ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Aug; 20 (8): 715-719
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Supraspinal D_2 receptor involved in antinociception induced by *l*-tetrahydropalmatine[1]

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KEY WORDS tetrahydropalmatine; dopamine receptors; quinpirole; spiperone; SKF38393; Sch-23390; naloxone; antinociception; non-narcotic analgesics

ABSTRACT

AIM: To study the role of dopamine (DA) receptors in *l*-tetrahydropalmatine (*l*-THP)-induced antinociception. **METHODS**: The intraperitoneal (ip) and intrathecal (ith) injections were used to give the drugs. The tail-flick test was used to assess the nociceptive threshold of rats. **RESULTS**: By ip injection, *l*-THP (10, 20, 40 mg·kg^{-1}) as well as D_2 receptor antagonist spiperone (1, 2, 3 mg·kg^{-1}) produced dose-dependent antinociceptive effects on the nociception of rats, while D_2 receptor agonist quinpirole, D_1 receptor agonist SKF38393, and D_1 receptor antagonist Sch-23390 showed no antinociception. Moreover, *l*-THP- or spiperone-induced antinociception was markedly attenuated by quinpirole (2, 3 mg·kg^{-1}) but not SKF38393 or naloxone. On the other hand, ith quinpirole (20, 30, 40 μg·kg^{-1}) also induced a dose-dependent antinociception, while ith *l*-THP, spiperone, SKF38393, and Sch-23390 did not exhibit any antinociception. Furthermore, ith spiperone (20, 30, 40 μg·kg^{-1}) but not Sch-23390 dose-dependently antagonised the antinociception induced by quinpirole. *l*-THP (ith, 100, 200, 300 μg·kg^{-1}) also dramatically attenuated the quinpirole-induced antinociception with a dose-dependent relationship. **CONCLUSION**: Activating the spinal D_2 receptor or blocking the supraspinal D_2 receptor produces antinociception. D_1 receptor might be not directly involved in the antinociception. *l*-THP (as a D_2 antagonist) as well as spiperone produces antinociception via blocking the supraspinal D_2 receptor.

INTRODUCTION

Tetrahydropalmatine (THP) is the main active ingredient of the *Corydalis ambigua* Cham et Sch (or called *Corydalis turtshaninovii* Bess *f Yanhusuo*, YH Chou et CC Hsu), a famous analgesic of traditional Chinese medicine. Its levo-enatiomer (*l*-THP) possesses the analgesic action with remarkable sedative tranquilizing effect[1]. *l*-THP has been acted as a remedy for analgesic or sedation listed in the Chinese Pharmacopoeia and textbooks of pharmacology. However, the exact analgesic mechanism of *l*-THP still remains unclear up to now. In the 1980s, *l*-THP was verified as a dopamine (DA) receptor antagonist with behavioral, biochemical, and electrophysiologic experiments[2,3], and *l*-THP had no affinity for opiate receptors[4]. Now, a very attractive task is how the DA antagonistic effect of *l*-THP elicits the analgesic action in clinic. To elucidate this mechanism not only will explain the analgesic mechanism of *l*-THP, but also will understand the role of DA nervous system involved in analgesia.

Much evidence has showed that dopaminergic system is directly or indirectly involved in the processes of nociception or antinociception. In general, most scholars agree that the processes of nociception at the spinal or supraspinal level might be influenced by the different DA receptors subtypes[5,6]. However, many reports about D_1 and D_2 receptor are involved in nociception were discordant. Some scholars demonstrated that D_1 and D_2 receptor antagonists could

[1] Project supported by the National Natural Science Foundation of China, № 39670829 and № 39600179, and State Key Laboratory of Drug Research, K 016.
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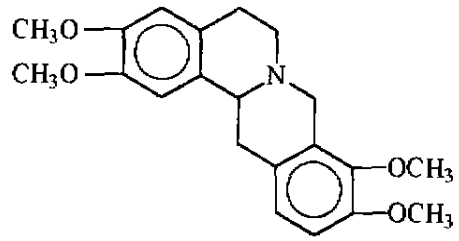
Received 1998-11-10 Accepted 1999-02-02

