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Effects of (−)-stepholidine on firing activity of dopamine neurons in ventral tegmental area of rats[1]

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ABSTRACT Extracellular single-unit recording techniques were used to evaluate the effects of (−)-stepholidine (SPD) on the firing activity of ventral tegmental area (VTA) dopamine (DA) neurons. SPD reversed the DA agonist apomorphine (Apo)-induced inhibition of VTA DA cell firing rate (ED_{50} = 4.9, 4.5-5.3 $\mu g \cdot kg^{-1}$), and the reversal was more rapid than that of a classic DA antagonist haloperidol (Hal) (ED_{50} = 11.2, 9.1-13.8 $\mu g \cdot kg^{-1}$). Pretreatment with SPD or Hal 0.5 $mg \cdot kg^{-1}$ attenuated Apo-induced inhibition, and SPD rendered the VTA DA cells less sensitive to larger doses of Apo (1024-4096 $\mu g \cdot kg^{-1}$) than Hal did. Pharmacological analysis indicated that the effects of SPD were mainly mediated through D_2 subtype receptors. In addition, SPD increased the firing rate of VTA DA cells, while higher doses (1.4, 0.6-3.3 $mg \cdot kg^{-1}$) of SPD dramatically inactivated 4 / 6 of the VTA DA cells sampled. This inhibition was considered to be due to depolarization inactivation. These results suggest that SPD is a DA receptor antagonist and can effectively block the D_2 autoreceptors located in the VTA DA cells.

KEY WORDS berbines; stepholidine; tegmentum mesencephali; dopamine receptors; electrophysiology

(−)-Stepholidine (SPD), isolated from *Stephania intermedica* Lo, is a well recognized dopamine receptor antagonist[1]. SPD has high affinity to dopamine (DA) D_1 and D_2 receptors in the striatum with preference to D_1 receptors[2]. It antagonizes DA agonists-induced behavioral responses[3], blocks the DA autoreceptor-mediated feed-back regulation in striatum[2,4-6], and reverses and / or antagonizes mixed DA agonist apomorphine (Apo)-induced inhibition of the firing activity of substantia nigra pars compacta (SNC) DA neurons[7]. All these data support that SPD is a DA receptor antagonist. But results from rotational behavior showed that SPD induced contralateral rotation in rats with unilaterial 6-hydroxydopamine (6-OHDA) lesions of SNC similar to DA agonists[8,9]. This feature is quite different from that of any other known DA receptor antagonists. Thus further investigation is warranted.

Ventral tegmental area (VTA) is one of the main regions containing DA neuron bodies in the brain[10]. VTA DA neurons innervate the mesolimbic areas and mesocortical sites, and play a very important role in the psychomotility and locomotion, including rotational behavior[10,11]. In addition, DA antagonists have been used to treat schizophrenia, which is supposed to be due to overactivation of mesolimbic and mesocortical dopaminergic system. Our previous investigations focused on the effects of SPD on nigrostriatal system. Although SNC and VTA DA cells have similar electrophysiological properties, some papers indicated that the 2 groups of DA cells were regulated by some different mechanisms, especially some atypical neuroleptic (eg, clozapine) had different influences on the 2 dopaminergic systems[12]. So the present paper aimed to study the effects of SPD on the firing activity of VTA DA neurons and to seek for some lights in elucidating the mechanisms of SPD on the dopaminergic systems.

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MATERIALS AND METHODS

Chemicals SPD (mp 161–2℃, $[\alpha]_D$ −440° in pyridine), isolated in this Institute, was dissolved in 10% H_3PO_4, then diluted and neutralized with NaOH 0.1 mol · L^{-1} to pH 5–7. Apomorphine–HCl (Apo, Shenyang First Pharmaceutical Co); haloperidol (Hal, Haipu Pharmaceutical Co, Shanghai); SKF 38393 and Sch 23390 (Research Biochemicals Incorporated, USA); N–0437 (Nelson Research, USA). Doses of the drugs given were in terms of the weight of their salts.

Rat surgery Sprague–Dawley rats ♂ (Shanghai Laboratory Animal Center), weighing 244 ± *s* 20 g, were anesthetized with chloral hydrate (0.4 g · kg^{-1}, ip) and mounted in a stereotaxic apparatus. A plastic cannula was inserted into a lateral tail vein for administration of drugs and additional anesthetic. A burr hole was drilled over the VTA and the dura was retracted. Coordinates for the VTA were: 3–3.1 mm anterior to lambda, 0.5–1.0 mm lateral to the middle suture, and 6.5–8 mm ventral to the dura, according to the atlas[13]. Rectal temperature was maintained at 36–38℃.

