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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

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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*-deacetyllappaconitine and morphine

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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) produced 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 superspinal sites, especially the PAG and NRM, are involved in the analgesic action

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induced by LA, DLA and morphine.

KEY WORDS aconitine; lappaconitine; *N*-deacetyllappaconitine; morphine; analgesia; periaqueductal gray; raphe nuclei; kainic acid; naloxone; spinal cord

Aconite roots have been employed for centuries as an important anodyne in China and Japan. Lappaconitine (LA), isolated from *Aconitum sinomontanum* Nakai, and *N*-deacetyllappaconitine (DLA), isolated from *A. finetianum* Hand-Mazz and *A. sinomontanum* Nakai, were characterized as analgesic principles in rodents by our institute[1-4]. In our previous reports, we have demonstrated that LA belongs to the non-narcotic analgesic and the analgesic effect of LA is not antagonized by naloxone[3,5]. The periaqueductal gray (PAG) microinjection or icv injection of $CaCl_2$ abolished the analgesia of LA[6]. We also hypothesized that the central 5-HT system might be involved in the analgesia induced by LA and DLA[5]. The PAG and the nucleus raphe magnus(NRM)have long been implicated as having critical roles in central pain inhibition system and morphine analgesia[7-9]. Accordingly, by using the electrolytic and chemical lesions and microinjection methods, we studied the effects of the PAG and NRM on the analgesia mediated by LA and DLA. The spinal origin analgesia by ith injection of drugs was also investigated. Morphine was chosen as the positive control in the experiments.

MATERIALS AND METHODS

Male rats purchased from the Shanghai Experimental Animal Center and weighing 248 ± SD 31 g were used.

LA and DLA alkaloids were isolated and purified by the Department of Phytochemistry of our institute. The alkaloid was dissolved in distilled water and neutralized with HCl (1 mol/L) to pH 6.0. Kainic acid(Sigma) was dissolved in 0.9% saline and neutralized with NaOH (1 mol/L) to pH 7.0. Morphine HCl (Qinghai Pharmaceutical Factory) and naloxone HCl (Endolab) were dissolved in 0.9% saline.

Intrathecal cannulation and injection[10] Under anesthesia of sodium pentobarbital (40 mg/kg ip), a catheter (PE 10) was inserted (7.5 cm) into the spinal subarachnoid space from an incision in the atlanto-occipital membrane to the lumbar space and secured by suturing a preformed loop between this membrane and overlying muscle. The free end of the cannula was placed beneath the skin and allowed to exit in the parietal area where it was relatively inaccessible to the paws. Only rats showing no evidence of spinal damage were used. The injection system consisted of a 30 cm PE 10 tubing connecting the permanent indwelling cannula to a 50 μl syringe which was driven by an infusion pump. The injection of the drug solution was monitored by observing the movement of an air bubble in the tubing. Drug in 10 μl saline was injected over 2 min and the tubing was flushed by an additional 10 μl saline.

Intracerebral cannulation and microinjection Under sodium pentobarbital anesthesia, a guide cannula (stainless steel tubing of 0.6 mm od) was stereotaxically implanted with the tip positioned 3 mm above the target. The coordinates used were: AP −10.3 mm posterior to the bregma, H 10.2 mm below the dura matter and L 0 mm for the NRM: AP −6.3, H 5.8 and L ±0.5 mm unilaterally for the PAG[11]. The cannula was anchored in the skull by dental acrylic cement. A stylet was placed in the guide cannula to keep it patent. Under ether anesthesia,the drug solution 0.5 μl was injected at a rate of 0.2 μl/min using syringe cannula which projected 3 mm beyond the tip of the guide cannula. The injection cannula was left in place for an additional 2 min to minimize the backflow of the drug.

Electrolytic and kainic acid lesions Under sodium pentobarbital anesthesia, rats were immobilized in stereotaxic apparatus. Electrolytic lesions were made by passing 0.2 mA anodal direct current 15 s to either PAG or NRM through a stainless steel needle (0.1 mm od) insulated except for 0.3 mm at the tip. In control rats the needle was lowered in the brain without any passage of current. Kainic acid lesions were made by stereotaxic injection of kainic acid (0.8 μg in 0.2 μl) into either PAG or NRM. As a control, 0.9% saline 0.2 μl was injected into the same site as kainic acid.