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Aug; 20 (8)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

enhance the antinociceptive effect of rats[7], but other scholars showed that mixed D_1/D_2 or D_2 agonists produced a dose-dependent antinociceptive effect, while D_1 agonist SKF38393 was devoid of such effect[8]. Recent report indicated that D_2 agonist quinpirole prolonged nociceptive threshold[9]. Therefore, the role that D_1 and D_2 receptors are involved in nociception or antinociception needs to be clarified. For this aim, the present study attempted to compare the effects of *l*-THP, selective DA receptor agonists and antagonists on nociception of rats by intraperitoneal (ip) and intrathecal (ith) injection. In addition, opioidergic receptor antagonist naloxone was also used to determine whether or not opioid receptors influenced the antinociceptive effect of *l*-THP.



Tetrahydropalmatine

MATERIALS AND METHODS

Materials Adult Sprague-Dawley rats (♀, 180 – 200 g) were supplied by Shanghai Animal Center, Chinese Academy of Sciences (Grade Ⅱ, Certificate № 005). *l*-THP (mp 141 – 142 ℃, $[\alpha]_D$ – 289°), isolated by Shanghai Institute of Materia Medica, was dissolved in H_2SO_4 0.1 mol·L^{-1}, and adjusted to pH 5.5 with NaOH 0.1 mol·L^{-1}. SKF38393, Sch-23390, quinpirole, spiperone, and naloxone, purchased from Research Biochemicals International Company (USA), were diluted with normal saline (NS).

Intrathecal (ith) injection of drugs Catheters were installed into the lumbar subarachroid space for ith injection[10]. Briefly, rats were anesthetized with sodium pentobarbital 30 mg·kg^{-1}, a PE-10 polyethylene catheter of 75 mm long was implanted into the lumbar enlargement of the spinal cord. Those rats showing any motor deficit resulting from the surgical procedure were excluded from the study. Experiments with ith injection of drugs were carried out 48 h after operation. Drugs were injected slowly via the ith catheter at a volume of 10 μL, followed by NS 10 μL to flush the catheter.

Tail flick test The nociceptive threshold was assessed using tail-flick test. The latency for a rat to flick its tail away from a source of radiant heat was measured with Tail Flick Timer 1.1 (IITC Inc, USA) through applying noxious radiant heat to stimulate the blackened undersurface of middle third portion of the tail. Tail-flick latency (TFL) was recorded by the digital timer. The baseline latency (BL) in each rat was kept from 3.0 s to 5.0 s. A BL was established by three trails at 5-min intervals. The TFL of trails at 10-min intervals was measured after injection of drugs. Each group consisted of 6 rats.

Statistical analysis TFL was converted % of the maximal possible effect. The % change of TFL was calculated according to the formula: % C = $(T_{TFL} - T_{BL})/T_{BL} \times 100\%$. Data ($x \pm s$) were analyzed by ANOVA followed by Bonferroni *t*-test.

RESULTS

Antinociceptive effects of *l*-THP and D_2 receptor antagonist spiperone by ip injection Administration of ip *l*-THP (10, 20, 40 mg·kg^{-1}) and D_2 receptor antagonist spiperone (1, 2, 3 mg·kg^{-1}) produced significant and dose-dependent antinociceptive effects on the nociception of rats, while ip D_2 receptor agonist quinpirole (2, 3 mg·kg^{-1}), D_1 receptor agonist SKF38393 (2, 3 mg·kg^{-1}) and antagonist Sch-23390 (2, 3 mg·kg^{-1}) showed no antinociception. Administration of ip the same volume of vehicle or NS also exhibited no effect on nociception of rats (Tab 1, Fig 1).

