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Effects of tetrahydroprotoberberines on dopamine D_2 receptors in ventral tegmental area of rat

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KEY WORDS tetrahydroprotoberberines; ventral tegmental area; dopamine D_2 receptors; action potentials; apomorphine; Sch-23390; haloperidol

AIM: To compare the actions of tetrahydroprotoberberines (THPB) on dopamine (DA) D_2 receptors in the ventral tegmental area (VTA) of rat. **METHODS:** Extracellular single unit recording technique was used in iv gallamine-paralyzed rats. **RESULTS:** Eleven THPB analogs tested completely attenuated the apomorphine (Apo, 20 $\mu g \cdot kg^{-1}$)-induced inhibition on VTA DA cell firing activity. The OH group on C_2 at THPB was linked with the reversal of Apo-induced inhibition. Their reversal potencies (ED_{50}, $\mu g \cdot kg^{-1}$) for D_2 receptors were: THPB-143 (5.6) > SPD (8.5) > Iso (17.0) > THP (33) > THB (48) > THPB-18 (66) > THPB-1 (179) > THPB-19 (408) > THPB-126 (510) > THPB-104 (1019) > THPB-10 (4815). **CONCLUSION:** Among these 11 THPB, the 2-hydroxyl-THPB (THPB-143) showed the strongest antagonistic action on D_2 receptors.

The dopamine (DA) neurons in the ventral tegmental area (VTA) and their ascending cortical and limbic projections are involved in the etiology or symptomatology of a variety of neuropsychiatric illnesses such as schizophrenia, the VTA becomes a very important action site of antipsychotic drugs[1,2]. Considerable evidence indicates that D_2 autoreceptors on the soma-dendrites of the VTA have negative regulation on the firing activity of DA neurons. The D_2 autoreceptors are 5 to 100 times more sensitive to DA agonists than the postsynaptic DA receptors[3]. The low dose of DA agonists, ie apomorphine (Apo), preferentially displays its inhibition on DA neuron firing activity via D_2 autoreceptors, while D_2 antagonists attenuate the Apo-induced inhibition[3]. Thus D_2 autoreceptors in the VTA DA cells are used to evaluate the effects of selective D_2 DAergic drugs.

Tetrahydroprotoberberines (THPB) are the alkaloids isolated from Chinese traditional medicine *Corydalis* and Chinese herb *Stephania*, or chemical synthesized compounds. They share a common chemical structure (Tab 1) with hydroxyl or methoxyl groups at C_2, C_3, C_9 and C_{10} positions. Previous studies have verified that THPB are the novel DA active compounds on the brain[4-5], but these studies mainly focused on the effects of THPB on the nigrostriatal system, which is thought to be related to the regulation of the extrapyrimal motor activity. However, the action characterizations of THPB on the VTA has not been studied systemically yet. Therefore, the present work aimed to investigate the effect of THPB on the firing activity of VTA DA neurons and to characterize their actions on D_2 receptors.

MATERIALS AND METHODS

Chemicals Tetrahydroberberine (*THB*), THPB-1, THPB-10, THPB-18, THPB-19, THPB-104, THPB-126, THPB-143 were synthesized in Shanghai Institute of Materia Medica, *l*-tetrahydropalmatine (THP), *l*-isocorypalmine (Iso), and *l*-stepholidine (SPD) were isolated from *Corydalis* and *Stephania* (Tab 1).

They were dissolved in H_2SO_4 0.1 $mol \cdot L^{-1}$, adjusted with NaOH 0.1 $mol \cdot L^{-1}$ to pH 5-5.5, and then diluted. Apo-HCl (Shenyang Pharmaceutical Co); gallamine triethiodide (Sigma); lidocaine-HCl (Haipu Pharmaceutical Co, Shanghai) were used.

Rats Sprague-Dawley rats ♂ ($n = 82$, $275 \pm s$ 38 g) were anesthetized with ether for surgery and mounted on a stereotaxic apparatus. A plastic cannula was inserted into the lateral tail vein for infusion of gallamine triethiodide (20 mg $\cdot kg^{-1}$, iv) and injection of test compounds. A tracheal cannula was connected to a respirator. A burr hole was drilled over the skull (3.2 mm anterior to lambda, 0.8 mm lateral to the sagittal sinus, and 6.5-8 mm ventral to the dura for VTA[6]). Rectal temperature was maintained at 36-38 ℃.

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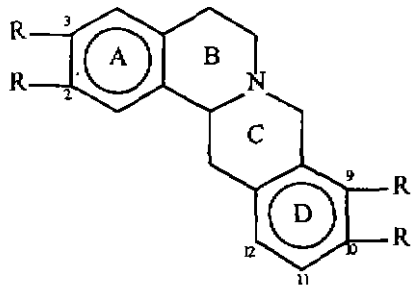
Tab 1. Chemical structures of tetrahydroprotoberberines (THPB) and their reversal potencies on Apo-induced inhibition of VTA DA neuron firing activity for D_2 receptors.



