

Influence of morphine microinjected into head of caudate nucleus on electric activities of nociceptive neurons in parafascicular nucleus of rat thalamus

LI Bai-Yan¹, XU Tun

(Department of Physiology, Harbin Medical University, Harbin 150086, China)

ABSTRACT Morphine (8 µg/µl) was injected into the head of the caudate nucleus (CN). The pain-discharges of the pain-excitation neurons (PEN) in the parafascicular nucleus (Pf) were inhibited, ie, decreasing in frequency of pain-discharges and lengthening in latent period of pain-discharges. The inhibitory effect of the pain-inhibition neurons (PIN) in the Pf induced by noxious peripheral stimulation were relieved, ie, increasing the firing rate and shortening the inhibitory duration. The opiate receptor antagonist naloxone (0.75 mg/kg, ip) blocked the above effects of morphine. These results suggest that the intracaudate opioid peptide system play an important role in the modulation of pain information in the Pf of thalamus.

KEY WORDS morphine; naloxone; caudate nucleus; thalamus; pain; electrophysiology; excitation; inhibition; neurons

The parafascicular nucleus (Pf) of thalamus is closely involved in the production of pain and plays an important role in integrating pain information from the spinal cord⁽¹⁾. The caudate nucleus (CN) is an important structure of the extrapyramidal system in the regulation of motor function and plays role in pain modulation and in acupuncture analgesia. Caudate stimulation increases the pain threshold⁽²⁾ and reduces the nociceptive responses⁽³⁾. Electroacupuncture elicits an increase in pain threshold and rise in endogenous opioid

peptides level in the perfusate from the anterior part of the head of CN, and microinjection of naloxone into CN blocks the effect of electroacupuncture⁽⁴⁾. CN participates actively in acupuncture analgesia, and the analgesia produced by caudate stimulation seems to be similar to that by acupuncture⁽⁵⁾. The local electric stimulation of CN inhibits the nociceptive responses of Pf neurons^(6,7). The systemic application of naloxone produces the facilitated effect on the pain-discharges of Pf neurons, and the inhibitory effect of caudate stimulation is removed⁽⁶⁾. Both the caudate stimulation and microiontophoretic morphine into Pf inhibit the pain-discharges of Pf neurons and this effect can be blocked by naloxone applied microiontophoretically^(8,9). These results demonstrate a possible involvement of CN in modulatory process of pain information in Pf, and suggest that the possible involvement be via the activities of intracaudate opioid peptide system. In this paper, the techniques of microinjection and standard microelectrode were used to observe the effect of morphine on the electric activities of pain-excitation neurons (PEN) and pain-inhibition neurons (PIN) in Pf of thalamus.

MATERIALS AND METHODS

42 adult ♂ Wistar rats, 267 ± SD 28 g, were anesthetized with a mixture (10 ml/kg, ip) of 1% chloralose (Beijing Chemicals Factory) and 10% urethane (Shang-

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¹ Now in Department of Pharmacology, Harbin Medical University, Harbin 150086, China

hai Chemicals Factory) and placed in a stereotaxic apparatus. The electrode and cannula were placed in the Pf thalami and CN head through a 3 mm hole in the skull. *D*-Tubocurarine chloride (Colbrook Bucks, England) was given (1 mg/kg, ip) to immobilize the rat and experiments were conducted under artificial respiration. The body temperature was kept at 36–38°C. The experiments were divided in 3 groups: morphine HCl (Shenyang Pharmaceuticals, 8 µg/µl, 2 min, intracaudate injection); naloxone HCl (Endo. Lab., USA, 0.75 mg/kg, ip) + morphine HCl; and saline group (NS) (1 µl, intracaudate injection).

The single unit discharges of Pf neurons were recorded by microelectrode (0.5–1.0 µm, 10–30 MΩ) filled with KCl 3 mol/L. According to the Pellegrino's atlas, the microelectrode was inserted into Pf (RC = -2.2–-2.8 mm; R = 1.2–1.6 mm; DV = 5.5–6.5 mm) and microinjection site was determined in the CN head (RC = 2.0–3.0 mm; L = 2.5–3.5 mm; DV = 4.5–5.5 mm).