D_2 receptor agonist quinpirole attenuating the antinociception induced by ip *l*-THP or spiperone To evaluate the possible interaction of *l*-THP-induced antinociception with DA and opioidergic receptors, quinpirole, SKF38393, and naloxone were ip injected 5 min after ip *l*-THP (40 mg·kg^{-1}) or spiperone (3 mg·kg^{-1}). Quinpirole (2, 3 mg·kg^{-1}) produced a dose-dependent antagonistic effect on *l*-THP- or spiperone-induced antinociception, while SKF38393 (2, 3 mg·kg^{-1}), naloxone (2, 4 mg·kg^{-1}), and NS exhibited no effect on the antinocicep-

Tab 1. Effects of *l*-THP, DA receptor agonists and antagonists on tail-flick latency (TFL) of rats.

Drugs	Dose	% change of TFL
ip		
D_1 agonist SKF38393	2, 3 mg·kg^{-1}	–
D_1 antagonist Sch-23390	2, 3 mg·kg^{-1}	–
D_2 agonist quinpirole	2, 3 mg·kg^{-1}	–
D_2 antagonist spiperone	1, 2, 3 mg·kg^{-1}	↑
NS	0.4 mL	–
l-THP	10, 20, 40 mg·kg^{-1}	↑
Vehicle	0.4 mL	–
l-THP 40 mg·kg^{-1}		
+ quinpirole	2, 3 mg·kg^{-1}	⊖
+ NS	0.4 mL	+
+ SKF38393	2, 3 mg·kg^{-1}	+
+ naloxone	2, 4 mg·kg^{-1}	+
ith		
D_1 agonist SKF38393	20, 40 μg·kg^{-1}	–
D_1 antagonist Sch-23390	20, 40 μg·kg^{-1}	–
D_2 agonist quinpirole	20, 30, 40 μg·kg^{-1}	↑
D_2 antagonist spiperone	20, 40 μg·kg^{-1}	–
NS	10 μL	–
l-THP	100, 200, 300 μg·kg^{-1}	–
Vehicle	10 μL	–
Quinpirole 40 μg·kg^{-1}		
+ spiperone	20, 30, 40 μg·kg^{-1}	⊖
+ NS	10 μL	+
+ *l*-THP	200, 300 μg·kg^{-1}	⊖
+ vehicle	10 μL	+
+ Sch-23390	20, 40 μg·kg^{-1}	+

↑: denote significant increase of TFL; –: denote no change of TFL; ⊖: denote significant antagonistic effect; +: denote no antagonistic effect.

tion induced by *l*-THP or spiperone (Tab 1, Fig 1).

Antinociceptive effects of ith D_2 receptor agonist quinpirole at the spinal level Administration of ith D_2 agonist quinpirole (20, 30, 40 μg·kg^{-1}) induced a significant and dose-dependent antinociception, but ith *l*-THP (100, 200, 300 μg·kg^{-1}), D_2 antagonist spiperone (20, 40 μg·kg^{-1}), D_1 agonist SKF38393 (20, 40 μg·kg^{-1}), and D_1 antagonist Sch-23390 (20, 40 μg·kg^{-1}) showed no effect on nociception. The same administration of vehicle or NS also exhibited no effect on nociception (Tab 1, Fig 2).

D_2 receptor antagonist spiperone and *l*-THP antagonizing ith quinpirole-induced antinociception at the spinal level To explore whether quinpirole-induced antinociception is mediated

