

Effect of (-)-stepholidine on serum prolactin level of female rats¹

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KEY WORDS (-)-stepholidine; prolactin; radioimmunoassay; dopamine antagonists; pergolide

AIM: To study the effect of (-)-stepholidine (SPD) on serum prolactin (PRL) level and elucidate its pharmacological action on dopamine D₂ receptors. **METHOD:** After ip administration of dopamine receptor agonist, antagonist, or SPD, the serum PRL levels were determined by radioimmunoassay. **RESULTS:** SPD (24 mg·kg⁻¹, ip) caused a rapid rise in serum PRL level, lasting more than 1 h. SPD 0.2 - 40 mg·kg⁻¹ raised serum PRL level in a dose-dependent manner with ED₅₀ of 3.7 mg·kg⁻¹ (95 % confidence limits, 2.6-4.3 mg·kg⁻¹) and PRL maximal level of 448 ± 64 µg·L⁻¹. Pergolide 2 mg·kg⁻¹ ip caused a decrease (*P* < 0.01 vs saline) of PRL level, which was partially attenuated by SPD of 5 mg·kg⁻¹ and completely abolished by 10 mg·kg⁻¹. **CONCLUSION:** SPD is a dopamine D₂ receptor antagonist.

Prolactin (PRL) is a polypeptide hormone secreted from anterior pituitary. Its secretion is regulated by many factors, among which dopamine (DA) is a major inhibitory factor. DA is released from hypothalamic tuberoinfundibular neurons, transported through the portal vessels to anterior pituitary and exerts tonic suppression of PRL secretion via D₂ dopamine receptors in PRL secreting cells^[1,2,3]. Like dopamine, D₂ agonists suppress PRL secretion and thus lower serum PRL level, while D₂ antagonists enhance it^[2,4]. On the other hand, D₁ agents have no effects owing to lack of D₁ receptors in PRL secreting cells. Therefore, PRL secretion is a good model to evaluate D₂ receptor activity and interaction between D₂ receptor and its

drug.

(-)-Stepholidine (SPD), isolated from *Stephania*, possesses the affinity for D₁ and D₂ receptors^[5], and shows a D₂ antagonistic action in behavioral experiments^[6,7], striatal levodopa accumulation^[8] as well as firing regulation of nigral dopamine neurons^[9]. However, SPD behaves as a D₁ agonist in rats with unilateral nigral lesions^[10]. D₁ and D₂ receptors concomitantly exist in striatum or substantia nigra (SN), and interaction between the two receptors is inevitable during determination of D₁ or D₂ receptor functions in striatum or SN. What pharmacological action of SPD will display in the tissue lack of D₁ receptors such as pituitary remains unclear. The present work aims to study the action of SPD on pituitary D₂ receptors.

MATERIALS AND METHODS

Reagents and drugs Radioimmunoassay kits, rat prolactin antigen (NIDDK-rPRL-1-6, for iodination), rat PRL antiserum (NIDDK-anti-rPRL-S-9), and rat PRL reference preparation (NIDDK-rPRL-RP-3, for cold standard, holding a potency of 3×10^4 IU·g⁻¹) were provided by National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK). NIDDK-rPRL-1-6 was labeled with ¹²⁵I in Shanghai Institute of Nuclear Research, yielding a specific activity of 1.1 TBq·g⁻¹. Sheep anti-rabbit antiserum and normal rabbit serum were obtained from Shanghai Traditional Medical University. SPD (mp 161 - 162 °C, $[\alpha]_D - 440^\circ$ in pyridine), supplied by Shanghai Institute of Materia Medica, was dissolved in H₂SO₄ 0.1 mol·L⁻¹, then diluted and neutralized with NaOH 0.1 mol·L⁻¹ to pH 4 - 5. Haloperidol (Hal, Haipu Pharmaceutical Co, Shanghai) was diluted with distilled water. Dopamine agonist pergolide mesylate (Tianjin Institute of Materia Medica) was dissolved in distilled water. Saline as control was adjusted to pH 4 - 5.

