ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 May；21 (5)：439－444
http：//www.chinaphar.com

Arcuate nucleus of hypothalamus involved in analgesic action of *l*-THP[1]

HU Jiang-Yuan[2], JIN Guo-Zhang[3]
(*Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China*)

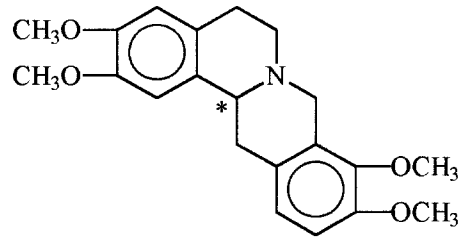
KEY WORDS tetrahydropalmatine; horseradish peroxidase; arcuate nucleus; PAG; corpus striatum; nucleus accumbens; analgesia; naloxone; endorphins

ABSTRACT

AIM: To study the role of the arcuate nucleus of hypothalamus in analgesic action of *l*-tetrahydropalmatine (*l*-THP). **METHODS**: The horseradish peroxidase (HRP) retrograde tracing, HRP retrograde tracing combined with immunohistochemistry, lesion of nucleus, tail-flick test, and intra-PAG injection were used in the present study. **RESULTS**: HRP retrograde tracing results showed that the striatum or accumbens nucleus connect with PAG by two pathways: 1) striatum or accumbens nucleus → arcuate nucleus → PAG; 2) striatum or accumbens nucleus →habenula → PAG. It was found that neurons in the arcuate nucleus projecting to PAG were mainly β-endorphin neurons as observed by HRP retrograde tracing combined with immuno-histochemistry. After lesion of the arcuate nucleus, the analgesic action of *l*-THP (40 mg·kg^{-1}, ip) was abolished, while lesion of the habenula had no such effect. Moreover, intra-PAG injection of naloxone (2, 3 μg) could markedly attenuate the analgesic action of *l*-THP (40 mg·kg^{-1}, ip) in a dose-dependent manner. **CONCLUSION**: β-Endorphin neurons in the arcuate nucleus play an important role in the analgesic action of *l*-THP.

INTRODUCTION

l-Tetrahydropalmatine (*l*-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 Chinese traditional medicine which possesses an analgesic action associated with remarkable sedative and tranquilizing effect[1,2]. *l*-THP's use as an analgesic or sedative has been listed in the Chinese Pharmacopoeia in the 1977, 1985, 1990, and 1995 editions. However, the exact analgesic mechanism of *l*-THP still remains unclear.



***l*-Tetrahydrophalmantine**

Previous studies in our laboratory have shown that *l*-THP is a dopamine D_2 receptor antagonist[3,4], and it can enhance the activity of descending brainstem pain modulation system, especially periaqueductal gray (PAG) by blocking D_2 receptors in the striatum and accumbens nucleus, and subsequently inhibiting the inputs of peripheral pain afferent message at the spinal cord level[5,6]. It is necessary to be clarified whether a direct descending neural pathway exists from the striatum or accumbens nucleus to the PAG or an indirect neural pathway which relays at other nuclei. The present study attempted to investigate the pathway from the striatum or accumbens nucleus to PAG, by observing the influences of destroying the nuclei in the pathway of the analgesic action of *l*-THP. In addition, naloxone, an antagonist to μ, δ, κ opioidergic receptors, was used to ascertain whether or not opioid peptides were involved in the analgesic action of *l*-THP.

[1] Supported by the National Natural Science Foundation of China, № 39770853 and 39670829.
[2] Now in *Shanghai Institute of Physiology, Chinese Academy of Sciences, Shanghai 200031, China*.
[3] Correspondence to Prof JIN Guo-Zhang.
Phn 86-21-6431-1833, ext 402. Fax 86-21-6437-0269.
E-mail: gzjin@mail.shcnc.ac.cn
Received 1999-09-10 Accepted 1999-12-30

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 May；21 (5)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

MATERIAL AND METHODS

Materials Adult Sprague-Dawley rats (♂, 180 – 200 g) were supplied by Shanghai Animal Center, Chinese Academy of Sciences (Grade Ⅱ, Certificate No 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}. HRP and naloxone, purchased from Research Biochemicals International Company (USA), were diluted with normal saline.