For microinjection, a stainless steel cannula consisting of an inner tube 0.3 mm diameter and an outer tube 0.45 mm diameter was used. The tip of the inner tube protruded out 2.0 mm. Morphine HCl and NS were injected into the head of CN by a motor-driven continuous slow injector at a rate of 0.5 µl/min.

The train of electric impulses to sciatic nerve (wave width 0.3 ms, frequency 200 Hz, train length 25 ms and intensity 40 V) and pinching tail with clamp were used as the noxious peripheral stimulation, while the flexion of joint and the touch of back skin were employed as the non-noxious stimulation.

At the end of experiments, the recording and injecting site were marked by 2% solution of pontamine sky blue dye in sodium acetate buffer 0.5 mol/L from the microelectrode with an anodal current (30 µA, 20 s) or microinjection (1 µl, 2 min). The brain was removed and stored in 10% formalin solution for 3–5 d. Frozen section (40 µm) were cut and stained with neutral

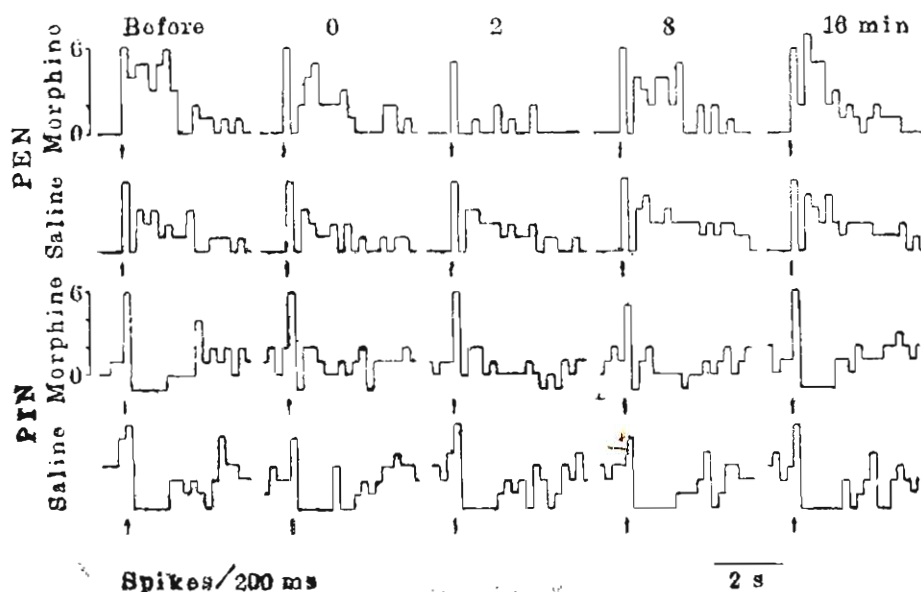


Fig 1. Inhibition of pain-discharges of pain-excitation neuron (PEN) and excitation of pain-discharges of pain-inhibition neuron (PIN) in the parafascicular nucleus (Pf) induced by morphine microinjected into the head of the caudate nucleus (CN). † Stimulation of sciatic nerve.

red for verification of the position of the tip of electrode and cannula.

The unit discharges of Pf neurons were displayed on oscilloscope and recorded by tape recorder, and the data were processed through KDS-1 A medical processor. The histogram was drawn by Z 3-304 function recording instrument. Experimental data were treated statistically by *t* test. Only the data in which the electrodes were confirmed to be in Pf were used. The locations of the cannulae were histologically examined by dye marks.

