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Inhibition of formalin-induced responses of spinal dorsal horn neurons by stimulation of lateral reticular nucleus in rats

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ABSTRACT The effects of stimulation of the lateral reticular nucleus (LRN) on responses of spinal dorsal horn neurons produced by formalin (5%, 50 μl, sc) were investigated. In 12 out of 19 neurons tested, formalin induced 2 distinct phases of response. The first phase was initiated immediately after the injection of formalin and lasted for 3—8 min. The second phase started gradually 20—35 min after administration of formalin and lasted 30—65 min. Both phases of the response were inhibited by LRN stimulation.

KEY WORDS formalin; nociceptors; spinal cord; reticular formation; neural inhibition

The medullary lateral reticular neucleus (LRN) was involved in the descending inhibitory control of spinal nociception. Hall *et al* first reported that LRN was the main area of brain stem tonically inhibiting the C-fiber evoked responses of dorsal horn neurons in cats[1]. Lesion of the LRN abolished the PAG-induced inhibition of the firing of dorsal horn neuron produced by excitation of unmyelinated fiber of the tibial nerve[2]. Focal electric stimulation or microinjection of glutamate into LRN inhibited the transmission of nociceptive information in cats[3] and rats[4—6].

Noxious heating on skin or electric stimulation of peripheral nerve eliciting phasic (acute) pain was employed in all the studies mentioned above. However, there are many differences between the acute and chronic pains. Formalin injected subcutaneously (sc) in several species induced 2 phases of pain responses in behavioral tests, ie, a transient phasic response followed by a long-lasting tonic response, which was widely used as a model of chronic pain[7,8]. Formalin produced the 2 phases of firing of dorsal horn neuron in rats, equivalent to the formalin-induced 2 phases of behavioral activity[9—11]. Since no report has ever shown the involvement of LRN in long-lasting nociception, the aim of the present work was to study the effects of the LRN on both formalin-induced phasic and tonic responses of spinal dorsal horn neurons as a model of acute and chronic pain.

METHODS

Experiments were performed on Wistar rats (280 ± *s* 20 g) anesthetized with urethane (1.1 g · kg^{-1}, ip), paralyzed with gallamine triethiodide (30—40 mg · kg^{-1}, iv) and artificially ventilated. The spinal cord was exposed at the level of T12-L2 and the occipital bone covering the left part of the dorsal surface of cerebellum was removed. The rat was fixed in a stereotaxic apparatus. The body temperature, ECG and blood pressure were continuously monitored and kept within physiological ranges.

Glass micropipettes filled with sodium acetate 0.5 mol · L^{-1} containing 2% pontamine sky blue and with a resistance of 5—10 MΩ were used for recording the firing extracellularly from the spinal dorsal horn neurons. A pair of stainless steel needles were transcutaneously inserted into the left hindpaw and a stimulus (2 ms, 5 mA pulse, repeated at 0.5 Hz) was routinely applied. A volume of 50 μl 5% formalin was injected sc to the center of the receptive field of the neuron. A bipolar concentric electrode (od 0.2 mm) was vertically introduced into the LRN from the surface of cerebellum, according to the coordinates (AP 4.2 mm, L 2.0 mm, V 9.5—10.0 mm) described in the Atlas of

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Paxinos and Watson[11]. The following parameters were used for LRN stimulation : a train of 10 pulses (100 μs, 100 μA, 100 Hz), repeated at 3 Hz, lasting 5 —10 s. All the spikes were discriminated and analyzed immediately using a SMU-1 data processing system.

RESULTS

Nineteen wide dynamic range (WDR) neurons, which received both A- and C-fiber afferents and responded to both innocuous and nocuous stimuli, were recorded extracellularly in the laminae IV-V of spinal dorsal horns. The receptive field of these neurons were usually distributed in the first 3 toes of the hindlimb. Following sc injection of formalin in the center of the receptive field, the 2 phases of response were seen in 12 of 19 neurons (Fig 1). The first phase of response was immediately evoked at the onset of sc formalin which lasted 3—8 min. The second one occurred 20 — 35 min after the injection of formalin and lasted for 30—65 min. There was a silent period of 5—10 min between the 2 phases.

The effects of LRN stimulation on formalin-induced response were observed. Following LRN stimulation (100 μA), the first phase of responses was inhibited by 81.7± *s* 10.4% of control, ranging 73.7—98.9%, in all 9 neurons tested (Fig 2). Usually, the inhibition appeared concurrently with the period of stimulation. In 3 cases, however, a relatively prolonged inhibition after stimulation was seen, lasting for 1—3 min. The second phase of response in 10 neurons tested was also markedly reduced by 82.4± *s* 11.6% of control, ranging 61.7 — 98.3%. The histogram in Fig 3 shows LRN-induced inhibition of the second phase of response. Compared with LRN-induced inhibition of the first phase, the LRN-induced inhibition of the second phase exhibited a relatively prolonged after-effect, lasting 3-9 min. In 3 neurons the LRN-induced inhibition of the second phase lasted more than 10 min.

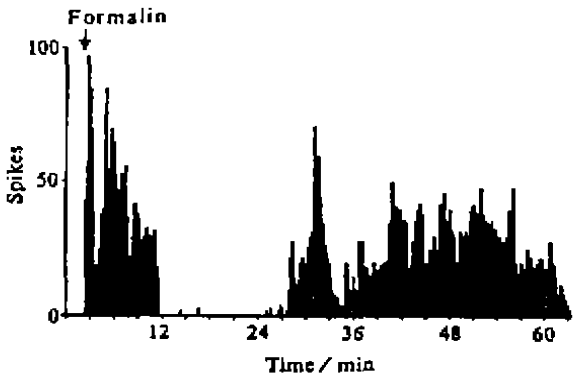

Fig 1. Formalin-induced responses of a WDR neuron in the spinal dorsal horn. Formalin (5%, 50 μl, sc) produced 2 phases of responses, i. e., the phasic (first) and the tonic (second) responses.