HRP retrograde tracing Rats were anaesthetized with sodium pentobarbital (40 mg·kg^{-1}), then 0.5 μL of 30 % HRP was injected into the PAG, arcuate nucleus, and habenula respectively using a glass micropipette with a tip diameter of 10 μm. The sites of injection were located in the PAG (Bregma: – 6.04 mm, R: 0.5 mm, H: 5.8 mm), arcuate nucleus (Bregma: – 3.8 mm, R: 0.25 mm, H: 11.5 mm), and habenula (Bregma: – 3.8 mm, R: 0.7 mm, H: 4.8 mm) respectively according to the rat brain atlas of Paxinos and Watson (1997 edition) (Fig 1). After surviving for 40 h rats were anaesthetized and then perfused intracardially for 25 min with 2 % paraformaldehyde and 0.25 % glutaraldehyde solution preceded by rapid saline flush. The brains were removed and postfixed in 20 % sucrose fixative for 12 h, and sunk in 30 % sucrose overnight at 4 ℃. The brains were cut into 30 μm thick transverse sections with a cryostat. HRP reaction product in the brain sections were stained following the tetramethyl benzidine-sodium tungstate (TMB-ST) procedure[7,8].

Immunohistochemistry HRP-labeled brain sections were pre-incubated in 10 % normal goat serum for 1 h, and incubated for 48 h at 4 ℃ in β-endorphin antibody at a dilution of 1∶1000 in 0.01 mol·L^{-1} phosphate buffer (pH 7.4) with 1 % normal goat serum and 0.3 % triton X-100. Sections were washed with 0.01 mol·L^{-1} phosphate buffer saline (PBS) and incubated for 1 h at 37 ℃ in biotinylated goat anti-rabbit immunoglobulin G solution (1∶200). Sections were washed with PBS and incubated for 1 h at 37 ℃ in avidin-biotin-peroxidase complex solution (1∶200). After three washes with PBS, the sections were then washed with Tris-HCl buffer (pH 7.4). The immune product was stained following glucose oxidase-diaminobenzidine-nickel (GDN) procedure[9], then brain sections were mounted, dehydrated, and coverslipped with neutral balsam.

Lesion of nucleus After rat was anaesthetized with sodium pentobarbital (40 mg·kg^{-1}), an insulated stainless steel electrode was inserted into the arcuate nucleus or habenula (the sites of electrolytic lesion were the same as the sites of HRP injection), with its tip extending 0.5 mm beyond the insulation. The arcuate nucleus or habenula was bilaterally destroyed by passing an anodal current of 5 mA for 20 s. Experiments were performed one week after lesion. At the end, the extent of brain tissue lesion was carefully checked by crysyl violet stain.

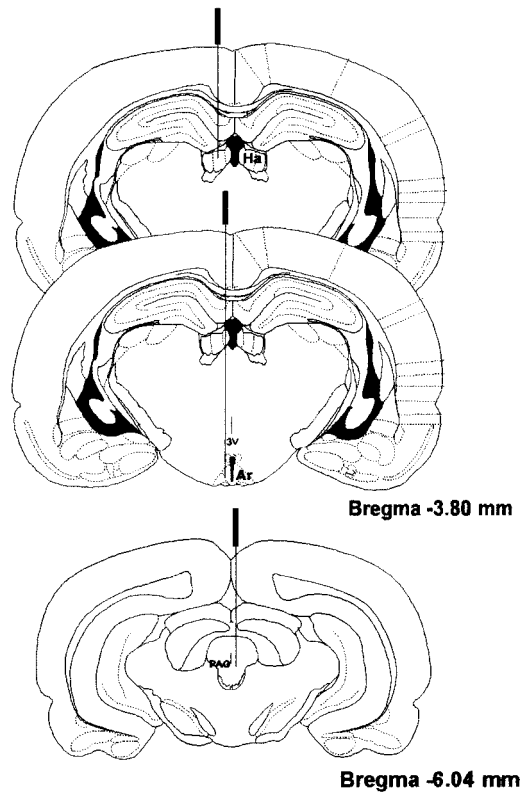


Fig 1. Sites of HRP injected in habenula (Bregma – 3.8 mm, L 0.7 mm, H 4.8 mm), arcuate nucleus (Bregma – 3.8 mm, L 0.25 mm, H 11.5 mm), and PAG (Bregma – 6.04 mm, L 0.5 mm, H 5.8 mm). Ha, habenula; Ar, arcuate nucleus.

