K_H	K_i (nM)
APO	2.7 ± 0.45	38.2 ± 4.9	378 ± 62	61.8 ± 4.9	140	155 ± 39
SPD	3.9 ± 1.1	59.1 ± 3.6	126 ± 25	40.9 ± 3.6	32.3	85.7 ± 13.3
SCH-23390	1.7 ± 0.29					

The competition experiments were performed in the absence and presence of GTP (450 μM). In the absence of GTP, the competition curves for APO and SPD fitted best to a two-site model ($P < 0.05$). In the presence of GTP, the competition curves for APO and SPD fitted best to a one-site model. Each value is the mean ± SEM of 4 experiments.

observed in the rats treated with the selective D_1 antagonist SCH-23390 (10 μmol/kg, i.p.). The rotation induced by SPD (10 μmol/kg, i.p.), however, was antagonized completely by pretreatment with SCH-23390 (5 min prior to SPD, $P < 0.01$). However, haloperidol only slightly reduced SPD-evoked rotational behavior in the same dose ($P > 0.05$) (Fig. 7). Thus, the agonistic action of SPD is characterized clearly as D_1 receptors.

DISCUSSION

It has been demonstrated that SPD and its analogs are a novel type of DA receptor antagonists [1, 2]. In the rotational behavior model, however, SPD shows agonist action on D_1 DA receptors [11, 12]. The mechanism of this paradoxical effect of SPD has not been clarified. The present study further elucidated that the agonist action of SPD originated from D_1 receptors, while the antagonist action of SPD originated from D_2 receptors.

The present study has shown that SPD as well as APO (a DA receptor agonist) could induce remarkable contralateral rotation in 6-OHDA-lesioned rats. At the same molar dose (10 μmol/kg, i.p.), however, the potency of SPD was weaker than that of APO ($P < 0.05$). The selective D_1 DA receptor antagonist SCH-23390 could not induce rotational behavior, but it antagonized the rotation induced by SPD. Consistent with previous reports [9–12], this result indicates that SPD can exhibit D_1 receptor agonist activity in the supersensitive DA receptors by 6-OHDA lesion.

Moreover, in the receptor binding assays, both SPD and APO competitively inhibited [^{3}H]SCH-23390 binding to D_1 receptors, and their shallow competition curves fitted best to an equation modeling two sites with a high affinity (R_H) and a low affinity (R_L). However, the ratio value of APO (K_L/K_H = 140) was larger than that of SPD (K_L/K_H = 32.3). This suggests that the intrinsic agonist effect of SPD is smaller than that of APO. When the competition curves were shifted to the right by GTP, the high-affinity components of both SPD and APO were abolished. Under the same conditions, the competition curve of SCH-23390 to D_1 receptors fitted best to a one-site model. These results with APO and SCH-23390 are similar to those of other reports [14, 16]. Thus, SPD possesses D_1 agonist action consistent with the rotational behavior. Interestingly, as to D_2 receptors, SPD demonstrated only one binding site in competition with [^{3}H]spiperone. This result indicates that

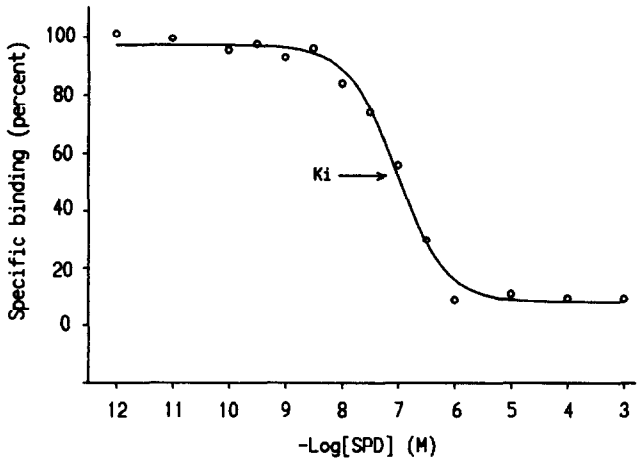


FIG. 4. Competition curve of SPD for specific [^{3}H]spiperone binding in calf striatal membranes from 3 separate experiments. Competition curves of SPD (N = 3) in the absence of GTP fitted best to a one-site model ($P > 0.05$) with a K_i value of 81.9 ± 14.4 nM.

