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## 左旋千金藤立定对大鼠纹状体酪氨酸羟化酶活性的影响

R963

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**提要** 本文研究 SPD 对大鼠纹状体 DOPA、DA 和 DOPAC 含量及酪氨酸羟化酶活性的影响. ip SPD 2.5 和氟哌啶醇 1.0 mg · kg$^{-1}$ 增强 NSD 1015 引起的大鼠纹状体 DOPA 和 DOPAC 累积 SPD 不改变 GBL 增加大鼠纹状体 DA 含量的作用，但阿扑吗啡显著抑制 GBL 的作用. ip SPD 5 或氟哌啶醇 2.5 mg · kg$^{-1}$ 显著增强大鼠纹状体酪氨酸羟化酶的活性. 结果提示 SPD 对突触前 DA 受体表现阻滞作用.

**关键词** 左旋千金藤立定；酪氨酸羟化酶；纹状体；氟哌啶醇；阿扑吗啡

SPD

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# Comparison of effects of tetrahydropalmatine enantiomers on firing activity of dopamine neurons in substantia nigra pars compacta[1]

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**ABSTRACT** Extracellular single unit recording techniques were used to elucidate the effects of enantiomers of tetrahydropalmatine (THP) on the firing activity of dopamine (DA) neurons in substantia nigra pars compacta (SNC). (−)–THP rapidly reversed the apomorphine (Apo)–induced inhibition of the SNC DA cell firing activity ($ED_{50}$ = 0.77, 0.52–1.14, mg · kg$^{-1}$), while much larger doses of (+)–THP were required to reverse the Apo–induced inhibition ($ED_{50}$ = 23, 15.2–34.7, mg · kg$^{-1}$) and the maximal reversal caused by (+)–THP was 79 ± 9% of the basal firing rate. In paralyzed rats, (−)–THP (0.5–16 mg · kg$^{-1}$) significantly increased the spontaneous firing rate of SNC DA neurons dose–dependently, while (+)–THP did not until the dose reached 16 mg · kg$^{-1}$. Pretreatment with (−)–THP 4 mg · kg$^{-1}$ attenuated Apo–induced inhibition of SNC DA cell firing rate, while (+)–THP 32 mg · kg$^{-1}$ revealed a similar potency to block the Apo–induced inhibition. In addition, (+)–THP did not potentiate the effect caused by *d*–amphetamine (Amp) as some behavioral experiments have shown, but large dose of (+)–THP (32 mg · kg$^{-1}$) blocked the Amp–induced inhibition of SNC DA cell firing activity as (−)–THP (4 mg · kg$^{-1}$) did. These results sug–

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gest that the interaction between $D_2$ receptors and THP enantiomers has stereoselectivity and that (−)−THP is a $D_2$ antagonist while (+)−THP seems to be not.

**KEY WORDS** tetrahydropalmatine; berbines; substantia nigra; dopaminergic agents; electrophysiology

It has been found that (±)−tetrahydropalmatine (THP) is the main active ingredient of the Chinese herb *Corydalis turtschaninovii* Bess. f. yanhusuo Y.H. Chou et C.C. Hsu, a famous analgesic in Chinese traditional medicine, and that levo−enantiomer of THP ((−)−THP) possesses both sedative−tranquilizing effect and analgesic action, while dextro−enantiomer ((+)−THP) has no such effect at the same dose except a transient excitation[1]. Behavioral, biochemical and electrophysiological experiments have shown that opiatergic system and PGs are not involved in the mechanism of analgesia of (−)−THP directly. (−)−THP is verified as a dopamine (DA) receptor antagonist[2,3]. However, (+)−THP differs much from its levo−isomer, it is supposed to be a prototype of DA depletor[4−6], and behavioral experiments have shown that (+)−THP potentiates the *d*−amphetamine (Amp) induced rotation in rats with 6−hydroxydopamine (6−OHDA) lesions of substantia nigra pars compacta (SNC) and / or spontaneous activity in mice[4].

It is generally agreed that there are autoreceptors on DA neuron somato−dendrites, which are mainly $D_2$ receptor subtype and play an important role in the regulation of impulse flow and synthesis and release of DA. Plenty of experiments have demonstrated that these autoreceptors are more sensitive to DA agonists than postsynaptic DA receptors[7,8]. In order to further compare the different effects between (−)−THP and (+)−THP and clarify the mechanism of action the 2 isomers in a sensitive model, the present work attempts to investigate the effects of (−)−THP and (+)−THP on the firing activity of SNC DA neurons.