Tail flick test The pain 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

drugs injection.

Intra-PAG injection For implantation of intracranial cannulae, the rat was mounted on a stereotaxic instrument under pentobarbital anesthesia (40 mg·kg^{-1}, ip). Stainless steel guide cannulae of 0.3 mm outer diameter were directed to PAG (Bregma：−6.04 mm, R：0.5 mm, H：5.8 mm). The cannulae were fixed to the skull with dental acrylic. One week was allowed for surgical recovery. Bilateral injection into PAG was performed through a stainless injection tube of 0.25 mm outer diameter which was inserted into the guide cannula, with the former extending 0.1 mm beyond the tip of the latter. Naloxone solution (5 μL) was gradually injected into PAG via a slow injection apparatus over a period of 5 min, followed by NS 5 μL to flush the stainless injection tube.

Statistical analysis HRP-labeled, β-endorphin-labeled, HRP and β-endorphin-double labeled neurons were counted under light microscopy (×100). TFL was converted into % of the maximal possible effect. The % change of TFL was calculated according to the formula：$\%C = (T_{TFL} - T_{BL}) \cdot T_{BL}^{-1} \times 100\ \%$. Data ($\bar{x} \pm s$) were analyzed by ANOVA followed by Bonferroni *t*-test.

RESULTS

HRP retrograde tracing After injecting HRP into PAG, HRP-labeled neurons were observed as green granules in the arcuate nucleus and habenula by TMB-ST stain (Tab 1, Fig 2), but not in the striatum and accumbens nucleus. The result indicates that the striatum or accumbens nucleus do not directly connect with PAG, and that neurons projecting to PAG exist in arcuate nucleus or habenula. Moreover, HRP-labeled neurons could be observed in the striatum and accumbens nucleus by the arcuate nucleus or habenula HRP retograde tracing (Tab 2). This result indicates that there are direct nerve fiber connection between the striatum or acumbens nucleus and the arcuate nucleus or habenula, but there is no direct pathway from the striatum or accumbens nucleus to PAG. The arcuate nucleus or habenula may be presumed a relay station.

Tab 1. Number of three kinds of labelled neurons in the arcuate nucleus (Ar) and habenula (Ha) by PAG HRP retograde tracing. $n = 4$.

Brain area	HRP-labelled neurons	Endorphin-labelled neurons	Neurons double-labelled by HRP and endorphin
Ar	16.3 ± 2.6	28.3 ± 2.8	12.8 ± 0.8
Ha	6.3 ± 2.2	no	no

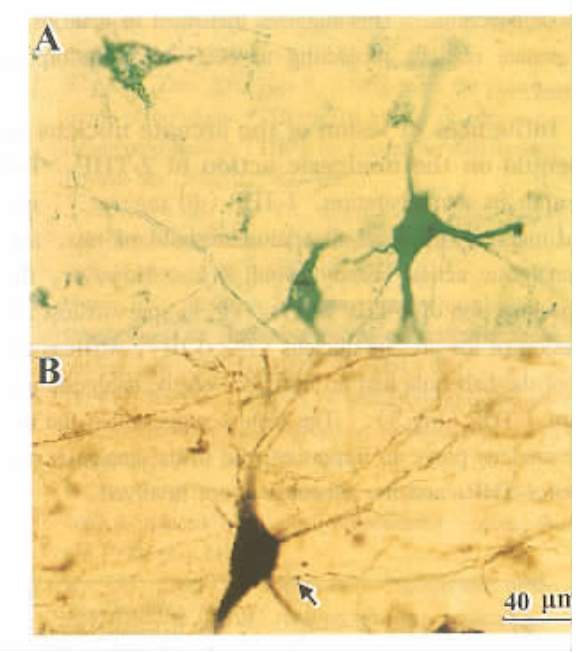


Fig 2. Light micrograph showing HRP-labeled neurons in the arcuate nucleus by PAG HRP retograde tracing and staining with TMB-ST (A), the double-labeled neurons (↑) of HRP, and β-endorphin in the arcuate nucleus (B). Bar = 40 μm.