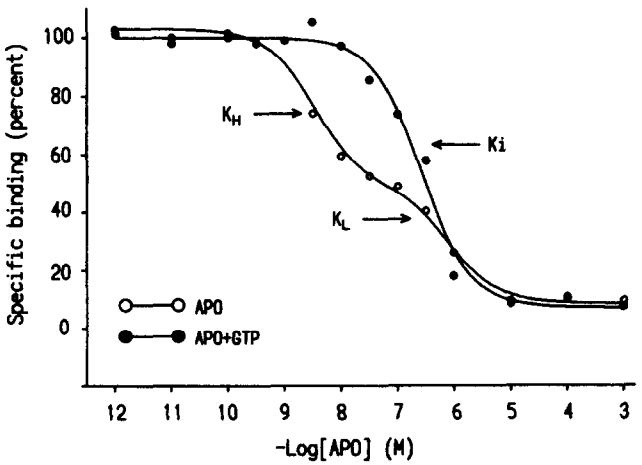


FIG. 5. Competition curve of APO for specific [^{3}H]spiperone binding in calf striatal membranes from 3 separate experiments. Competition curves of APO (N = 3) in the absence of GTP fitted best to a two-site model ($P < 0.05$) with K_H and K_L values of 2.9 ± 0.69 and 721.8 ± 141.5 nM. In the presence of 450 μM GTP (N = 3), the competition curves of APO fitted best to a single-site model ($P > 0.05$), with a K_i value of 227.9 ± 23.5 nM.

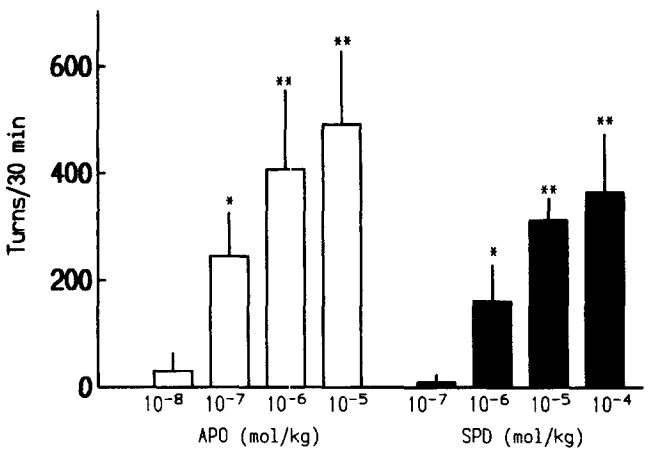


FIG. 6. Effects of APO and SPD on rotational behavior in a supersensitive model. The rats were lesioned unilaterally by microinjection of 6-OHDA into the right SNC. Sixty days later, the animals were screened for the ability to turn with APO and SPD given i.p. in a range of 10 nmol/kg to 100 μmol/kg. Both APO (open bars) and SPD (filled bars) induced leftward turning in a dose-dependent manner. Each column represents the mean ± SD of 6 experiments. Key: (*) $P < 0.05$, and (**) $P < 0.01$, compared with the saline control group. Values for the saline control group are 5 ± 3 turns/30 min, N = 6.