Single–unit recording The techniques have been described elsewhere[7,14]. Identification of neurons as VTA DA cells was ascertained on the basis of previously established criteria[12,14]: 1) position within 2 mm ventral to the red nucleus, 2) wide spike duration (>2.3 ms) with bi– or tri–phasic waveforms, 3) slow firing rate (1–10 Hz) with regular or bursting firing pattern, 4) low pitch sound produced on the audio amplifier.

Drug administration Steady baseline firing rate was recorded for 3–5 min before iv of any drugs. In cumulative dose experiments, the drugs were given in such a way that each dose equaled the previous cumulative dose at an interval of 1.5–2 min. Average firing rate during the last 40 s after each dose was counted as the function of each dose. Only one cell was studied in each rat.

Statistics Data were expressed as arithmetical $\bar{x} \pm s$, but the doses required to change the firing rate to a certain level were presented as geometric $\bar{x} \pm s$. Statistical significance of the differences between means was analyzed by two–tail *t* test. The ED_{50} values for drugs were determined by polynomial regression probit analysis. Significant differences between ED_{50} values or dose–response curves were detected by ANCOVA (analysis of covariance) with basal firing rate as a confounding variable.

RESULTS

Basal firing activity of VTA DA cells All the VTA DA cells studied here showed a firing rate of 2.9 ± 1.4 spikes · s^{-1} with a prolonged duration of action potential 2.9 ± 0.5 ms (n = 56). Data were expressed as the % of baseline firing rate as the function of each doses.

Reversal of SPD on Apo–induced inhibition of VTA DA cell firing activity Apo always fully inhibited the firing activity of VTA DA cells (ID_{95} = 27.2, 15.1–49.0 μg · kg^{-1}; n = 17). This inhibition required about 1 h to recover completely. SPD (1–64 μg · kg^{-1}) dose–dependently reversed the inhibition (ED_{50} = 4.9, 4.5–5.3 μg · kg^{-1}), and in comparison with Hal (ED_{50} = 11.2, 9.1–13.8 μg · kg^{-1}), SPD showed a more rapid reversal effect (P < 0.01) (Fig 1, 2)

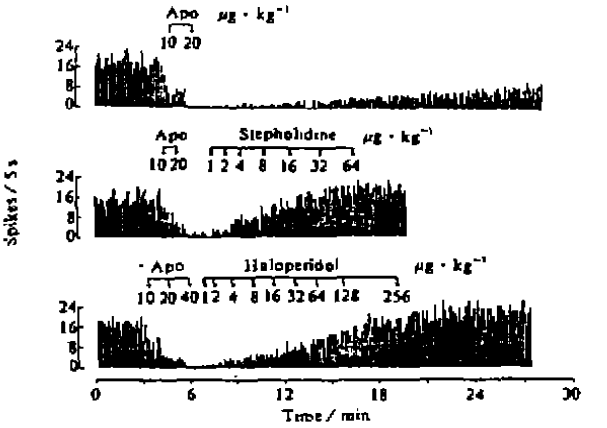

Fig 1. Reversal effects of (−)–stepholidine (SPD) and haloperidol (Hal) on apomorphine (Apo)–induced inhibition of ventral tegmental area (VTA) dopamine (DA) cell firing rate. Doses were represented as cumulative ones.

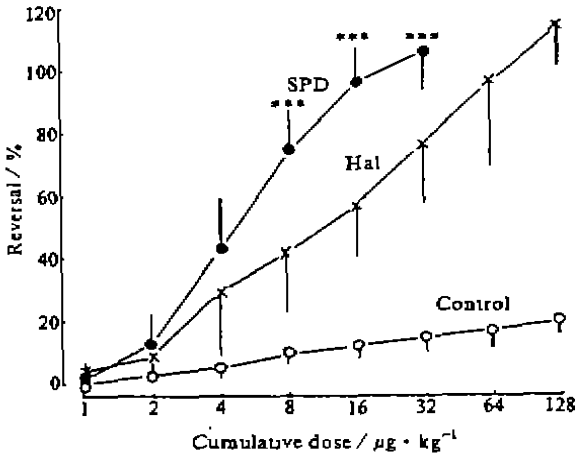

Fig 2. Reversal of SPD ($n=7$) and Hal ($n=5$) on Apo-induced inhibition of VTA DA cell firing activity. $\bar{x} \pm s$, $^{***}P<0.01$ *vs* Hal group. Basal firing rate (100%) for control ($n=5$), SPD and Hal group was 3.1 ± 0.9, 2.9± 1.2, and 3.0± 0.4 spikes · s^{-1}, respectively.