Rats Sprague-Dawley rats (clean, ♀, *n* = 200, 195 ± s 23 g, Shanghai Experimental Animal Center, Shanghai. Certification No 005 conferred by Animal Management Committee, Chinese Academy of Sciences) were housed in an air-conditioned room and fed a standard rat chow with water *ad lib*.

Experimental protocol SPD or saline was injected ip to

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rats in experiments studying the time-course or dose-response. For determining the effect of SPD on pergolide-induced PRL suppression, rats were first given pergolide ($2 \text{ mg} \cdot \text{kg}^{-1}$) and then SPD 1 h later. All rats were decapitated 20 min after the last injection. Serum was separated and stored at $-20 \text{ }^\circ\text{C}$ until radioimmuno-assay (RIA) for PRL.

RIA PRL was determined by double antibody RIA. The dilution of PRL antiserum was 1:7000. Serum PRL level was assayed in duplicate.

Statistics Data were processed with an analysis program EIARIA. Significances were evaluated using *t* test and ANOVA. The ED_{50} value and maximal effect of SPD were estimated by rectangular hyperbola fitting analysis.

RESULTS

Time course of SPD on serum PRL Five groups of 8 rats each were given SPD $24 \text{ mg} \cdot \text{kg}^{-1}$ ip, and killed at 20 min, 1, 3, 5, and 20 h. A rapid rise in serum PRL was observed and a sharp decrease at 1 h. At 3 h, it returned to the control level of $41.3 \pm 2.2 \text{ } \mu\text{g} \cdot \text{L}^{-1}$. At 5 h, the PRL levels in both SPD and saline rats displayed a slight increase (Fig 1).

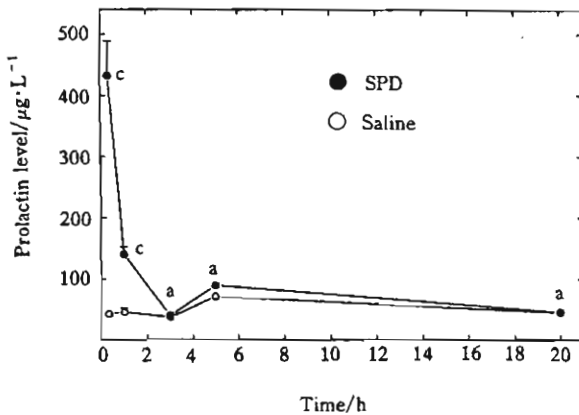


Fig 1. Effect of a single ip injection of SPD on serum PRL. *n* = 8 rats. ^a*P* > 0.05, ^c*P* < 0.01 vs saline.

Dose-response relationship of SPD on serum PRL Five groups of rats were given SPD 0.2, 1, 5, 10, 20, and $40 \text{ mg} \cdot \text{kg}^{-1}$. SPD $0.2 \text{ mg} \cdot \text{kg}^{-1}$ did not lead to any essential change in PRL level (*P* > 0.05 vs saline control). SPD 1 - $20 \text{ mg} \cdot \text{kg}^{-1}$ caused a dose-dependent increase of serum PRL level up to $448 \pm 64 \text{ } \mu\text{g} \cdot \text{L}^{-1}$, with ED_{50} of $3.7 \text{ mg} \cdot \text{kg}^{-1}$

(95 % confidence limits, $2.6 - 4.3 \text{ mg} \cdot \text{kg}^{-1}$). SPD $40 \text{ mg} \cdot \text{kg}^{-1}$ gave the increase of serum PRL no more. Hal $10 \text{ mg} \cdot \text{kg}^{-1}$ increased serum PRL level to $642 \pm 104 \text{ } \mu\text{g} \cdot \text{L}^{-1}$ (Tab 1).