## MATERIALS AND METHODS

**Drugs** (−)−THP was isolated from *Stephania intermedia* Lo. mp. 141−2℃, $[\alpha]_D = -287.5°$ (C, 1.0, $CHCl_3$) and (+)−THP was resolved from (±)−THP by optic tartaric acid. mp. 140−141℃, $[\alpha]_D = +285°$ (C, 0.49, $CHCl_3$). They were dissolved in 10% $H_3PO_4$, adjusted by NaOH 0.5 mol · $l^{-1}$ to pH 4−5. The other drugs used were: apomorphine−HCl (Shengyang Pharmaceutical Co); Haloperidol (Shanghai 12th Pharmaceutical Co); gallamine trithioidide and Amp (Sigma); chloral hydrate (Shanghai Baihe Chemical Co); lidocaine−HCl (Haipu Pharmaceutical Co, Shanghai).

**Surgery** Sprague−Dawley rats ♂ (264 ± *s* 33 g) were used in all experiments. Rats were anesthetized with chloral hydrate (400 mg · $kg^{-1}$, ip); or anesthetized with short−acting anesthetic ether for surgery, then paralyzed (gallamine triethiodide, 20 mg · $kg^{-1}$, iv) and locally anesthetized (lidocaine−HCl) during recording. The later procedure was sometimes used because the anesthesia significantly attenuated the activation of spontaneous firing activity of DA neurons caused by DA antagonists, while DA agonists−induced responses are only slightly affected by anesthetics[7,9]. A cannula was intubated into the trachea and then connected to an artificial respirator in paralyzed rats. In each rat, a plastic cannula was inserted into a lateral tail vein for drug administration. All experiments were carried out in strict accordance with the "Guiding Principles in Care and Use of Animals" approved by the Council of American Physiologic Society.

**Single−unit recording procedure** Recording procedures were conducted according to well established methods[7,9]. Briefly, a small burr hole was drilled through the skull (3.0−3.5 mm anterior to lambda and 2.0−2.5 mm lateral to middle suture) of rats fixed into stereotaxic apparatus[11]. Electrical signals from the glass microelectrode (the impedance was 3−9 MΩ *in vitro*) having inserted through the burr hole into the SNC (6.0−7.5 mm ventral to the dura) were amplified, then displayed on an oscilloscope and simultaneously calculated with an IBM computer

recorded by a cassette data recorder and monitored by an audio amplifier.

Identification of neurons as DA cells was based on previously well-established criteria[7,10]: (1) low firing rate with two firing patterns, regular single firing or burst firing; (2) long spike duration (>2.2 ms) with bi- or tri-phasic and usually a notch in the rising phase; (3) low pitch sound produced on the audio amplifier; (4) sensitive to DA agonist such as apomorphine (Apo).

In the cumulative dose-response experiments, after 5-7 min of stable firing, the drugs were given iv in a way that each dose equaled the previous cumulative dose at an interval of 90 s for the same drug. Only 1 cell per rat was tested. At the end of each experiment, the recording site was marked by passing a 25 μA cathodal current through the electrode for 20-30 min to deposit a spot of dye (Pontamine sky blue). Then the recording site was verified histologically.

**Statistics** The doses of agonist producing a 50% reduction in activity ($ID_{50}$) or antagonist reversing 50% of agonist-induced inhibition ($ED_{50}$) were determined by logit method. Significant difference between $ED_{50}$ values was detected by ANCOVA (analysis of covariance) with basal firing rate as a confounding variable[12]. All other data were expressed as $\bar{x} \pm s$ and evaluated by $t$ test for the 2-tail paired value.

## RESULTS

**Basal firing activity of SNC DA neurons** A total of 101 DA neurons studied fired at range of 1.1-8.2 (3.4 ± 1.9) spikes · $s^{-1}$ with prolonged duration of action potential (2.3-4.5 ms). Data shown below were expressed as percentage of pre-medication firing rate as a function of the dose administration.