Attenuation of SPD on Apo-induced inhibition of VTA DA cell firing activity Apo-induced inhibition on the firing activity of VTA DA cells was also in a dose-dependend manner ($ID_{50}=5.9$, 5.2–6.7 μg · kg^{-1}). Following pretreatment with SPD or Hal 0.5 mg · kg^{-1}, extraordinarily high doses of Apo were necessary to suppress the VTA DA cell firing ($ID_{50}=245$, 229–262, and 242, 174–338 μg · kg^{-1}, respectively). There was no significant difference between the ID_{50} values of Apo in SPD and Hal pretreatment groups ($P>0.05$), but SPD rendered the VTA DA cells less sensitive to larger doses of Apo (1024–4096 μg · kg^{-1}) than Hal did ($P<0.05$) (Fig 3).

D_2 receptors mediated the effects of SPD on VTA DA cell firing activity D_1 agonist SKF 38393 (8 mg · kg^{-1}) had little effect on the firing activity of VTA DA cells, while D_2 agonist N-0437 (10–80 μg · kg^{-1}) sensitively inhibited the firing activity. SPD rapidly reversed the N-0437 and / or Apo induced

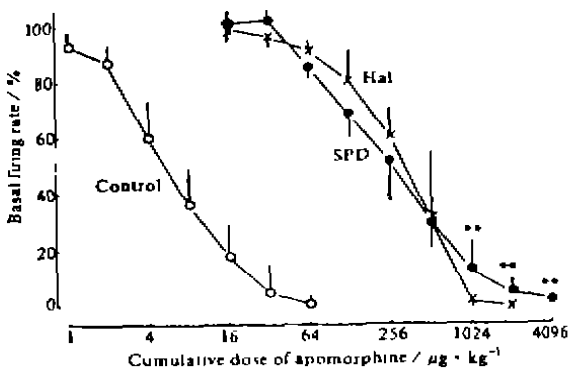

Fig 3. Attenuation of pretreatment with SPD ($n=6$) or Hal ($n=5$) on the Apo-induced inhibition of firing activity of VTA DA cells. $\bar{x} \pm s$, $^{**}P<0.05$ *vs* Hal pretreatment group. Basal firing rate (100%) for control ($n=6$), SPD, and Hal group were 2.9 ± 1.0, 3.6 ± 0.8, and 3.0 ± 1.0 spikes · s^{-1}, respectively.

inhibition, while D_1 antagonist Sch 23390 (up to 1 mg · kg^{-1}) showed only slight reversal effects (Fig 4).

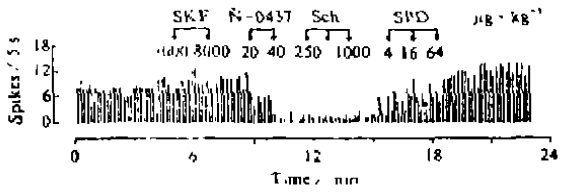

Fig 4. SPD affected firing activity of VTA DA neurous mainly via D_2 subtype receptors. SKF: SKF 38393; Sch: Sch 23390. Doses were represented as cumulative ones.

Inactivation of SPD on spontaneous firing activity of VTA DA cells SPD enhanced the spontaneous firing rate of 4 / 6 VTA DA cells at relatively low doses (Fig 5). The dose required for producing at least a 20% increase of basal firing rate was 0.14 (0.05–0.44) mg · kg^{-1}. The maximal firing rate after SPD administration was 39 ± 29% over the baseline ($n=6$) ($P<0.05$). But larger doses of SPD (1.4, 0.6–3.3 mg · kg^{-1}; $P<0.01$ *vs* doses

required for rate enhancement) dramatically drove 4/6 of VTA DA neurons into inactive, including the 2 cells with no significant elevation in firing rate after iv SPD. After the administration of SPD, the amplitude of the spikes became smaller and smaller. In these 3 sensitive cells, bursting activity began immediately before the onset of inactivation, and the amplitude of many of the spikes near the end of the burst was too near the noise to be picked up by the window discriminator. Consequently, the records for some cells indicate no rate elevation prior to inactivation. Thus, increased firing rate and/or inactivation afterwards took place in all the 3 VTA DA cells. The SPD-induced inhibition was reversed by DA agonist Apo or N-0437 (Fig 5).