RESULTS

Influence on PEN Electric activities of 34 PEN before and after microinjecting morphine were made in 31 rats. The pain-discharges of 94% (32/34) PEN were inhibited obviously and enduringly by morphine, ie, decrease and disappearance in pain-discharges and lengthening in latent period of nociceptive responses (Fig 1). The net value of increase in frequency was regarded as the standard of change in frequency of pain-discharges (difference of the average frequency of discharges after and within 2s before noxious stimulation). The net value of discharges decreased obviously from 15.2 ± 5.0 to 3.2 ± 0.8 Hz after injecting morphine. The effect of morphine was most obvious at 2 min after injection. The inhibitory % of pain-discharges (decrease in % of net value of frequency of pain-discharges after injection) increased from 0 up to $78.6 \pm 9.9\%$. Morphine microinjected into CN head not only inhibited the pain-discharges but also lengthened the latent period in 88% (30/34) PEN, the latent period increased from 129 ± 34 to 274 ± 65 ms at 2 min after microinjection. The difference between the data after and before microinjecting morphine and between the date microinjecting morphine and NS were statistically significant ($P < 0.01$) The morphine effect disappeared at 10 min

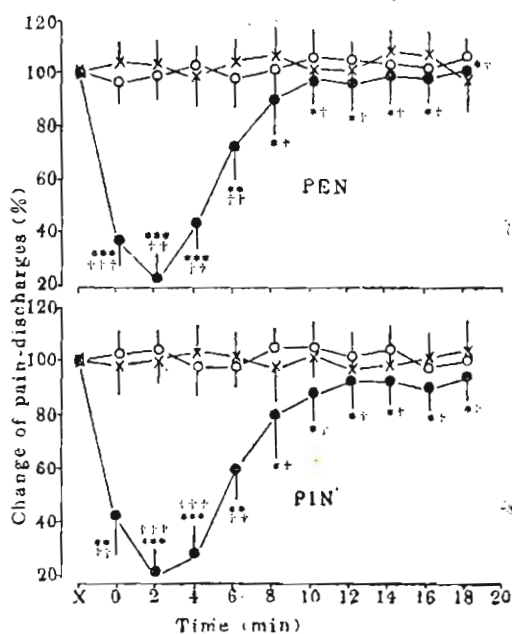


Fig 2. Effect of morphine microinjected into the CN head on the pain-discharges of PEN ($n=34$) and PIN ($n=27$) in Pf. (○) normal saline (NS), (●) morphine, (▲) naloxone + morphine. (×) shows the beginning of microinjection. $\bar{x} \pm SD$, * $P > 0.05$, ** $P < 0.05$, *** $P < 0.01$ vs before injection † $P > 0.05$, †† $P < 0.05$, ††† $P < 0.01$ vs NS.

after microinjection (Fig 2).

NS microinjected into CN head had no effect on the pain-discharges and latent period of PEN. The naloxone administrated ip 2 min before microinjecting morphine blocked completely the effect of morphine.

Influence on PIN The morphine relieved the inhibitory effect in 89% (24/27) PIN, ie, inhibitory duration shortened obviously or disappeared and the frequency of discharges increased (Fig 1). Before microinjecting morphine, the inhibitory duration was 2.1 ± 0.5 s and the firing rate was -11.4 ± 2.1 Hz. At 2 min after microinjecting morphine, the inhibitory duration shortened to 372 ± 48 ms and the firing rate increased up to 1.0 ± 0.3 Hz, the % of change in inhibitory duration (shortening in % of inhibitory duration after injection) increased from 0 to 79%.

The difference between the data after and before microinjecting morphine and between the data microinjecting morphine and NS were statistically significant ($P < 0.01$). The morphine effect disappeared at 12 min after microinjection (Fig 2).

NS likewise had no effect on the electric activities of PIN in the Pf and the morphine effect also blocked by naloxone.