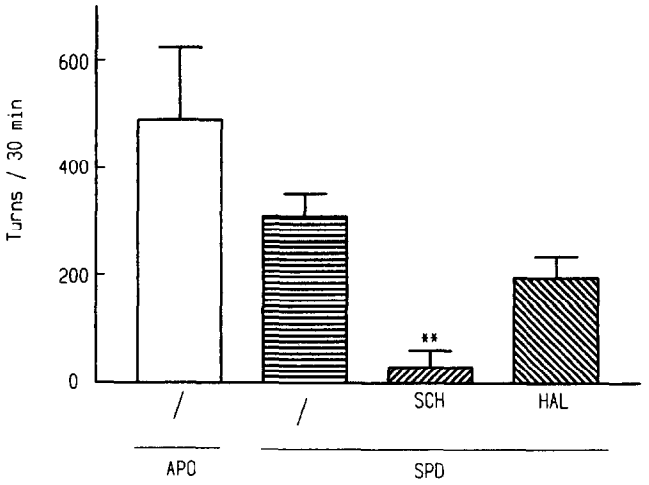


FIG. 7. Antagonist effects of SCH-23390 (SCH) and haloperidol (HAL) on SPD-induced rotational behavior. Rats pretreated i.p. with SCH-23390 or haloperidol in a dose of 10 μmol/kg were injected with SPD (10 μmol/kg, i.p.). Each column represents the mean ± SD of 17 experiments in the APO group and of 8 experiments in each SPD group. The dose of each compound was 10 μmol/kg. Key: (**) $P < 0.01$, as compared with the SPD alone group.

SPD exhibits an antagonist action at D_2 receptors, which is consistent with previous work [2, 4–6, 8, 10–12].

Based on the theory of the ternary complex model (ARN) [13, 17], although agonists (A) and antagonists can bind the receptor (R) recognition site, only agonists, but not antagonists, promote or stabilize the interaction between R and the guanine nucleotide regulatory subunit (N). Thus, the formation of two binding states, AR and ARN, in the ternary complex model represents the agonist action. The ARN state represents the R_H of agonist, and the binding of GTP to N promotes the reduction of R_H. The agonist action of SPD and APO on D_1 DA receptors obeyed this theory.

From the above-mentioned results, we could draw the conclusion that SPD is a D_1 agonist, although the present work was performed in different experiments (rotation and receptor binding assay) and different animals or tissues (rat and calf striatum). However, this idea has been supported by the following experiments by means of both lesioned-rat rotation and denervated striatal tissue of lesioned rats.

(1) In the AC activity test with the rat striatal membrane preparation under blockade with a D_2 receptor antagonist, butaclamol, SPD stimulated the AC activity and enhanced the cyclic AMP level [18]. This indicates that SPD is an agonist at D_1 receptors. In contrast to D_1 receptors, SPD showed antagonist action at D_2 receptors on AC activity which was measured under prior forskolin stimulation and blockade by SCH-23390.

(2) The D_1 agonist action of SPD is also observed in both the rotation induced by SPD in the 6-OHDA-lesioned rats and D_1 receptor supersensitivity seen with a binding assay in the denervated striatum of unilaterally 6-OHDA-lesioned rats [19].

(3) In a recent experiment with DARPP-32 protein (DA receptor phosphoprotein-32), which is a selective marker protein of D_1 receptors, SPD increased the phosphorylation of DARPP-32 in the striatum of the lesioned side, but not in the unlesioned side [20]. It is indicated that the D_1 agonistic action of SPD increases the phosphorylation on D_1 receptor marker protein.

Clearly, all experiments have established SPD as a D_1 receptor agonist. Previously, its agonist action on D_1 receptors has been found only in the rotational behavior. Interestingly, the present study and other work provide more evidence to support SPD as an agonist at D_1 receptors. Why does SPD hardly express its agonist action on D_1 receptors except in the rotation behavior? One explanation might be that SPD possesses weak intrinsic activity and is a D_1 partial agonist, as elucidated in very recent work [19, 21,*].

In conclusion, SPD has agonist action at D_1 receptors and antagonist action at D_2 receptors. This dual action of SPD on DA receptors is exactly opposite to the actions of bromocriptine and lisuride on DA receptors [22]. Both of the latter are agonists at D_2 receptors with weak antagonist action at D_1 receptors.

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* Zhang XX and Jin GZ, Manuscript submitted for publication.