**Reversal of the Apo-induced suppression of firing activity of SNC DA cell by THP enantiomers** Apo, a mixed DA agonist, feedbackly inhibited the firing activity of SNC DA neurons. In anesthetized rats, the suppression (>90% of basal firing rate) caused by Apo (10-40 μg · $kg^{-1}$) took 1 h or more to recover spontaneously to basal level. Two min after the Apo-induced inhibition, systemic (-)-THP (0.5-32 mg · $kg^{-1}$, cumulative dose, iv) rapidly reversed the suppression ($ED_{50}$=0.77, 0.52-1.14, mg · $kg^{-1}$, $n$=9) and drove the firing rate over the basaline, 3 out of 9 cells increased the firing rate to more than 35% over the basal rate. The single firing pattern often converted into a burst firing as larger doses of (-)-THP were given. (+)-THP, however, failed to significantly reverse the Apo-induced suppression until the dose reached 32 mg · $kg^{-1}$ ($ED_{50}$=23, 15.2-34.7, mg · $kg^{-1}$, $n$=7, 29.9-fold that of (-)-THP) ($P$<0.01) and the maximal reversal rate was 79 ± 9% of the basal firing rate (Fig 1).

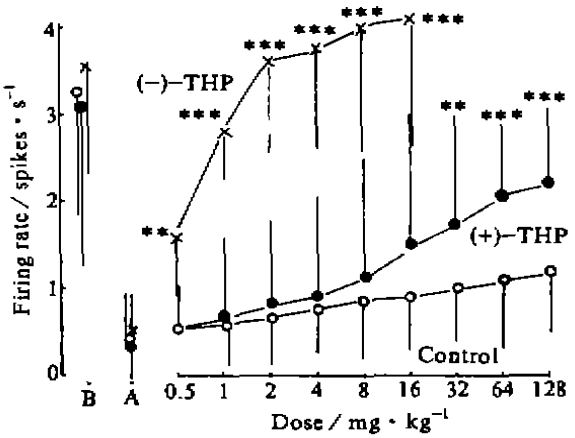


**Fig 1. Reversal of Apo-induced inhibition of firing activity of SNC DA neurons by (-)-THP ($n$=9) and (+)-THP ($n$=7) in anesthetized rats.** $\bar{x} \pm s$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control ($n$=5). B: basal firing rate; A: firing rate after Apo.**

**Effect of THP enantiomers on the spontaneous firing activity of SNC DA neurons** In the paralyzed rats, (-)-THP (0.5-32 mg · $kg^{-1}$, iv) markedly increased the basal firing rate in a dose-dependent way and the maximal increment was 119 ± 47% over the basal firing rate ($n$=6). All neurons recorded (6/6) showed excitatory response to systemic (-)-THP, and the requirement of (-)-THP to increase the firing rate of 20 and 100% over the basal firing rate was 0.85 ± 0.2 and 12 ± 3

mg · $kg^{-1}$, respectively (Fig 2). However, (+)–THP at the same dose scale did not significantly increase the spontaneous firing rate until the dosage reached 16 mg · $kg^{-1}$, and the maximal increment of the firing was only 30± 28% over the basal firing rate ($n=8$). The maximum responsible dose of (+)–THP was about 32–fold higher than that of (−)–THP and the same response required a 8–32–fold dose of (+)–THP higher than (−)–THP (Fig 2).

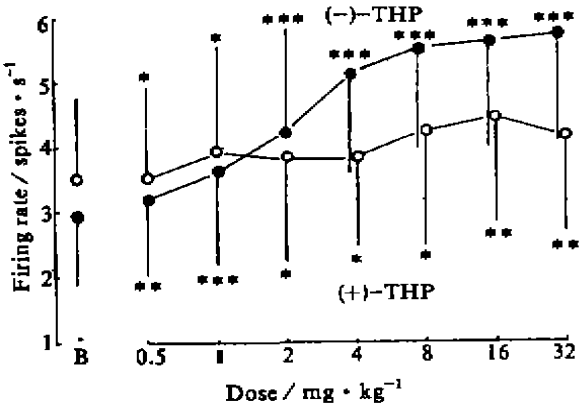


**Fig 2. Effects of (−)–THP ($n=6$) and (+)–THP ($n=8$) on spontaneous firing rate of SNC DA cells in gallamine–paralyzed, local–anesthized rats.** $\bar{x} \pm s$, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **before (B).**

**Effects of pretreatment with THP enantiomers on the Apo–induced inhibition of the SNC DA cell firing activity** In anesthetized rats, Apo dose–dependently inhibited the firing activity of SNC DA neurons ($ID_{50}=7.8$, 6–10.1 μg · $kg^{-1}$, $n=12$). Pretreatment with (−)–THP 4 mg · $kg^{-1}$ (3–5 min before iv Apo) significantly attenuated the inhibitory effect of Apo ($ID_{50}=95$, 68–133 μg · $kg^{-1}$, $n=6$, $P<0.01$ *vs* control). However, the same dose of (+)–THP did not significantly affect the Apo–induced suppression of SNC DA neuron firing ($ID_{50}=9.2$, 6.1–13.8 μg · $kg^{-1}$, $n=6$, $P>0.05$ *vs* control) (Fig 3).