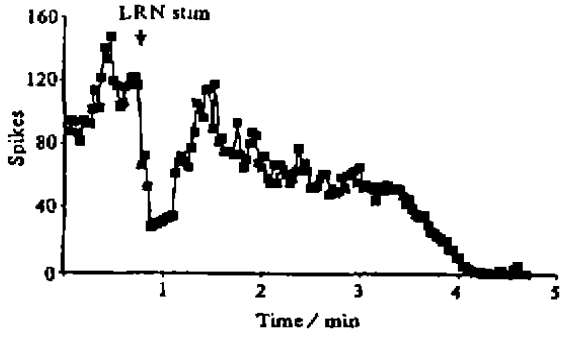

Fig 2. Effect of LRN stimulation on formalin-induced first response of a WDR neuron of the spinal dorsal horn.

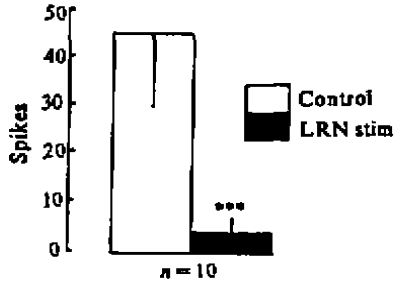

Fig 3. Effect of LRN stimulation on formalin-induced second responses of 10 neurons. *** $P<0.01$.

DISCUSSION

The present study clearly demonstrated that sc formalin produced the 2 phases of response of spinal dorsal horn neurons consistent with those in other reports[9—11]. Many behavioral studies in several species (including humans) have revealed that sc formalin can produced 2 phases of pain, i. e., the first short-lasting (phasic) pain and the second prolonged (tonic) pain[7-15]. Both the time course and the properties of the 2 pains were essentially identical to the 2 phases of neuronal responses observed in electrophysiological studies. It has been reported that neurons responsive only to innocuous stimulation exhibited no response to the injection of formalin whereas the typical 2 phases of response were frequently observed in WDR neurons of the spinal cord[9,10], which convey nociceptive information. Since the first phase of response of WDR neurons was likely to be elicited by direct stimulation of nociceptive receptors and the second phase resulted mainly from local inflammation. The former could contribute to the acute or phasic pain while the latter to the chronic or tonic pain.

LRN stimulation produced significant inhibition of both phases of response by injection of formalin, suggesting that LRN may not only be implicated in the descending control of the acute pain, but also in that of the chronic pain. The fact that the inhibition of the second phase is more prolonged with a remarkable after-effect seemed to indicate that the LRN had a more profound inhibitory effect on the chronic pain. In previous studies, it has been demonstrated that the LRN-induced inhibition was reduced by alpha-2 adrenoceptor antagonist[14]. It is reasonable, therefore, to assume that involvement of alpha adrenoceptors in the mediation of LRN-induced inhibition of spinal nociception was produced by formalin.

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电刺激大鼠外侧网状核抑制福尔马林诱发的脊髓背角神经元反应

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摘要 观察了刺激外侧网状核(LRN)对皮下注射5%福尔马林诱发的背角神经元反应的作用. 在记录的19个神经元中，福尔马林诱发12个神经元产生双时相反应. 第一时相反应是在注射后立即产生，持续3—8 min，第二时相是在注射后20—35 min逐渐产生，持续30—60 min 刺激外侧网状核对两个时相反应均有抑制作用.

关键词 福尔马林；伤害性感受器；脊髓；网状结构；神经抑制

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Protective effects of 3, 6-dimethylaminodibenzopyriodonium edetate on global ischemia reperfused isolated rat hearts

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ABSTRACT The effects of 3,6-dimethylamino-dibenzopyriodonium edetate (IHC-72) on global ischemia reperfused rat hearts were investigated. In the isolated working rat heart, 40-min global ischemia followed by 30-min reperfusion resulted in increases of ventricular tachycardia (VT) and ventricular fibrillation (VF), increases of creatine kinase (CK) release and malondialdehyde (MDA) contents, but decreased superoxide dismutase (SOD) activity. Following ischemia and reperfusion, the accumulation of myocardial calcium increased. IHC-72 50 $\mu mol \cdot L^{-1}$ given 10 min before ischemia and during reperfusion decreased the cardiac CK release, VT, and VF, reduced the MDA contents, prevented the reduction of SOD activity and attenuated the accumulation of myocardial calcium and sodium *vs* control. These results indicated that IHC-72 protected myocardial reperfused injury.

KEY WORDS 3, 6-dimethylamino-dibenzopyriodonium; myocardial reperfusion injury; arrhythmia; superoxide dismutase; malondialdehyde; calcium

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It has been known that ischemia reperfused myocardial injury is related to the production of oxygen free radicals. The lipid peroxidation of oxygen free radicals leads to cell membrane damage, then the disturbances of intracellular calcium, sodium, and potassium[1]. Ischemia itself also leads to disorder of active and passive ionic homeostasis, and reperfusion may allow the calcium and sodium ions to flow along their concentration gradients into the myocardial cells[2]. There are evidences that the oxygen free radical scavengers or some calcium antagonists can protect ischemic reperfusion hearts from injury[3,4]. 3, 6-Dimethylamino-dibenzopyriodonium edetate (IHC-72) possesses anti-arrhythmic action in experimental models[5]. Our previous study showed that it inhibited the transmembrane movement of calcium and sodium in myocardial cells[6,7]. The present study is to