Tail flick test The rat was gently wrapped in a towel so that the tail was freely mobile. The tail flick test was to measure the latency for a rat to withdraw its tail from a radiant heat source. A rheostat was used to adjust the lamp (12 V, 75 W) intensity to give a pre-drug tail flick latency of approximate 3 s. Once adjusted, the intensity was not changed. Baseline tail flick latencies were determined at least three times 5 min apart and averaged to give a single pre-drug baseline. A cutoff time of 8 s was used to minimize tissue damage to the tail.

General procedure Rats with cannulae implanted were housed in individual cage with food and water *ad lib* and given 1 wk for recovery from surgery. Rats lesioned by electrolysis and kainic acid were used in the experiments on d 7 and d 4, respectively. After completion of the tests, the location of the microinjection or lesion was checked histologically. Only data referring to lesions or cannulae correctly placed in the PAG or NRM were included in the results. All data were presented as mean ± SD. Statistical comparisons were made by *t* test.

RESULTS

Effect of naloxone on analgesia induced by LA, DLA and morphine Injections of LA, DLA and morphine exhibited markedly analgesic effects in the rat tail flick test. Rats given icv injection of naloxone (4 μg/rat) showed a significant reduction in the tail flick latencies elevated by morphine. But icv naloxone (4 μg/rat) failed to attenuate the analgesia induced by LA and DLA as compared with icv saline (Fig 1).

Intrathecal injections of LA, DLA and morphine On d 7 of spinal cannulation, ith morphine (5 μg) produced a high increase in the rat tail flick latency. The mean maximum increase in the latency was

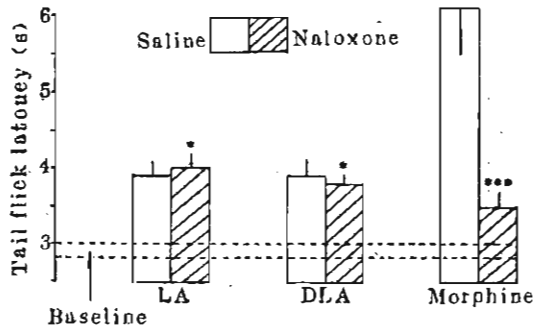

Fig 1. Effect of naloxone on the analgesia induced by lappaconitine (LA), *N*-deacetyllappaconitine (DLA) and morphine in the rat tail flick test. LA (6 mg/kg ip), DLA (60 μg icv) and morphine (5 μg icv) were injected 30 min before test. Naloxone (4 μg) and saline (10 μl) were icv injected 15 min before test. $n=5$. $\bar{x}\pm SD$. $^{*}P>0.05$, $^{***}P<0.01$ *vs* saline.

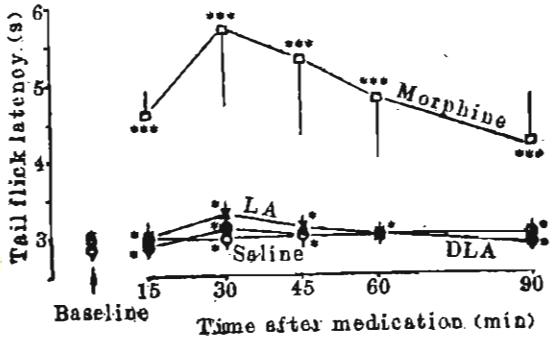

Fig 2. Effects of ith injections of LA (40 μg), DLA (60 μg), morphine (5 μg) and saline (10 μl) on the rat tail flick latency. $n=5$. $\bar{x}\pm SD$. $^{*}P>0.05$, $^{***}P<0.01$ *vs* baseline.

at 30 min after medication. In contrast, ith LA (40 μg), DLA (60 μg) and saline did not give any significant change in tail flick latency (Fig 2).

Effect of microinjection of DLA or morphine into PAG or NRM Microinjection of saline 0.5 μl into PAG or NRM had no effect on the tail flick latency in the rat tested. That DLA 20 μg or morphine 5 μg per rat were injected into either PAG or NRM produced a markedly analgesic action. The analgesic effect of DLA appeared within 30 min and disappeared within 90 min. The analgesic activity and duration of morphine were more potent and longer, respectively, than those of DLA(Fig 3).