DISCUSSION

Pf of thalamus has been known as one of the most important structure in pain integration⁽¹⁾. The involvement of the head of CN in pain modulation and processing the pain information in Pf has been demonstrated by recent studies^(6,7). In the present work, the opiate receptor agonist morphine microinjected into the head of CN inhibits significantly the nociceptive responses of Pf neurons, including PEN and PIN, and the inhibitory effect of morphine can be blocked by naloxone administrated ip. This result was similar to that of caudate stimulation and that of microiontophoretic morphine into Pf directly, in addition, the inhibitory effects of caudate stimulation and microiontophoretic morphine on the nociceptive responses of Pf neurons can also be antagonized by microiontophoretic naloxone into Pf⁽⁹⁾. The results from the present work and the previous studies suggest that the inhibitory effect of CN on the nociceptive responses of Pf involve in endogenous opiate peptides. When CN excites, the activities of the nociceptive neurons in Pf are inhibited. So it is considered that CN play a modulatory role in the pain information in the thalamus.

The present work has proved that naloxone can antagonize the effect of morphine, but, under the conditions of present work, a definite conclusion has not been drawn whether naloxone blocks the opiate receptors in CN⁽¹⁰⁾ or in Pf⁽¹¹⁾. So the further study will be needed.

On the other hand, the inhibitory effect of morphine on the nociceptive responses of Pf neurons can take place directly, so the faster action of morphine post-microinjection is observed in present experiments. And this result has been supported by electrophysiological⁽⁷⁾ and morphological^(12,13) studies. It is not clear that why the analgesia of morphine in the brain is shorter in spite of its $T_{1/2}$ is over 1 h, but the similar experimental results^(9,14) have been reported. The result of present work is also consistent with that of ip morphine⁽¹⁵⁾, but ip morphine is via periaqueductal gray area (PAG) to exert inhibitory effect on the nociceptive responses of Pf and the analgesia of ip morphine lasts longer than that of intracaudate injecting morphine. The reason is probably because of the different administrating road.

In CN, many kinds of neurotransmitters and neuromodulators exist, for example, acetylcholine, dopamine and endogenous opiate peptides. Currently, it has been suggested that these neurotransmitters participate in pain modulatory process of thalamus and the interrelationships among them in analgesia of CN is to be clarified.

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尾核头部微量注射吗啡对大鼠丘脑束旁核痛反应神经原电活动的影响

李柏岩、徐 屯 (哈尔滨医科大学 生理教研室, 哈尔滨 150086, 中国)

提要 本实验观察了尾核头部微量注射吗啡(8 μg/μl)对丘脑束旁核痛兴奋神经原和痛抑制神经原电活动的影响。结果表明,束旁核痛兴奋神经原的痛放电被抑制,即痛放电的频率下降,潜伏期延长。束旁核痛抑制神经原对伤害性刺激的抑制作用解除,即痛放电的频率增加,抑制时程缩短。吗啡的上述作用可被纳洛酮(0.75 mg/kg, ip)阻断。本文结果证明尾核头部内阿片肽系统在丘脑束旁核痛觉信息的调制方面发挥重要作用。

关键词 吗啡; 纳洛酮; 尾核; 丘脑; 痛; 电生理学; 兴奋; 抑制; 神经原

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Roles of periaqueductal gray and nucleus raphe magnus on analgesia induced by lappaconitine, N-deacetylappaconitine and morphine

GUO Xin, TANG Xi-Can

(Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China)

ABSTRACT In the rat tail flick test, ip LA 6 mg/kg, icv DLA 60 μg and icv or ith morphine 5 μg exhibited significant analgesia. But ith either LA 40 μg or DLA 60 μg was inactive. Naloxone (4 μg icv) which antagonized morphine analgesia failed to alter the analgesia induced by LA and DLA. Microinjection of DLA 20 μg or morphine 5 μg into the periaqueductal gray (PAG) or nucleus raphe magnus (NRM) pro-

duced markedly analgesic activity. The effects of electrolytic and kainic acid (0.8 μg) lesions of the PAG and NRM on the analgesia elicited in the rat from ip LA, icv DLA and morphine were also evaluated. No change in baseline tail flick latency was observed following lesions of the PAG and NRM. But lesions of the PAG and NRM significantly attenuated the analgesia mediated by LA, DLA and morphine. These results suggest that supraspinal sites, especially the PAG and NRM, are involved in the analgesic action

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