Tab 1. Effect of SPD and Hal on serum PRL level in rats. ^a*P* > 0.05, ^b*P* < 0.05, ^c*P* < 0.01 vs saline. ^f*P* < 0.01 vs $10 \text{ mg} \cdot \text{kg}^{-1}$ SPD.

Dose/ $\text{mg} \cdot \text{kg}^{-1}$	Rats	Serum PRL/ μg
Saline	8	47 ± 10
SPD 0.2	8	44 ± 6 ^a
SPD 1	8	63 ± 5 ^b
SPD 5	8	304 ± 45 ^c
SPD 10	7	380 ± 52 ^c
SPD 20	8	448 ± 64 ^c
SPD 40	8	433 ± 46 ^c
Hal 10	8	642 ± 104 ^f

SPD antagonizes the action of D_2 agonist on serum PRL Pergolide $2 \text{ mg} \cdot \text{kg}^{-1}$ ip induced a remarkable decrease of serum PRL level (*P* < 0.01), indicating an inhibition of PRL secretion. This inhibition of pergolide could be antagonized by administration of SPD. SPD $5 \text{ mg} \cdot \text{kg}^{-1}$ attenuated pergolide inhibition, and SPD $10 \text{ mg} \cdot \text{kg}^{-1}$ completely abolished it (*P* < 0.01). SPD $40 \text{ mg} \cdot \text{kg}^{-1}$ not only abolished the action of pergolide but also induced higher PRL level than that of control rats received saline alone. SPD $2 \text{ mg} \cdot \text{kg}^{-1}$ hardly attenuated the effect of pergolide. Hal abolished the pergolide inhibition and SPD did and displayed more potent effect than Hal at the same dose of $10 \text{ mg} \cdot \text{kg}^{-1}$ (Tab 2).

Tab 2. Effects of SPD and Hal on serum PRL level in rats pretreated with pergolide $2 \text{ mg} \cdot \text{kg}^{-1}$. ^a*P* > 0.05, ^c*P* < 0.01 vs saline.

Dose/ $\text{mg} \cdot \text{kg}^{-1}$	Rats	Serum PRL
Saline	8	24 ±
SPD 2	8	31 ±
SPD 5	8	43 ±
SPD 10	8	60 ±
SPD 20	8	86 ±
SPD 40	8	112 ±
Hal 10	8	99 ±

DISCUSSION

Based on the above results it should be concluded that SPD is a D_2 antagonist in pituitary tissues. These results are consistent with previous studies of experiments performed in striatal tissues⁽⁸⁾, hippocampus⁽⁹⁾, and peripheral cardiovascular tissues⁽⁷⁾. As a D_2 antagonist, SPD displays a weaker action than Hal at the dose of $10 \text{ mg} \cdot \text{kg}^{-1}$, which is consistent with the receptor binding data⁽⁶⁾.

After ip injection SPD exhibits a short action with a half-life less than 180 min and its maximal effect is at 15 min, which is in agreement with the pharmacokinetic results⁽¹¹⁾ reported SPD with a peak absorption time of 15 - 30 min and an overall elimination phase $T_{1/2\beta}$ as short as 168 min. A slight increase in serum PRL level at 5 h might be attributed to the diurnal variation because the rise is observed in control SPD and control rats. A circadian rhythm of PRL secretion that serum PRL levels peak at 8:00 AM and reach a nadir (40 % of peak value) at 10:00 PM, was indeed reported⁽¹²⁾. In the time-course experiment rats of 5 h group were killed at 10:00 PM due to unexpected of the rhythm, which resulted in a higher PRL baseline than other groups of rats killed at 8:00 - 10:00 AM.

The present results, in combination with the previous experimental⁽¹⁰⁾ in which SPD displays a D_1 agonistic action, support SPD a dualist which exerts different action on heterotypic receptors. However, receptor sensitivity discrepancy (D_1 receptor supersensitivity in 6-OHDA-lesioned rats and D_2 receptor normosensitivity in the present study) should be considered. Whether SPD also displays D_2 agonistic action under supersensitive D_2 receptor remains to be studied.