Tab 2. Number of HRP-labelled neurons in the striatum and accumbens nucleus by Ar or Ha HRP retograde tracing. $n = 4$.

Brain area	Ar HRP tracing	Ha HRP tracing
Striatum	36.5 ± 5.9	19.6 ± 4.7
Accumbens nucleus	22.3 ± 6.1	14.9 ± 6.4

HRP retrograde tracing combined with immunohistochemistry By combining HRP retrograde tracing with immunohistochemistry technique, three kinds of labeled neurons were observed in the arcuate nucleus：① green HRP-labeled neurons by TMB-ST stain；② gray β-endorphin-immunoactivity neurons by GDN stain；③ dark brown HRP and β-endorphin double-labeled neurons, which constituted 78.5 % of the HRP-labeled neurons (Tab 1, Fig 2). However, only one kind of HRP-labeled neurons, without double-labeled or β-endorphin neurons, were observed in the striatum, accumbens nu-

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 May；21 (5)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

cleus or habenula. This suggests that most of neurons in the arcuate nucleus projecting to PAG are β-endorphin neurons.

Influences of lesion of the arcuate nucleus or habenula on the analgesic action of *l*-THP Ten min after its administration, *l*-THP (40 mg·kg^{-1}, ip) could markedly increase the pain threshold of rats, and the analgesic action lasted beyond 3 h. However, the analgesic action of *l*-THP (40 mg·kg^{-1}, ip) was lost after lesion of the arcuate nucleus ($P < 0.01$), whereas lesion of the habenula had no influence on the analgesic action of *l*-THP (Fig 3). The results suggest that the arcuate nucleus plays an important role in the analgesic action of *l*-THP, and the habenula is not involved.

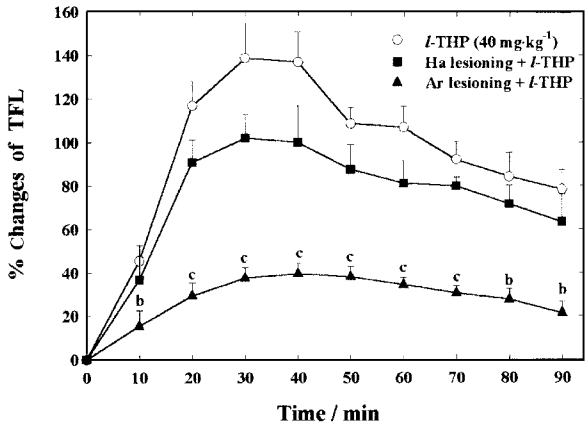


Fig 3. Effect of lesioned arcuate nucleus (Ar) or lesioned habenula (Ha) on the percentage changes of TFL induced by *l*-THP (40 mg·kg^{-1}, ip). Ar lesion could attenuate the increase of TFL induced by *l*-THP, while Ha lesion had no effect. $n = 6$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* *l*-THP.

Effect of intra-PAG injection of naloxone on the analgesic action of *l*-THP Twenty min after administration of *l*-THP (40 mg·kg^{-1}, ip), rats were injected naloxone (1 μg, 2 μg, 3 μg) or NS into PAG bilaterally. Ten min after injecting *l*-THP there was an increase in the pain threshold in the *l*-THP group. The pain threshold went on increasing in rats receiving intra-PAG injection of 1 μg naloxone or NS, which showing no significant differences with the *l*-THP group. When rats received intra-PAG injection of 2 μg or 3 μg naloxone, there was a dose-related attenuation of the analgesic action of *l*-THP ($P < 0.01$, Fig 4). The results suggest that β-endorphin neurons in the arcuate nucleus are involved in the analgesic action of *l*-THP.