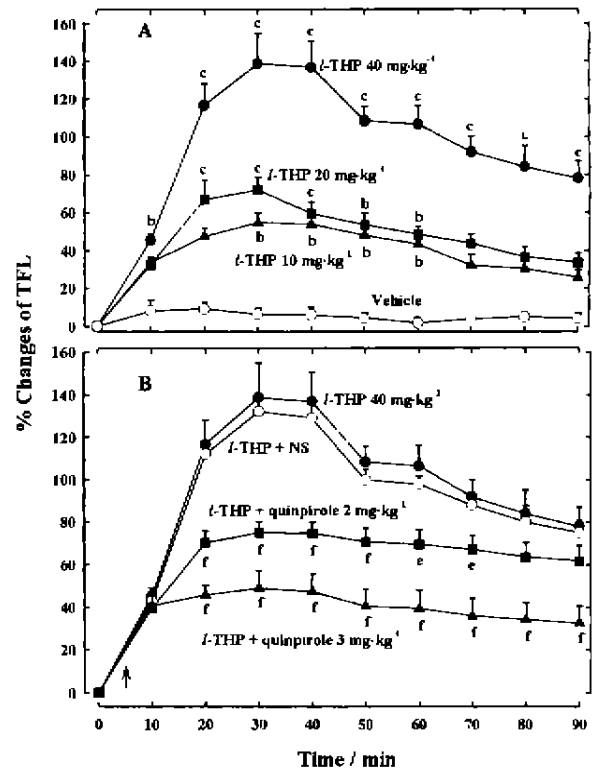


Fig 1. Percentage changes of TFL after ip *l*-THP (A), $^bP<0.05$, $^cP<0.01$ *vs* vehicle; and ip D_2 receptor agonist quinpirole attenuated antinociception induced by ip *l*-THP (B). $n = 6$. $\bar{x} \pm s$. Arrow denotes time at which ip quinpirole or NS. $^eP<0.05$, $^fP<0.01$ *vs* *l*-THP.

via D_1 or D_2 receptors at the spinal level, D_1 antagonist Sch-23390 and D_2 antagonist spiperone were ith injected 5 min after ith quinpirole (40 μg·kg^{-1}) respectively. Quinpirole-induced antinociception was dose-dependently antagonised by spiperone (20, 30, 40 μg·kg^{-1}) but not Sch-23390 (20, 40 μg·kg^{-1}) or NS. Moreover, ith *l*-THP (200, 300 μg·kg^{-1}) also exhibited an antagonistic effect against quinpirole-induced antinociception with a dose-dependent relationship, while ith vehicle had no antagonistic effect (Tab 1, Fig 2).

DISCUSSION

In the present experiments, the nociceptive threshold of rats was measured with tail-flick test applying noxious radiant heat stimuli. The evidences of *l*-THP, D_1 and D_2 agonists or antagonists ith injected directly into spinal level have presented a conception that at the spinal only D_2 agonist quinpirole, but not

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Aug; 20 (8)
E-mail aps@server.shcnc.ac.cn Phn Fax 86-21-6474-2629

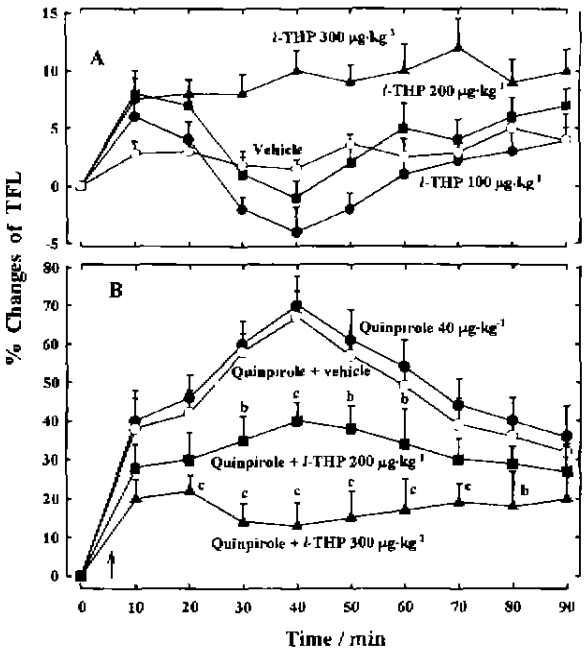


Fig 2. Percentage changes of TFL after ith *l*-THP (A); and ith *l*-THP attenuated antinociception induced by ith D_2 receptor agonist quinpirole (B). $n=6$. $\bar{x} \pm s$. Arrow denotes time at which ith *l*-THP or vehicle, $^bP<0.05$, $^cP<0.01$ *vs* quinpirole.

l-THP, D_2 antagonist, D_1 agonist and antagonist, participates in antinociception. Moreover, our experiment still showed that ith D_2 antagonist spiperone and *l*-THP but not D_1 antagonist Sch-23390 could block the antinociception induced by ith D_2 agonist quinpirole. The results are similar to some reports indicating that ith DA agonist inhibited the spinal nociception, and ith apomorphine could prolong the TFL of rats [6,8,11,12]. Obviously, our findings strongly support the idea that D_2 receptor is involved in the modulation of nociception transmission, and that activating the spinal D_2 receptor contributs to the antinociception. Thus, it is presumed that analgesic site of *l*-THP is not at the spinal level due to its D_2 antagonistic action.

