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关键词 精氨酸; 加压素类; G 蛋白类; 加压素受体类; 海马; 蛋白激酶类; 信号转导**目的:** 研究精加压素片段(4-8)在大鼠海马中的信号跨膜转导。 **方法:** 比较大鼠海马切片在药物刺激后的 MAPK 活性(MK)和 CaMK II 自身磷酸化水平(K II)的变化。 **结果:** (4-8)的拮抗剂ZDC(C)PR 及 GPCR 的抑制剂 PTX 分别都能阻断(4-8)引起的(MK)和(K II)的增高, 但都不影响 AVP 诱导的(MK)变化; PMB 抑制(4-8)诱导的(MK)增高而对(K II)无影响; TPA 单独可以刺激(MK)增高达(4-8)的(MK)水平, 同时使(K II)停留在对照水平; (MK)的增高不被 KN-62 阻断; 与 AVP 不同, (4-8)不影响 cAMP 水平。 **结论:** 精加压素片段(4-8)通过未知 GPCR 和 G₀ 介导一支信号途径。

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Involvement of medullary tail-flick related neurons in descending facilitation evoked by chemical stimulation of rat lateral habenular nucleus¹

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nociceptive spinal defensive reflex. This effect is brought out by the cooperation of on- and off-cells.

AIM: To study effects of sodium L-glutamate microinjection into lateral habenular nucleus (LHN) of rats on the firing of medullary tail-flick related neurons and tail-flick reflex (TF). **METHODS:** Using synchronous recording of unitary neuronal discharges and TF induced by noxious heat. **RESULTS:** Chemical stimulation of LHN induced an excitement of the on-cell spontaneous activity, an inhibition of the off-cell spontaneous activity with an enhancement of their TF related responses. The spontaneous firing rate of on-cells increased from 5.8 ± 2.2 Hz to 10.9 ± 3.4 Hz while the spontaneous firing rate of off-cells decreased from 11.8 ± 2.2 Hz to 6.1 ± 2.2 Hz. Meanwhile the TFL was shortened from 4.04 ± 0.17 s to 2.97 ± 0.13 s. **CONCLUSION:** The chemical stimulation of LHN produced a facilitating action onHabenular nucleus (HN) may participate in central mechanism of pain and nociceptive modulation. Electric or chemical stimulation of the rat HN produced an inhibition for noxious heat-induced tail-flick reflex (TF)⁽¹⁾. But there were some opposite results which indicated that HN activation by both electric stimulation and L-glutamate microinjection induced a decrease in pain threshold^(2,3,4). The activation of medial habenlar (MHN) increased the tail-flick latency (TFL), whereas the activation of lateral habenlar (LHN) decreased TFL⁽⁴⁾. The effect of HN on behavior is probably mediated via some nuclei of brain stem. The TF related cells, ie, on- and off-cells in the rostral ventromedial medulla (RVM), were the output elements of RVM which modulated nociceptive transmission at the spinal cord⁽⁵⁾. The off-cells exerted an inhibitory effect⁽⁶⁾ and the on-cells were likely to exert a facilitating action on spinal nociceptive transmission. In the present study we observed the effect of LHN activation by microinjection of sodium¹ Project supported by the National Natural Science Foundation of China, No 3880906.² Ptn 86-21-6564-3924. Fax 86-21-6534-0149.

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L-glutamate on the spontaneous firing and TF related responses of on- and off-cells and the TFL.

MATERIALS AND METHODS

Rats Sprague-Dawley rats (Certificate No 08-005) of either sex, weighing $0.27 \pm s 0.03$ kg (0.23–0.32 kg), were supplied by Center of Experimental Animals, Xi'an Medical University.