References

1. Jin GZ, Progress in the pharmacological study of (−)-tetrahydropalmatine and (−)-stepholidine. *Acta Pharm Sin* **22:** 472–480, 1987.
2. Jin GZ, (−)-Tetrahydropalmatine and its analogues as new dopamine receptor antagonists. *Trends Pharmacol Sci* **8:** 81–82, 1987.
3. Xu SX, Yu LP, Han YR, Chen Y and Jin GZ, Effects of tetrahydroprotoberberines on dopamine receptor subtypes in brain. *Acta Pharmacol Sin* **10:** 104–110, 1989.
4. Zhang ZD, Jin GZ, Xu SX, Yu LP and Chen Y, Effects of (−)-stepholidine on central nervous and cardiovascular systems. *Acta Pharmacol Sin* **7:** 522–526, 1986.
5. Huang KX and Jin GZ, The antagonistic effects of tetrahydroprotoberberines on dopamine receptors: Electropharmacological studies. *Sci China B* **35:** 689–696, 1992.
6. Sun BC and Jin GZ, Effects of (−)-stepholidine on the firing activity of dopamine neurons in ventral tegmental area of rats. *Acta Pharmacol Sin* **13:** 395–399, 1992.
7. Chen LJ, Guo X, Wang QM and Jin GZ, Feed-back regulation of presynaptic D_2 receptors blocked by (−)-stepholidine and (−)-tetrahydropalmatine. *Acta Pharmacol Sin* **13:** 442–445, 1982.
8. Jin GZ, Han YR, Gonon FG, Yu LP, Xie Y and Xia Y, Inhibition of (−)-stepholidine on feedback regulation of striatal presynaptic DA receptors. *Chin J Physiol Sci* **7:** 195–203, 1991.
9. Shi WX, Chen Y and Jin GZ, Effects of (−)-stepholidine on rotational behavior in rats. *Acta Pharmacol Sin* **5:** 395–399, 1984.
10. Jin GZ, Wang XL and Shi WX, Tetrahydroprotoberberine—A new chemical type of antagonist of dopamine receptors. *Sci Sin* **29:** 527–534, 1986.
11. Huang KX, Sun BC and Jin GZ, (−)-Stepholidine: A dopamine receptor antagonist shows agonistic effect on rotational behavior in 6-hydroxydopamine-lesioned rats. *Acta Pharmacol Sin* **13:** 17–22, 1992.
12. Jin GZ, Huang KX and Sun BC, Dual actions of (−)-stepholidine on dopamine subtype receptors after nigral lesion. *Neurochem Int* **20** (Suppl): 175s–178s, 1992.
13. Leff SE, Hamblin MW and Creese I, Interactions of dopamine agonists with brain D_1 receptors labeled by [^{3}H]-antagonists: Evidence for the presence of high and low affinity agonist-binding states. *Mol Pharmacol* **27:** 171–183, 1985.
14. Hess EJ, Battaglia G, Norman AB, Iorio LC and Creese I, Guanine nucleotide regulation of agonist interactions at [^{3}H]SCH23390-labeled D_1 dopamine receptors in rat striatum. *Eur J Pharmacol* **121:** 31–38, 1986.
15. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
16. Seeman P, Ulpian C, Grigoriadis D, Pri-Bar I and Buchman O, Conversion of dopamine D_1 receptors from high to low affinity for dopamine. *Biochem Pharmacol* **34:** 151–154, 1985.
17. Leff SE and Creese I, Interactions of dopaminergic agonists and antagonists with dopaminergic D_3 binding sites in rat striatum: Evidence that [^{3}H]dopamine can label a high affinity agonist-binding state of the D_1 dopamine receptor. *Mol Pharmacol* **27:** 184–192, 1985.
18. Dong ZJ, Guo X, Chen LJ, Jin GZ and Han YF, Dual actions of (−)-stepholidine on the dopamine receptor-mediated adenylate cyclase activity in rat corpus striatum. *Life Sci*, in press.
19. Zou LL, Liu J and Jin GZ, Involvement of receptor reserve in D_1 agonistic action of (−)-stepholidine in lesioned rats. *Biochem Pharmacol* **54:** 233–240, 1997.
20. Guo X, Liu J, Dai H and Jin GZ, Effect of (−)-stepholidine on the phosphorylation of DARPP-32 protein in reserpinized and 6-OHDA-lesioned rats. *Information Chin Pharmacol Soc* **13:** 30, 1996.
21. Sun BC, Zhang XX and Jin GZ, (−)-Stepholidine acts as a D_1 partial agonist on firing activity of substantia nigra pars reticulata neurons in 6-hydroxydopamine-lesioned rats. *Life Sci* **59:** 299–306, 1996.
22. Kopin IJ, The pharmacology of Parkinson's disease therapy: An update. *Annu Rev Pharmacol Toxicol* **32:** 467–495, 1993.