On the other hand, the present study results still showed that ip D_2 antagonist spiperone could induce the antinociception, while ip D_2 agonist quinpirole had no antinociception. This fact indicates that the peripherally administration of spiperone produces the antinociception at the supraspinal level but not at the spinal level, its action sites of antinociception are just opposite to those of D_2 agonist quinpirole. The results are similar to the previous report[7] or recent report [5]. Moreover, ip spiperone-induced antinociception was markedly attenuated by ip D_2 agonist quinpirole but not ip D_1 agonist Sch-23390. In other words, the present results indicate that D_2 receptor involved in antinociception is dependent on the action sites, both activating the spinal D_2 receptor and blocking the supraspinal D_2 receptor exert the analgesic effect.

As to the possible role of D_1 receptor in nociception or antinociception, our experiment showed that ip or ith D_1 agonist and antagonist did not have any antinociception. Our observation is agreement with other report[6,13]. The results suggest that D_1 receptor might be not directly involved in the antinociception.

l-THP, a novel DA receptor antagonist, which acts as an analgesic drug, also produced antinociceptive effect by ip but not ith. Interestingly, ith *l*-THP could also dramatically attenuate the antinociception induced by ith quinpirole. This effect was very similar to D_2 antagonist spiperone. It suggests that the analgesic effect of *l*-THP is mediated by blocking the supraspinal D_2 receptor. In addition, the result that ip *l*-THP-induced antinociception is not attenuated by naloxone indicates opioidergic receptors not directly involved in the antinociception of *l*-THP.

However, in the present study, it is difficult to ascertain the exact action sites of *l*-THP in the brain. Owing to that *l*-THP soluble in the acidic medium is difficult to be adjusted to pH 7.4 for intracerebroventricular or intro-nucleus injection, we deduce that *l*-THP-induced antinociception is mediated by blocking the supraspinal D_2 receptor based not only on present study results, but also on the other results[14]. In previous experiment, using Fos protein immunohistochemistry technique, we have demonstrated that *l*-THP acted on the striatum, accumbens nucleus, and sensorimotor cortex[14]. As we all know, Fos protein, a product of immediate early gene c-*fos* expression, is a marker of neuronal activity for tracing neuronal action sites [15]. Furthermore, *l*-THP showed its analgesic effect against formalin-pain based on its enhancing the activity of brainstem descending modulation system. Therefore, these data support that the analgesic sites of *l*-THP are at the supraspinal level.

In conclusion, *l*-THP acts as a D_2 antagonist involved in antinociceptive effect by blocking the

supraspinal D_2 receptor.

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脊髓以上多巴胺 D_2 受体介导左旋四氢巴马汀的镇痛作用[1]

R971.1

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关键词 四氢巴马汀；多巴胺受体；喹吡罗；螺哌隆；SKF38393；Sch-23390；纳洛酮；抗痛效应；非麻醉性镇痛药

R343.2

目的：研究 DA 受体与左旋四氢巴马汀（*l*-THP）镇痛作用的关系，以阐明 *l*-THP 的镇痛机制。**方法**：腹腔（ip）与鞘内（ith）给药，以大鼠甩尾反应观测热伤害性致痛阈。**结果**：ip *l*-THP 或 D_2 受体拮抗剂螺哌隆产生剂量依赖性镇痛效应，并能被 D_2 受体激动剂喹吡罗翻转，但不被纳洛酮翻转。而 ith *l*-THP或螺哌隆无镇痛效应，但它们能拮抗 ith 喹吡罗引起的镇痛效应。**结论**：激动脊髓 D_2 受体或阻滞脊髓以上水平 D_2 受体均产生镇痛效应；*l*-THP镇痛作用通过阻滞脊髓以上 D_2 受体实现。

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