In order to further elucidate the properties of THP enantiomers, gallamine–paralyzed rats were used. In this preparation, (+)–THP (32 mg · $kg^{-1}$) also significantly attenuated the Apo–induced suppression. The $ID_{50}$ value of Apo for (+)–THP pretreatment and control were 213 (143.9–315.2) μg · $kg^{-1}$ ($n=5$) and 25 (19.1–32.8) μg · $kg^{-1}$ ($n=11$) respectively ($P<0.01$). Under the same experimental conditions, (−)–THP 32 mg · $kg^{-1}$ showed more potent attenuation to Apo–induced suppression ($ID_{50}$ of Apo = 1488, 1041–2128 μg · $kg^{-1}$; $n=6$) ($P<0.01$, as compared to either control or (+)–THP group) (Fig 4).

**Effects of THP enantiomers on the Amp–induced inhibition of the SNC DA neuron firing** In gallamine–paralyzed rats, Amp, an indirectly–acting agonist which promotes release of DA from nerve terminals, dose–dependently inhibited SNC DA cell firing rate ($ID_{50}=1.21$, 0.85–1.70 mg · $kg^{-1}$, $n=6$). Pretreatment with (+)–THP (4 mg · $kg^{-1}$) did not potentiate the Amp–induced inhibition

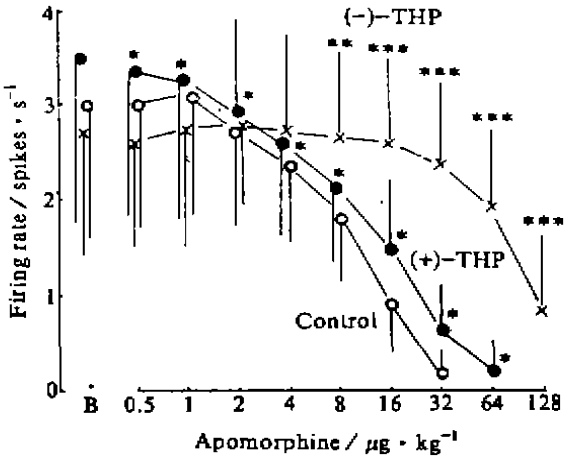


**Fig 3. Effects of pretreatment with (−)–THP ($n=6$) and (+)–THP ($n=6$) 4 mg · $kg^{-1}$ on Apo–induced inhibition of SNC DA cell firing rate.** $\bar{x} \pm s$, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control ($n=12$). B: basal firing rate.**

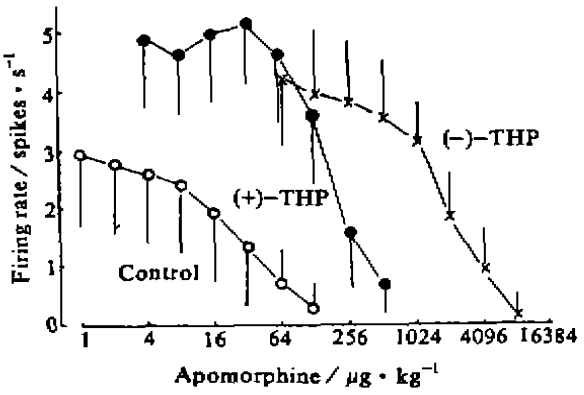


**Fig 4. Effects of (−)−THP ($n=6$) and (+)−THP ($n=5$) 32 mg · kg$^{-1}$ on Apo−induced inhibition of SNC DA cell firing activity in gallamine−paralyzed rats. $n=11$ in control group. $\bar{x}\pm s$.**

($ID_{50}$=2.4, 1.4–4 mg · kg$^{-1}$, $n=7$, $P>0.05$) as several behavioral experiments showed. On the other hand, (+)−THP 32 mg · kg$^{-1}$ significantly reduced Amp−induced inhibition ($ID_{50}$=6.3, 4.2–9.5 mg · kg$^{-1}$, $n=5$; $P<0.01$ *vs* control) similar as (−)−THP 4 mg · kg$^{-1}$ did ($ID_{50}$ of Amp=7.1, 4.7–10.7 mg · kg$^{-1}$, $n=4$, $P<0.01$ *vs* control; $P>0.05$ *vs* that of 4 mg · kg$^{-1}$ group) (Fig 5).