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REFERENCES

1. Meltzer HY. Biochemical properties of CNS dopamine receptors. In: Meltzer HY, editor. Psychopharmacology: the third generation of progress; New York: Raven, 1987: 257 - 64.
2. Weiner RI, Ganong WF. Role of brain monoamines and dopamine in regulation of anterior pituitary secretion.

Physiol Rev 1978; 58: 905 - 76.
3. Weiner RI, Ramsdell JS, Weisman AS, Beach JE. Characterization of anterior pituitary dopamine receptors. In: Mena F, Valerde-R CM, editors. Prolactin secretion: a multidisciplinary approach. New York: Academic, 1984: 225 - 37.
4. Cocchi D, Gil-Ad I, Parenti M, Stefanini E, Locatelli V, Müller EE. Prolactin-releasing effect of a novel anti-dopaminergic drug, domperidone, in the rat. Neuroendocrinology 1980; 30: 65 - 9.
5. Jin GZ. (-)-Tetrahydropalmatine and its analogues, as new dopamine receptor antagonists. Trends Pharmacol Sci 1987; 8: 81 - 2.
6. Xu SX, Yu LP, Han YR, Chen Y, Jin GZ. Effects of tetrahydroprotoberberines on dopamine receptor subtypes in brain. Acta Pharmacol Sin 1989; 10: 104 - 10.
7. Zhang ZD, Jin GZ, Xu SX, Yu LP, Chen Y, Jiang FY, et al. Effects of *l*-stepholidine on central nervous and cardiovascular systems. Acta Pharmacol Sin 1986; 7: 522 - 6.
8. Chen LJ, Guo X, Wang QM, Jin GZ. Feed-back regulation of presynaptic D_2 receptors blocked by *l*-stepholidine and *l*-tetrahydropalmatine. Acta Pharmacol Sin 1992; 13: 442 - 5.
9. Huang KX, Jin GZ. The antagonistic effects of tetrahydroprotoberberines on dopamine receptors: electrophysiological studies. Sci Sin (B) 1992; 35: 688 - 96.
10. 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.
11. Zhang ZD, Zhou CM, Jin GZ, Zhang X, Yang L. Pharmacokinetics and autoradiography of [³H] or [¹⁴C] stepholidine. Acta Pharmacol Sin 1990; 11: 289 - 92.
12. Dunn JD, Arimura A, Scheving LE. Effect of stress on circadian periodicity in serum LH and prolactin concentration. Endocrinology 1972; 90: 29 - 33.

左旋千金藤立定对大鼠血清中催乳素水平的影响¹

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关键词 左旋千金藤立定; 催乳素; 放射免疫测定; 多巴胺拮抗剂; 培高利特

目的: 观察左旋千金藤立定 (SPD) 对血清催乳素 (PRL) 水平的影响, 研究 SPD 的药理作用. **方法:** 成熟♀鼠 ip 多巴胺受体激动剂、拮抗剂或 SPD 后断头取血, 然后用放射免疫法测定血清中的催乳素水平. **结果:** SPD 引起血清 PRL 水平迅速而显著的增加, 效应持续约 1 h, 具有剂量依赖性. SPD 的半数有效剂量为 $3.7 \text{ mg} \cdot \text{kg}^{-1}$ (95% 可信限为 $2.6 - 4.3 \text{ mg} \cdot \text{kg}^{-1}$). 剂量为 $20 \text{ mg} \cdot \text{kg}^{-1}$ 时产

生最大效应, 使血清 PRL 水平达到 $448 \pm 64 \mu\text{g} \cdot \text{L}^{-1}$, $0.2 \text{ mg} \cdot \text{kg}^{-1}$ 则无效. 对于多巴胺受体激动剂培高利特 (pergolide) 引起的 PRL 水平低下,

SPD $5 \text{ mg} \cdot \text{kg}^{-1}$ 有部分对抗作用, $10 \text{ mg} \cdot \text{kg}^{-1}$ 够完全对抗. 结论: SPD 是 D_2 多巴胺受体拮抗剂.