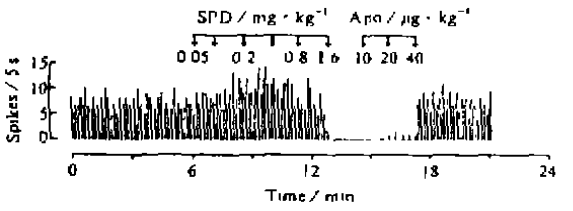

Fig 5. A example showing that SPD activated firing activity at small doses (0.05–0.8 mg · kg^{-1}) but larger dose (1.6 mg · kg^{-1}) dramatically inactivated the VTA DA cell and Apo reversed the SPD-induced inhibition. Doses were represented as cumulative ones.

DISCUSSION

This study demonstrated that SPD reversed and attenuated the DA agonist Apo-induced inhibition on the firing activity of VTA DA cells. In addition, the pharmacological analysis indicated that the effects of SPD were mainly mediated through D_2 receptors. These results suggest that SPD is a D_2 DA antagonist and it can block the DA autoreceptors (D_2) located in VTA DA cells to regulate the impulse flow.

In comparison with the classical potent DA antagonist Hal, SPD was more effective in reversing the Apo-induced inhibition on VTA DA cell firing activity, and pretreatment with SPD 0.5 mg · kg^{-1} rendered VTA DA cells less sensitive to high doses of Apo (1024–4096 μg · kg^{-1}) than Hal did. While previous binding assay experiments showed that the affinity of SPD to D_2 receptors was lower than that of Hal[2], Although it has been generally accepted that the firing activity of midbrain DA cells is mainly regulated by D_2 receptors, previous works and our further investigations showed that large doses of D_1 receptor agents were also involved in the modulation of the firing activity of midbrain DA cells [1,5], These results offered a possibility to explain the fact that SPD rendered the VTA DA cells less sensitive to large doses of Apo than Hal, as Apo is a mixed D_1 and D_2 agonist and SPD is a mixed D_1 and D_2 receptor antagonist with preferential affinity to D_1 receptors, while Hal is mainly a D_2 antagonist, thus SPD (0.5 mg · kg^{-1}) blocked the 2 components of Apo (when the dose was large) which both induced inhibition of VTA DA cell firing activity. In the reversal experiment, other reasons for more effectiveness of SPD may include differences between SPD and Hal in nucleus-selectivity, absorption rate and tissue distribution.

It is well-known that mixed DA receptor agonists or selective D_2 DA agonists inhibit the firing activity of midbrain DA cells. Does SPD-induced inhibition on firing rate of VTA cells represent an agonistic action on DA receptors as it possesses in 6-OHDA-lesioned behavior? The answer is no, since DA agonist Apo, but not DA antagonist, reversed the SPD induced inhibition; and the amplitude of spikes became smaller and smaller after administration of SPD, which was in a oppsite manner as DA agonist Apo. Our further observation demonstrated that inhibition challenged by SPD was due to excessive

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depolarization of VTA DA cells and the SPD-induced inactivation was similar to that of atypical neuroleptic clozapine while different from that of typical neuroleptic Hal (unpublished data).

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左旋千金藤立定对腹侧背盖区多巴胺神经元放电活动的作用

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提要 左旋千金藤立定(SPD)能翻转阿扑吗啡(Apo)对大鼠腹侧背盖区(VTA)多巴胺(DA)神经元的放电抑制($ED_{50}=4.9\ \mu g \cdot kg^{-1}$), 且比氟哌啶醇更迅速. SPD 显著减弱 Apo 对 DA 神经元的放电抑制, 它主要通过 D_2 起作用. SPD 能增加 DA 神经元自发放电, 大剂量($14\ mg \cdot kg^{-1}$)却使 4/6 的 DA 神经元失活. 提示: SPD 能阻滞 VTA D_2 DA 受体.

关键词 小檗因类; 千金藤立定; 中脑背盖; 多巴胺受体; 电生理学