## DISCUSSION

In the present study, the effects of enantiomers of THP on the firing activity of SNC DA cells were evaluated with single−unit recording techniques. (−)−THP significantly increased the spontaneous firing of SNC DA neurons, reversed Apo−induced inhibition of SNC DA cell firing activity and shifted the Apo−induced inhibitory curve to right in both anesthetized and paralyzed rats. Together with previous results[3,6,13,14], these results strongly support that (−)−THP is a DA receptor antagonist (mainly $D_2$). Compared to its levo−isomer, much larger doses of (+)−THP were required to produce similar effects on the SNC DA cell firing activity, and the extent of response was much less. In the experiment of effects on the spontaneous firing activity and the reversal experiment, (+)−THP was about 32−fold less potent than (−)−THP, and other expriments all showed (+)−THP was quite less potent. These results suggest that the interaction between THP enantiomers and $D_2$ receptors has stereo−selectivity. This is consistent with our previous results and strongly supports the conclusion that (−)−THP is a DA receptor antagonist while (+)−THP seems not.

Our previous works have clearly demonstrated that (+)−THP is a DA depletor, and it potentiated Amp−induced rotational behavior in rats with unilateral 6−OHDA lesions of SNC and spontaneous activities of mice[4−6]. In the present study, however, we didn't find any synergic effects of (+)−THP on Amp−induced inhibition of SNC DA cell firing. This suggested that behavioral and electrophysiological responses evoked by Amp and (+)−THP behaved in some different mechanisms. It has been reported that some characteristics of dendritic release process appear to differ from the release mechanism operative in

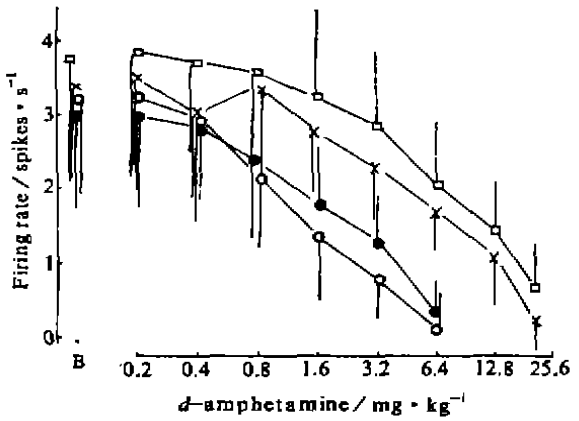


**Fig 5. Effects of (+)−THP and (−)−THP on Amp−induced inhibition of SNC DA cell firing activity in paralyzed rats. $\bar{x}\pm s$. B: basal firing rate. Control (○), $n=6$; (+)−THP 4 mg · kg$^{-1}$ (●), $n=7$; (+)−THP 32 mg · kg$^{-1}$ (×), $n=5$; (−)−THP 4 mg · kg$^{-1}$ (□), $n=4$.**

axon terminals[8] and Amp inhibits the firing activity of SNC DA cells partially through an action within the SN, perhaps at the DA dendrodendritic synapses[15]. Thus, the different influences of (+)-THP on Amp induced behavioral and electrophysiological effects may imply different mechanisms of Amp or (+)-THP on the DA neuron axon terminals and dendritic terminals.

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292-297

## 四氢巴马汀旋光异构体对黑质致密区多巴胺神经元放电活动的作用比较

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R966

**提要** 记录大鼠黑质致密区(SNC)多巴胺(DA)神经元单位放电活动, 左旋四氢巴马汀((-)-THP, 0.5-16 mg · kg$^{-1}$) 能翻转或阻滞阿扑吗啡(Apo)及苯丙胺(Amp)对 DA 神经元的放电抑制增加 DA 神经元的自发放电活动, 而(+)-THP 则需要 8-32 倍于(-)-THP 的剂量才能产生相似的作用. 提示: THP 旋光异构体与 DA 受体的作用有立体选择性: (-)-THP 是 DA 受体阻滞剂, 而(+)-THP 似乎不是.

**关键词** 四氢巴马汀; 小檗因类; 黑质; 多巴胺能药物; 电生理