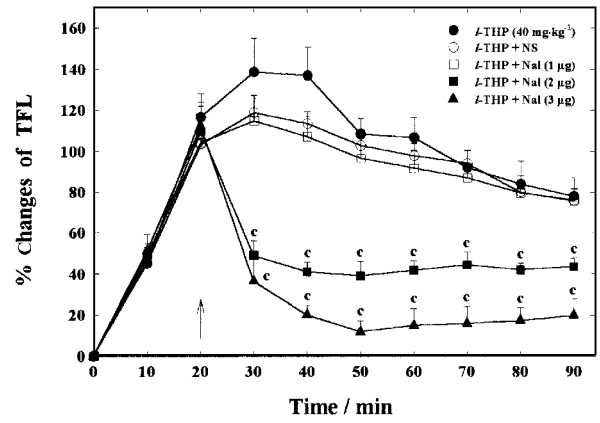


Fig 4. The increase of TFL induced by *l*-THP (40 mg·kg^{-1}, ip) was attenuated by intra-PAG injection of naloxone (Nal). Arrow denotes time of intra-PAG injection of Nal or NS. $n = 6$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* *l*-THP.

DISCUSSION

Our previous works have shown that after blocking D_2 receptors in the striatum and accumbens nucleus, *l*-THP can enhance the activity of descending brainstem pain modulation system, especially PAG, and subsequently inhibit the inputs of afferent peripheral pain message at the spinal cord level[5,6]. This suggests the existence of a descending pain modulatory pathway from the striatum or accumbens nucleus to PAG subserving the analgesic action of *l*-THP. Since previous morphological studies with HRP retrograde tracing did not show any evidence of direct fiber connection from the striatum or accumbens nucleus to PAG, so a neural relay is highly suggested. In the present study, it was revealed by the HRP retrograde tracing, that the striatum or accumbens nucleus sent nerve fibers to the arcuate nucleus or habenula, where the neurons projected to PAG. In other words, it is certain that the striatum or accumbens nucleus could connect indirectly with PAG via the arcuate nucleus or habenula. It is also considered that there are two neural pathways from the striatum or accumbens nucleus to PAG: one led from striatum or accumbens nucleus → arcuate nucleus → PAG, another from striatum or accumbens nucleus → habenula → PAG. Previous reports inferred that the arcuate nucleus or habenula may be a neural relay in the descending pain modulatory pathway from the striatum or accumbens nucleus to PAG[10,11]. The arcuate nucleus or habenula has been regarded as the major link between forebrain structures and midbrain nuclei[12]. Our morphological results provide the evi-

dence to support this hypothesis.

The present study attemptes to evaluate which neural pathway, from the striatum or accumbens nucleus to PAG, plays a relatively important role in the analgesic action of *l*-THP. If the pathway is indispensable, it would be expected that the analgesic action of *l*-THP be substantially attenuated or decreased after lesioning of arcuate nucleus or habenula. Our results showed that the analgesic action of *l*-THP was abolished after lesion of the arcuate nucleus, while lesion of the habenula had no effect on the analgesic action. The results suggest clearly that the following pathway, striatum or accumbens nucleus → arcuate nucleus → PAG, plays an important role in the analgesic action of *l*-THP, while the pathway via habenula to PAG is not involved in the analgesic action of *l*-THP. There are other reports which show that the above mentioned two pathways are all involved in the analgesic action induced by intra-accumbens nucleus injection of morphine[10,11,13], while electrostimulation of accumbens nucleus mainly elicited the analgesic effect via the accumbens nucleus → habenula → PAG pathway[14,15]. Thus, most researchers agree to the viewpoint that the analgesic action elicited by the forebrain (the striatum or accumbens nucleus) is finally exerted by PAG[12].

In the present study, another observation suggests that β-endorphin neurons in the arcuate nucleus are involved in the descending pain modulatory pathway leading from the striatum or accumbens nucleus to PAG. This conclusion was based mainly on the following findings: (1) most of neurons in the arcuate nucleus projecting to PAG were β-endorphin neurons as observed by HRP retrograde tracing combined with immunohistochemistry, (2) the analgesic action of *l*-THP could be attenuated by intra-PAG injection of naloxone. Therefore, β-endorphin neurons in the arcuate nucleus play an important role in the analgesic action of *l*-THP.

REFERENCES

1 Kin KC (Jin GZ), Hsu B. Studies on the pharmacological action of corydalis. I. Analgesic action of corydalis A, B and L. Acta Physiol Sin 1957；21：150－7.