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Anxiogenic effect of naltrexone in social interaction test in rats¹

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KEY WORDS naltrexone; animal behavior; morphine; fenclonine; 5-hydroxytryptophan; locomotion; anxiety disorders

AIM: To study the anxiogenic effect of naltrexone (Nal) on the emotional state of rats. **METHODS:** The duration of active interaction was measured in the social interaction test in rats. **RESULTS:** Without influence on the locomotor activity, Nal ($0.1 - 50 \text{ mg} \cdot \text{kg}^{-1}$) dose- and time-dependently decreased the duration of active interaction, which was antagonized by morphine ($5 \text{ mg} \cdot \text{kg}^{-1}$) or fenclonine (Fen, $150 \text{ mg} \cdot \text{kg}^{-1} \times 3 \text{ d}$) and was enhanced by 5-hydroxytryptophan (5-HTP, $50 \text{ mg} \cdot \text{kg}^{-1}$). **CONCLUSION:** Nal produced anxiety via its blockade of opioid receptors; central opioidergic neurons were involved in the regulation of anxiety through their tonic inhibitions in serotonergic neurons.

The studies of endogenous opioid peptides (EOP) have been focused on their analgesia and dependence since they were discovered more than 20 years ago, whereas their effects on anxiety are still unknown. This is because: (1) Exogenous opioids can not cross over the blood-brain barrier and are degraded quickly by the pertinent peptidase, so it is difficult to observe behavioral effects; (2) The anxiety studied is mostly of the state one which is characterized by instantaneousness⁽¹⁾. Fortunately, the function of EOP can be observed indirectly by

opioid antagonists, through which the effects of EOP on stress and analgesia have been studied successfully. Naltrexone (Nal) is a potent antagonist of opioid receptors, which acts mainly at μ subtype. In contrast to naloxone, Nal is orally effective and lasts longer⁽²⁾. Although Nal antagonizes exogenous opioids powerfully, Nal is hardly effective when given alone. However, we found recently that Nal had an anxiogenic effect in Vogel's conflict test⁽³⁾, which suggested that central EOP were involved in the regulation of anxiety. To verify such a result and explore its possible mechanism, we observed the effect of Nal in the social interaction test in rats.

MATERIALS AND METHODS

Fen, 5-HTP, and methyl-4-ethyl-6, 7-dimethoxy- β -carboline-3-carboxylate (DMCM) (Sigma, USA) were dissolved in 0.9% NaCl, but the solution of Fen containing Na_2CO_3 ($10 \text{ mmol} \cdot \text{L}^{-1}$ (pH 9) while those of 5-HTP and DMCM containing HCl $800 \text{ mol} \cdot \text{L}^{-1}$ (pH 5). Nal hydrochloride (Institute of Pharmacology and Toxicology, Beijing, China), morphine hydrochloride (Qinghai Pharmaceutical Factory, Qinghai, China), and clonidine hydrochloride (Changzhou Pharmaceutical Factory, Jiangsu, China) were dissolved in 0.9% NaCl. Drugs and vehicles were injected sc (for Nal and morphine) or ip, in a volume of $2 \text{ mL} \cdot \text{kg}^{-1}$ or $3 \text{ mL} \cdot \text{kg}^{-1}$ (for Fen and 5-HTP).

Male Wistar rats (Animal Center of Academy of Military Medical Sciences, Beijing, China) were grade-1 animals and weighed $250 \pm 25 \text{ g}$ ($n = 488$). The rats were housed in a room with temperature of $20 - 25 \text{ }^\circ\text{C}$, lights on from 07:00-19:00. Experiments were carried out daily during 08:00-13:00, keeping the room quiet. Blind observation was used to make the results more reliable.

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