Surgery preparation The rats were anesthetized with ip sodium pentobarbital $40 \text{ mg} \cdot \text{kg}^{-1}$, and placed in a stereotaxic frame after external jugular catheterization and tracheal cannulation. A small craniectomy was made to allow passage of a microelectrode into RVM. For the microinjection study, a 25-gage stainless-steel guide cannula was inserted towards the aspect of the LHN (3.6–4.3 mm posterior to bregma, 0.5–1 mm lateral from medline, 4.3–4.7 mm below dura) and lowered to a position 1.5 mm above the intended injection site. A 31-gage stainless-steel needle was inserted through the guide cannula so that it protruded 1.5 mm beyond the guide tip. As the effects of initial anesthetic diminished, the rats were maintained in a light anesthesia state by iv infusion of sodium pentobarbital to keep a stable baseline TFL of 3.5–4.5 s and to prevent any signs of discomfort.

Noxious stimulation and TF The TF was elicited by applying focal radiant heat from a projector lamp onto the blackened ventral surface of the tail. The tail was attached to a mechanoelectric bridge transducer. The current which turned on the lamp meanwhile triggered the scan of oscilloscope, so that the TF was recorded simultaneously with unitary discharges of RVM neurons. If no TF occurred, the heat was turned off at 8 s.

Recording from RVM Unitary neuronal discharges were extracellularly recorded from the RVM (9.8–11.3 mm posterior to bregma, 0.3 mm lateral from medline, 8–9.5 mm below dura) by glass microelectrodes. RVM neurons were classified according to the system of Fields *et al.*^[5]. On-cells showed an abrupt increase while off-cells showed a sudden pause in firing just before the TF. These neuronal responses were time-locked to the TF rather than to the noxious heat stimulus so they were regarded as the TF related responses. After identification of an on-cell or an off-cell, the spontaneous activity was monitored for a period of 20 min.

Microinjection Microinjections were carried out using a 0.5- μL syringe. Sodium *L*-glutamate (50 nmol, 0.3 μL) or equal volume saline was injected into the LHN in 4 min and the spontaneous activity was again recorded. The TF-related discharges of RVM neurons and TF were recorded every 5 min.

Histology At the end of the experiment, the microinjection site and recording site were marked by pontamine sky blue dye and were histologically localized^[7].

Statistics Data were expressed as $\bar{x} \pm s$ and analyzed by *t*-test for paired or unpaired values.

RESULTS

The effect of sodium *L*-glutamate microinjection in LHN was tested on 11 on-cells and 9 off-cells, which were all in the RVM (Fig 1).

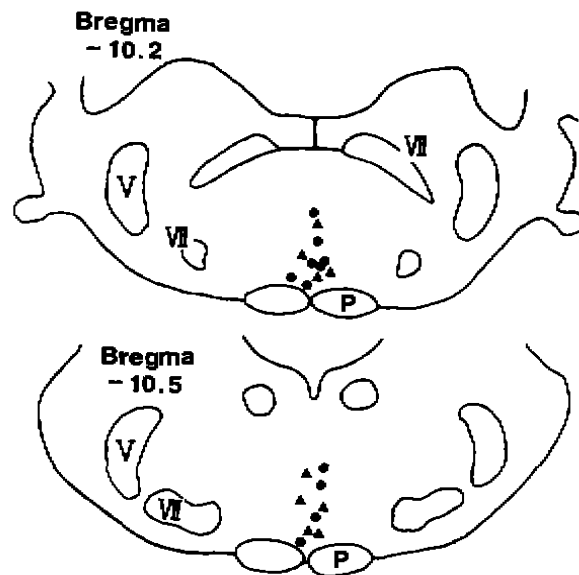


Fig 1. Coronal sections showing localization of 11 on-cells (●) and 9 off-cells (▲). P, pyramidal tract; V, trigeminal nucleus; VI, facial nucleus and nerve.

Following microinjection of sodium *L*-glutamate, the spontaneous firing rate of all on-cells (9/11) increased except two unchanged. The spontaneous firing rate increased from 5.8 ± 2.2 Hz (before) to 10.9 ± 3.4 Hz (10 min after microinjection, $P < 0.05$). In the contrast, the spontaneous firing rate of 8 off-cells decreased and only one off-cell was unchanged. The spontaneous firing rate of off-cells decreased from 11.8 ± 2.2 Hz (before) to 6.1 ± 2.2 Hz (10 min after microinjection, $P < 0.01$) (Fig 2).