Compound	R position						ED_{50} and 95 % fiducial
	2	3	9	10	11	12	limits/$\mu g \cdot kg^{-1}$
THB		O−CH_2−O	OCH_3	OCH_3	H	H	47.6 (40.9 − 55.6)
THP	OCH_3	OCH_3	OCH_3	OCH_3	H	H	32.9 (28.3 − 38.2)
SPD	OH	OCH_3	OCH_3	OH	H	H	8.5 (2.9 − 25.1)
THPB-1	OH	OH	OCH_3	OCH_3	H	H	179 (159 − 202)
THPB-18	OH	OCH_3	OH	OCH_3	H	Cl	65.8 (58.1 − 74.4)
THPB-19	OH	OCH_3	OH	OCH_3	H	Br	408 (341 − 488)
Iso	OH	OCH_3	OCH_3	OCH_3	H	H	17.0 (14.8 − 19.4)
THPB-143	OH	OCH_3	H	H	H	H	5.6 (5.1 − 6.2)
THPB-126	OCH_3	OCH_3	Cl	OH	H	H	510 (438 − 592)
THPB-10	OCH_3	OCH_3	OH	OCH_3	H	Cl	4 815 (4 066 − 5 702)
THPB-104	OCH_3	OCH_3	H	OH	OCH_3	Cl	1 019 (838 − 1 239)

Single unit recording[7] Extracellular neuronal signals were recorded from single glass microelectrode fulled with NaCl 2 mol · L^{-1} and Pontamine Sky Blue (the impedance was 3 − 10 MΩ measured *in vitro*) which had been inserted into the VTA. The signals were amplified, displayed on an oscilloscope, calculated with an IBM computer recorded by a cassette data recorder, and monitored by an audio amplifier.

Identification of DA neurons Neurons recorded were identified as VTA DA cells: (1) low spontaneous firing rates of 1 − 10 Hz, (2) either a regular or a burstig firing pattern with decreasing spike amplitude, and (3) long spike duration (>2.2 ms) with tri-phasic waveforms and usually with a notch in the rising phase[8].

After 4 − 6 min of stable spontaneous firing was recorded, test compounds were injected iv in a way that each dose equaled the previous cumulative dose at an interval of 90 s for the same drug in all cumulative dose-response experiments.

Histological examination At the end of experiment, the recording site was marked by padding a 25 μA cathodal current through the recording barrel for 20 − 30 min to deposit Pontamine Sky Blue dye. The rats were perfused with saline followed by 10 % buffered formalin. Frozen serial sections (50 μm) were cut and the dye site was verified under a light microscope.

Statistics The antagonists reversing 50 % of agonist-induced inhibition (ED_{50}) was determined by a logit method.

RESULTS

Basal firing activity of VTA DA neurons In paralyzed rats (n = 56), all the VTA DA neurons fired spontaneously in a rate of 3.1 ± 1.7 spikes · s^{-1} with the long duration of action potentials (>2.5 ms). Two types of firing pattern were recognized: (1) regular single spontaneous firing and (2) burst firing with diminishing amplitude separated by short intervals and followed by a subsequent pulse. No significant effect of gallamine

used to paralyze rats was seen on the basal firing activity of VTA DA neurons.

THPB reversing Apo-induced inhibition on firing activity of VTA DA neurons In paralyzed rats, Apo 1 – 16 $\mu g \cdot kg^{-1}$ profoundly inhibited the firing activity of VTA DA neurons. The suppression lasted 1 h or more to recover spontaneously to the basal level. The Apo-induced inhibition was not antagonized by Sch-23390 (D_1 selective antagonist), but was rapidly reversed by haloperidol (D_2 antagonist) (Fig 1).

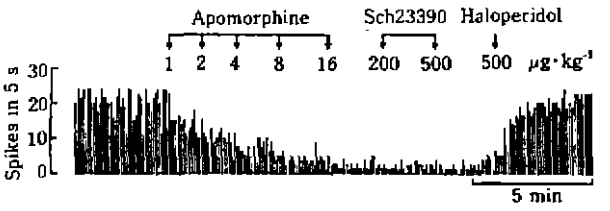


Fig 1. Effects of Sch-23390 and haloperidol on apomorphine-induced inhibition on VTA DA neuron firing activity.

The inhibitory effect of Apo on VTA DA neurons firing activity was mainly through D_2 receptors. Two or three minutes after the Apo-induced inhibition, the THPB rapidly reversed the inhibition with their cumulative doses. When the dose was larger than 2 times of ED_{50}, the firing rate was often driven over the baseline and the single spontaneous firing pattern converted into a burst firing one.