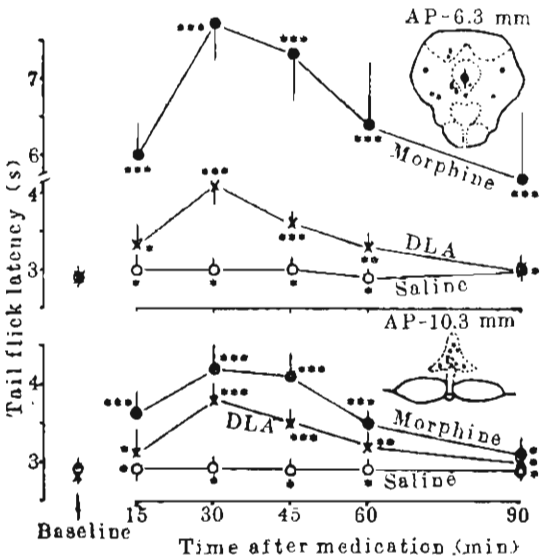

Fig 3. Effects of microinjections of DLA (20 μg) and morphine (5 μg) into periaqueductal gray (PAG) and nucleus raphe magnus (NRM) in the rat tail flick test. $n = 5$. $\bar{x} \pm SD$. $^*P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* baseline. The insets show the microinjected sites.

Effect of PAG lesions on analgesia induced by LA, DLA and morphine PAG lesions made by anodal direct current (DC) and kainic acid (KA) failed to alter significantly the baseline pain response as compared with control ($P > 0.05$). The baseline latencies were as follows: 2.8 ± 0.1 s for both DC and sham DC lesion groups and 2.9 ± 0.1 s for both KA and sham KA lesion groups. Following injections of LA, DLA and morphine, PAG sham lesion groups remained analgesic at 30 min. In contrast, PAG lesion groups demonstrated profound attenuations of analgesia induced by LA, DLA and morphine as compared with controls (Fig 4 A).

Effect of NRM lesions on analgesia induced by LA, DLA and morphine NRM lesions made by DC and KA did not change baseline tail flick latency from control ($P > 0.05$). The baseline latencies were 2.9 ± 0.1 s for DC, 2.8 ± 0.1 s for sham DC, 3.0 ± 0.1 s for KA and 2.9 ± 0.1 s for sham KA lesion groups. Following injections of LA, DLA and morphine, analgesia was seen at 30 min in sham lesion rats. But NRM lesioned groups markedly decreased the analgesia induced by LA, DLA and morphine as compared with controls (Fig 4 B).

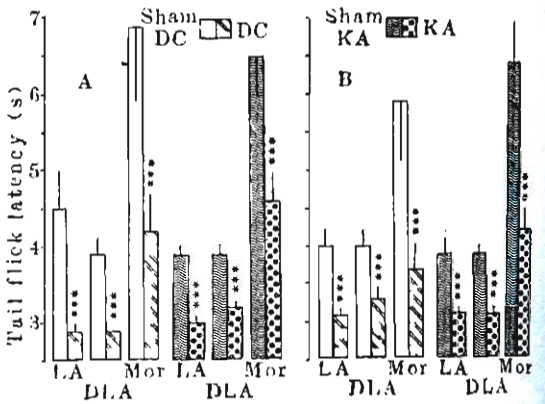

Fig 4. Effects of A) PAG and B) NRM direct current (DC) and kainic acid (KA, 0.8 μg) lesions on LA (6 mg/kg ip), DLA (60 μg icv) and morphine (Mor, 5 μg icv) induced analgesia in the rat tail flick test. Drugs were injected 30 min before test. $n = 5$. $\bar{x} \pm SD$. $^{***}P < 0.01$ *vs* sham groups.

DISCUSSION

In our previous studies, icv LA 20-40 μg had no analgesic effect and icv DLA

20–60 μg elicited dose-dependent analgesic action in the rat tail flick test[5]. Presently available results show that ith injection of LA 40 μg as well as DLA 60 μg did not exhibit any analgesic activity. Pretreatment of rats with narcotic antagonist, naloxone, failed to reduce the analgesia produced by LA and DLA either. We conclude that LA may not directly act on the central nervous system and DLA may act on superspinal sites without effects of opioid receptors. This conclusion is in accordance with our previous hypothesis that LA may metabolize into the active metabolite, DLA, then results in analgesia[5].

Our results as well as literature reports[7-9]demonstrated that morphine microinjection into the PAG or NRM produced an analgesic effect. Microinjection of DLA 20 μg into either PAG or NRM resulted in markedly analgesic action while the saline alone showed no such effect. These preliminary experiments indicate that the PAG and NRM may be two of the primary sites for the analgesic effects of DLA and morphine.