The onset of TF related responses of 9 on- and 8 off-cells, which reacted to sodium *L*-glutamate microinjection in LHN, were all advanced and the duration of the TF related responses were prolonged in some cases (Fig 3). Meanwhile the TFLs were shortened from 4.04 ± 0.17 s (before) to 2.97 ± 0.13 s (10 min after microinjection) ($n = 17$, $P < 0.01$).

All these effects occurred in 5 min, reached the peaks in 10–15 min, and returned to the control value

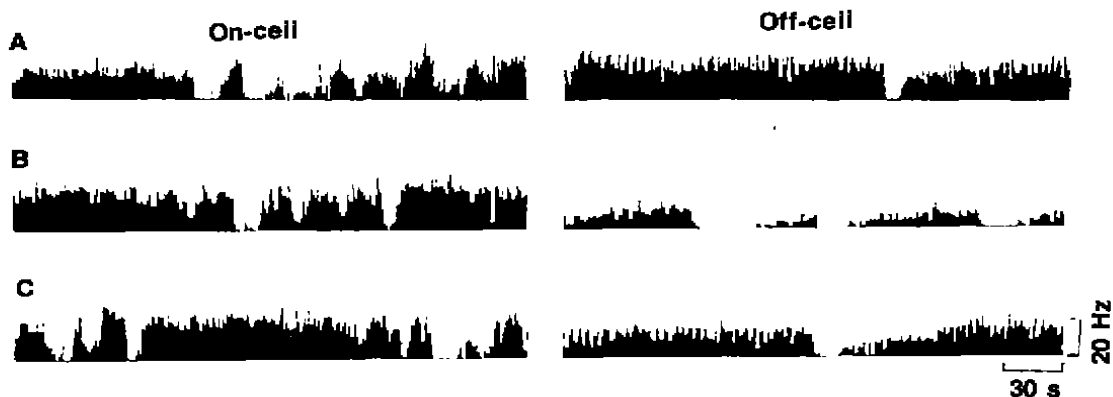


Fig 2. Spontaneous activity of on-cell and off-cell before and after sodium *L*-glutamate microinjection in LHN. A) before, B) 10 min, and C) 35 min after microinjection.