Comparison of reversal potencies of THPB The reversal potency was determined by the 50 % reversing of the Apo-induced inhibition (ED_{50}). It could be summarized: (1) THPB with an OH group on C_2, such as isocorypalmine and THPB-143, were more potent than those with OH group on C_9 or C_{10}, such as THPB-126, THPB-104, and THPB-10. (2) THPB with two OH groups attached to the A and D rings, respectively, on C_2 and C_{10} (SPD) or on C_2 and C_9 (THPB-18) were more potent than those with two OH groups on C_2 and C_3 of A ring (THPB-1). (3) When OH groups were substituted by methoxy or methylenedioxy group, the effects of THPB decreased, as in cases of THB and THP (Tab 1).

DISCUSSION

Using electrophysiological method *in vivo*, the present study demonstrated that THPB attenuated the DA agonist Apo-induced inhibition on the firing activity of VTA DA neurons. The attenuation was mediated via the D_2 receptors. Many studies also reported D_2 receptors located on the VTA DA neuronsoma and dendrites in detail[3,9]. THPB showed the characteristics of D_2 antagonist, but did not display agonistic effects. The results are consistent with those from biochemical[5] and behavioral studies (unpublished), which all suggested THPB to be D_2 receptor antagonists.

In addition, the relationship of structure-activity among THPB has demonstrated the action role of the OH group on C_2, C_9, and C_{10}. It appeared that the C_2 position is critically important to the potency of THPB for D_2 receptors. The results were compatible with that of our previous work on radioligand binding assay *in vitro*[5]. No matter what is mono-hydroxy THPB (such as THPB-143 and Iso) or dihydroxy-THPB (such as SPD and THPB-18) which have one hydroxy group at C_2 position, they would revealed the strong potency to reverse the Apo-induced inhibition. In other word, the effective potencies of THPB for D_2 receptors are inseparably linked with OH group on C_2 at THPB.

It is well known that DA antagonists, especially D_2 receptor antagonists have been widely used in clinic to treat schizophrenia, which is supposed to be due to the overactivation of the VTA DA neurons innervating mesolimbic and mesocortical areas. The present study has demonstrated that THPB are the DA antagonists on VTA DA neurons. Thus, THPB, especially the 2-hydroxyl-THPB, are warrant to be developed for the antipsychotic drugs.

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四氢原小檗碱同类物对大鼠中脑腹侧被盖区 D_2 多巴胺受体的作用

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关键词 四氢原小檗碱类；中脑腹侧被盖区；多巴胺 D_2 受体；阿扑吗啡；Sch-23390；氟哌啶醇

目的：阐明四氢原小檗碱同类物(THPB)对大鼠中脑腹侧被盖区(VTA)多巴胺(DA)受体的作用特性，并比较它们的作用强度. 方法：采用大鼠在体胞外单位放电记录. 结果：观察了 11 个 THPB 均可完全地翻转 DA 受体激动剂阿扑吗啡(20 $\mu g \cdot kg^{-1}$)所产生的放电抑制作用，为 D_2 受体拮抗剂的作用特性. THPB 对 D_2 受体的作用与 C_2 位上的 OH 基团有密切的关系. 它们的作用强度(ED_{50}，$\mu g \cdot kg^{-1}$)：THPB-143 (5.6) > SPD (8.5) > Iso (17.0) > THP (33) > THB (48) > THPB-18 (66) > THPB-1 (179) > THPB-19 (408) > THPB-126 (510) > THPB-104 (1019) > THPB-10 (4815). 结论：11 个 THPB 均为 VTA D_2 受体拮抗剂，以 C_2 位上有 OH 基团的 THPB-143 作用最强.

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Effects of clonidine on myocardial β-adrenergic receptor-adenyl cyclase-cAMP system after scalds in rats[1]

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KEY WORDS clonidine; burns; beta-adrenergic receptors; adenyl cyclase; phosphoric diester hydrolases; cyclic AMP; myocardium; yohimbine; prazosin

AIM: To study the role of clonidine (Clo) on the myocardial β-adrenergic receptor (β-AR)-adenyl cyclase (AC)-cAMP system after the scalds in rats. **METHODS**: A 30 % skin-full-thickness scald was produced by immersing rats in 95 ℃ water for 9 s. Clo 0.1 - 3.0 $mg \cdot kg^{-1}$ was injected ip to rats at 30 min before scalds, yohimbine (Yoh) 0.05 $mg \cdot kg^{-1}$ or prazosin (Pra) 0.03 $mg \cdot kg^{-1}$ to rats at 30 min before ip Clo. β-AR density and affinity, AC activity, phosphoric diester hydrolases (PDH) activity, and cAMP content were determined with

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