When injected into brain, kainic acid produced a lesion due to destruction of neurons and postsynaptic apparatus while sparing axons of passage[12]. Electrolytic lesions of the PAG and NRM had been reported to attenuate the analgesia induced by morphine[13,14]. Anatomical and behavioral evidence had confirmed the PAG-NRM pathway was crucial for the expression of systemic and cerebral morphine analgesia [15]. As observed in this study, two techniques employed in the PAG and NRM lesioning had no significant difference in the antagonism on LA, DLA and morphine analgesia. Our observation also demonstrated that lesion of either PAG or NRM abolished LA and DLA and reduced morphine analgesia. According to these results, the PAG-NRM pathway is as important in mediating analgesia of LA and DLA as that of morphine.

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水管周灰质和中缝大核在刺乌头碱、*N*-脱乙酰刺乌头碱和吗啡镇痛中的作用

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提要　大鼠光热甩尾法测痛表明，ip LA 6 mg/kg icv DLA 60 μg 和 icv 或 ith 吗啡 5 μg 有显著镇痛作用，ith LA 40 μg 或 DLA 60 μg 无作用．icv 纳洛酮 4 μg 拮抗吗啡但不改变 LA 和 DLA 的镇痛作用．水管周灰质或中缝大核注射 DLA 20 μg 或吗啡 5μg 也表现显著镇痛作用．电解和卡因酸(0.8 μg)损毁水管周灰质或中缝大核均拮抗 LA、DLA 和吗啡的镇痛作用．结果提示脊髓上部位特别是水管周灰质和中缝大核参与了 LA、DLA 和吗啡的镇痛作用．

关键词　乌头碱；刺乌头碱；*N*-脱乙酰刺乌头碱；吗啡；镇痛；水管周灰质；中缝核；卡因酸；纳洛酮；脊髓

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药物镇痛时不同脑区 ^{45}Ca 的摄取、线粒体蛋白质结合 Ca^{2+} 和亚微结构 Ca^{2+} 的分布[1]

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^{45}Ca-uptake, mitochondrial protein bound Ca^{2+} and ultrastructural distribution of Ca^{2+} in some brain regions of mice during drug-induced analgesia[1]

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ABSTRACT　After buprenorphine (Bup, 0.8 mg/kg ip) treatment ^{45}Ca-uptake (cpm/mg fresh brain) *in vivo* by brain slices decreased from 589 ± 12 and 436 ± 12 to 522 ± 14 and 408 ± 10 and mitochondrial protein bound Tb^{3+} (Tb^{3+} relative fluorescent intensity) reduced from 41 ± 5 and 32 ± 2 to 30 ± 3 and 22 ± 2 in periaqueductal grey and hypothalamus, respectively. A large amount dense precipitate occurred in the myelin sheath and mitochondria in both regions. The ^{45}Ca-uptake evoked by buprenorphine at 16 μg/40 μl *in vitro* has the similar tendency with that *in vivo*. Treated by ruthenium red (20 μg / mouse ip or icv) before buprenorphine, the above-mentioned effects were all abolished. Similar results were obtained with morphine (Mor, 10 mg/kg ip) and verapamil (Ver, 8 μg/mouse icv) instead of buprenorphine and ruthenium red, respectively. These results suggest that Ca^{2+} transport across neuroplasmic membranes plays a mediator role in drug-induced analgesia.

KEY WORDS　buprenorphine; morphine; calcium radioisotopes; mitochondria; ruthenium red; verapamil; neurochemistry; analgesia; calcium

提要　丁丙诺啡(0.8 mg/kg ip)使小鼠水管周灰质和下丘脑组织薄片的 ^{45}Ca 摄取减低，线粒体蛋白质结合 Ca^{2+} 增加，髓鞘和线粒体出现大量 Ca^{2+} 沉淀颗粒．预注钌红(20 μg/mouse ip 或 icv)则相反．用吗啡(10 mg/kg ip)和维拉帕米(8 μg/mouse icv)作同样处理亦获得类似的结果．提示，神经细胞质膜内外 Ca^{2+} 的移动可能在药物镇痛中起某种调节作用．

关键词　丁丙诺啡；吗啡；钙放射性同位素；线粒体；钌红；维拉帕米；神经化学；镇痛；钙

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