2 Kin KC, Zhen XF, Hsu B. Studies on the pharmacological action of corydalis. XII. The effects of optical isomers of tetrahydropalmatine (THP) on central nervous system. Acta Physiol Sin 1964；27：47－58.

3 Xu SX, Yu LP, Han YR, Chen Y, Jin GZ. Effects of tetrahydropotoberberines on dopamine receptor subtypes in brain. Acta Pharmacol Sin 1989；10：104－10.

4 Huang KX, Jin GZ. The antagonistic effects of tetrahydropotoberberines on dopamine receptors: electropharmacological studies. Sci Sin [B] 1992；35：688－96.

5 Hu JY, Jin GZ. Effects of tetrahydropalmatine analogs on Fos protein expression induced by formalin-pain. Acta Pharmacol Sin 1999；20：193－200.

6 Hu JY, Jin GZ. Supraspinal D_2 receptor involved in antinociception induced by *l*-tetrahydropalmatine. Acta Pharmacol Sin 1999；20：715－9.

7 Rye DB, Saper CB, Wainer BH. Stabilization of the tetramethylbenzidine (TMB) reaction product: application for retrograde and anterograde tracing, and combination with immunocytochemistry. J Histochem Cytochem 1984；32：1145－9.

8 Francisco O, Fernando MG, Carlos LG. A new stabilizing agent for the tetramethylbenzidine (TMB) reaction product in the histochemical detection of horseradish peroxidase (HRP). J Neurosci Methods 1985；13：131－8.

9 Shu SY, Ju G, Dan LZ. The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system. Neurosci Lett 1988；85：169－72.

10 Yu LC, Han JS. Involvement of arcuate nucleus of hypothalamus in the descending pathway from nucleus accumbens to periaqueductal grey subserving an antinociceptive effect. Int J Neurosci 1989；48：71－8.

11 Yu LC, Han JS. Habenular as a relay in the descending pathway from nucleus accumbens to periaqueductal grey subserving antinociception. Int J Neurosci 1990；54：245－51.

12 Eric HC, Willie KD. The role of the basal ganglia in nociception and pain. Pain 1995；60：3－38.

13 Han JS, Xuan YT. A mesolimbic loop of analgesia: I. Activation by morphine of a serotonergic pathway from periaqueductal grey to nucleus accumbens. Int J Neurosci 1986；29：109－18.

14 Wang RY, Aghajanian GK. Physiological evidence for habenular as a major link between forebrain and midbrain raphe. Science 1977；197：89－91.

15 Wang S, Fang JZ, Xia YH. The effect of stimulating nucleus accumbens on the discharge of pain-related neuron in the periaqueductal grey. Physiol Sci 1984；4：37－44.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 May；21 (5)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

下丘脑弓状核参与左旋四氢巴马汀的镇痛作用[1]

胡江元[2]，金国章[3]
（中国科学院上海药物研究所，上海 200031，中国）

关键词　四氢巴马汀；辣根过氧化物酶；弓状核；PAG；纹状体；伏膈核；镇痛；纳洛酮；内啡肽

目的：研究弓状核在左旋四氢巴马汀（*l*-THP）镇痛效应中的作用，以阐明 *l*-THP 的镇痛作用机制．方法：应用辣根过氧化物酶（HRP）逆行追踪术追踪纹状体或伏膈核与 PAG 之间的纤维联系，HRP 逆行追踪结合免疫组化观察投射神经元的性质，神经核团损毁和 PAG 核内注射药物观察对 *l*-THP 镇痛作用的影响．结果：纹状体或伏膈核通过弓状核或缰核间接与 PAG 联系，弓状核投射至 PAG 的神经元大部分是 β 内啡肽神经元．损毁弓状核后，*l*-THP 的镇痛作用消失，而损毁缰核对 *l*-THP 的镇痛作用无明显影响．PAG 核内注射纳洛酮能剂量依赖性翻转 *l*-THP 的镇痛作用．结论：弓状核的 β 内啡肽神经元在 *l*-THP 镇痛作用中起重要作用．

（责任编辑　李　颖）