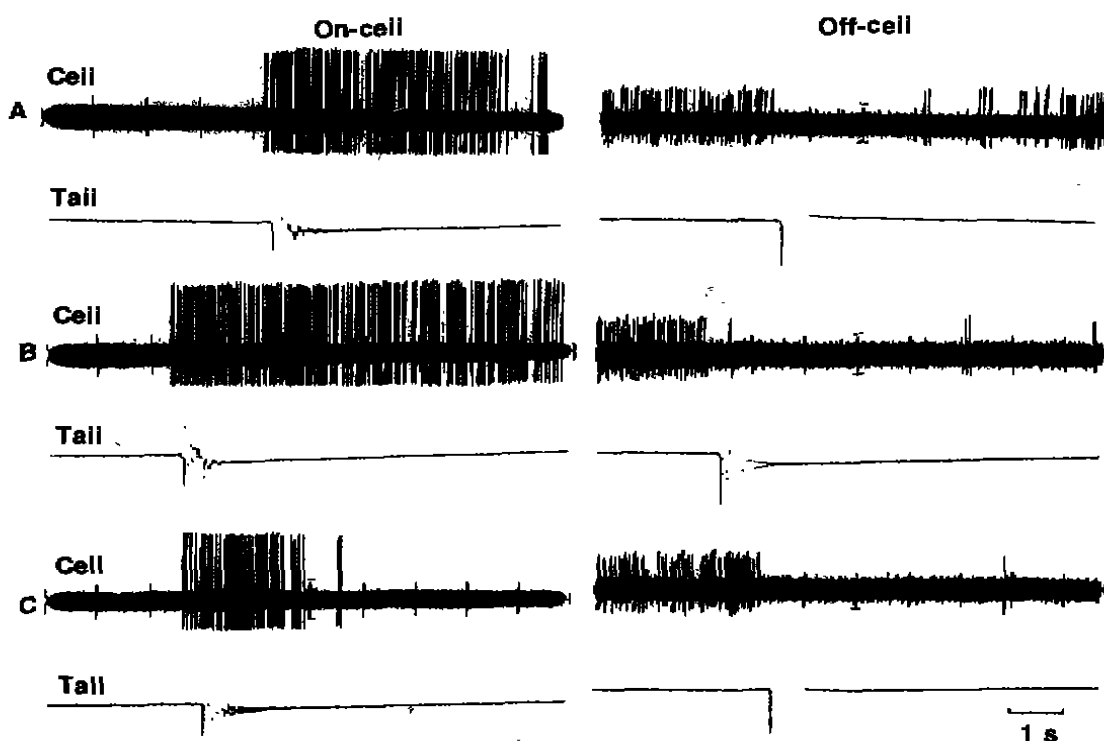


Fig 3. Effects of sodium *L*-glutamate in LHN on cell activity and tail movement. A) before, B) 10 min, and C) 35 min after microinjection.

in 35 min after the microinjection of sodium *L*-glutamate. Microinjection with equal volume of saline into LHN showed no significant change in on- and off-cell activity and the TFL for a period of 50 min after microinjection ($n = 5$, $P > 0.05$).

DISCUSSION

Microinjection of sodium *L*-glutamate into LHN

produce a decrease in TFL. This is consistent with our previous study^[2] and the results from some laboratories^[3,4]. But there are some opposite results from other laboratories^[1,9,10]. The different position of HN stimulation may account for this discrepancy. Different neural mechanisms may be activated by MHN and LHN stimulation. As a result, the activation of MHN increased TFL, whereas the activation of LHN

decreased TFL^[4].

On the other hand, sodium *L*-glutamate preferentially excites neurons but not the passing fibers, therefore decreasing the TFL. Thus it is suggested that increasing the activity of neurons in the LHN may lead to facilitating action on nociceptive spinal defensive reflex.

It is not very clear that the descending facilitation of LHN is mediated by which nuclei of brain stem. Some research indicated that the spontaneous firing rates of the nucleus raphe magnus (NRM) were decreased by electrical stimulation of HN and increased by HN lesion. Other results showed that the spontaneous firing rates of both pain-excitatory and pain-inhibitory neurons of NRM were decreased by LHN stimulation^[8]. This indicated there was a functional relation between HN and NRM. Now we recorded the TF related cell in RVM (included NRM). Its electrical-physiological characteristics consist with Fields' reports^[5]. The activation of LHN induced a significant excitement of the on-cell activity and inhibition of the off-cell activity with an enhancement of their TF-related responses and a decrease in TFL. Therefore we suggest that the facilitating effect of LHN activation on TF is brought out by the cooperation of on- and off-cells in RVM.

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延髓甩尾相关神经元参与化学性刺激大鼠
外侧缰核诱发的下行易化作用¹

R338.1

R441.1

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关键词 缰核; 谷氨酸钠; 延髓; 神经元; 电生理学; 尾; 痛测定

目的: 研究外侧缰核内微量注射 *L*-谷氨酸钠对大鼠延髓甩尾相关神经元活动和甩尾反射的影响。
方法: 用辐射热烫尾并同步记录延髓头端腹内侧区神经元单位放电和甩尾反射。
结果: 外侧缰核内微量注射 *L*-谷氨酸钠可加强给型甩尾相关细胞自发放电, 抑制撤型甩尾相关细胞的自发放电, 两类细胞的自发放电率分别从注射前的 5.8 ± 2.2 Hz 和 11.8 ± 2.2 Hz 变为注射后的 10.9 ± 3.4 Hz 和 6.1 ± 2.2 Hz。同时易化了两类细胞的甩尾相关反应和伤害刺激引起的甩尾反射, 甩尾反射潜伏期从注射前的 4.04 ± 0.17 s 缩短为注射后的 2.97 ± 0.13 s。
结论: 外侧缰核对节段性防御反射有下行易化作用, 这种易化作用是通过延髓内撤型和给型甩尾相关细胞的协同活动而实现的。