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Selective blockade by yohimbine of locus coeruleus-induced inhibition of nociceptive reflex but not that of C responses of spinal dorsal horn neurons in rats

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ABSTRACT The effect of α_2-adrenoceptor antagonist yohimbine (Yoh) on locus coeruleus (LC)-induced spinal antinociception was investigated in 18 anesthetized Wistar rats. Stimulation of LC markedly inhibited both nociceptive reflex of the posterior biceps semitendinosus (PBST) muscle and C responses of 16 wide-dynamic range (WDR) neurons of the dorsal horn. Application of Yoh (0.2 %, 5 — 10 μl) to the surface of spinal cord at L_{3-4} attenuated the LC-induced inhibition of nociceptive reflex without affecting that of C responses of 10 WDR neurons that were tested in 6 rats. The results suggested that LC may exert its inhibitory action on the nociceptive reflex via α_2 adrenoceptors somewhere other than the WDR neurons in the spinal dorsal horn.

KEY WORDS adrenergic receptors; pain measurement; electromyography; locus coeruleus; norepinephrine; spinal cord; yohimbine

Stimulation of LC selectively inhibits the nociceptive responses of the spinal dorsal horn neurons[1] and increases the latency of tail flexor reflex (TFR)[2]. The α_2-adrenoceptor antagonists, Yoh and/or idazoxan, reduced the norepinephrine-induced inhibition of nociceptive responses of the dorsal horn neurons[3] and attenuated the spinal antinociception to stimulation of LC[4]. It seemed that α_2-adrenoceptors played an important role in LC-induced spinal antinociception[4]. However, our previous results did not support this view, as α_2-adrenoceptor antagonists failed to block LC-induced inhibition of nociceptive responses of the dorsal horn neurons in cats[3] and rats[5]. Therefore, the differential effects of α_2-adrenoceptors on LC-induced inhibition of nociceptive reflex and the nociceptive responses of dorsal horn neurons merit further investigation.

MATERIALS AND METHODS

Experiments were performed on 18 ♂ Wistar rats (Shanghai Animal Center, Chinese Academy of Sciences), weighing 314 ± 29 g, anesthetized with urethane (1.1 g·kg^{-1}, ip). Trachea was canulated for artificial respiration. The spinal cord was exposed by laminectomy at L_1-L_6 and covered with warm agar. The rats were fixed in a stereotaxic frame. BP, body temperature and ECG were monitored and kept at physiological levels.

As a nociceptive flextion reflex (FR), the firings of electromyography (EMG) from the PBST muscle were evoked by peripheral electric stimulation according to Hoffer's description[6]. The electric stimuli (1 —2 ms, 100 V, 3 pulses, 100 Hz, at 5-min intervals) were transcutaneously applied to the ipsilateral hindpaw via a pair of stainless steel needles. A concentric bipolar stainless steel electrode (0.15 mm in diameter) was inserted into the LC at the stereotaxic coordinates of P 0.5, L 1.0, H 7.5 according to the atlas of Paxinos[7] for the electric stimulation (50—150 μA, 0.1—

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Abbreviations: BP = blood pressure; ECG = electrocardiogram; EMG = electromyogram; FR = flection reflex; L = lumbar; LC = locus coeruleus; NPY = neuropeptide Y; PBST = posterior biceps semitendinosus muscles; TFR = tail flexor reflex; WDR = wide-dynamic range; Yoh = yohimbine.

0.2 ms, 100 Hz, for 500—600 ms). The stable baseline of EMG was established for at least 30 min prior to the examination of the effects of LC stimulation and the application of drug.

After the observation of FR, the rat was paralyzed with gallamine triethiodide (2 %, 0.1—0.2 ml, iv) and artificially ventilated. The responses of the dorsal horn neurons were extracellularly recorded with a single micropipette filled with NaCl 4 mol·L^{-1}. A 0.02 % solution of Yoh (Sigma) in saline was topically applied to the dorsal surface of the spinal cord at segments of L_{3-4} (5—10 μl).

Statistical analysis: The firing rate of EMG of single or multiple muscle unites and the responses of spinal WDR neurons were represented by $\bar{x}\pm s$. Statistical significance was evaluated by t test.

RESULTS

The EMG of PBST reflex by peripheral electric stimulation exhibited 2 components, the early one with a latency of ≤10 ms, and a threshold of 9±5 V (range 5—20 V, n=7), the late one with a latency of 125±23 ms (range 80—160 ms, n=12), and a threshold of 19±7 V (range 10—32 V, n=7), corresponded to A- and C-afferent fibres evoked responses, respectively. Following the stimulation of LC, the C-afferent-volley induced FR was selectively inhibited to 28±16 % (range 65.4 %—1.8 %, n=18) of control level in all 18 rats tested. When LC stimuli were applied 200 ms before the onset of the peripheral stimuli, the maximal inhibition of FR was obtained without fluctuation of BP.

In 9 rats, after LC-induced inhibition of the FR was tested, responses of WDR neurons in the dorsal horn were recorded. The responses also exhibited typically 2 components which represented A- and C-afferent volley induced responses with the latency of ≤10 ms and 138±79 ms (n=10), respectively. Electric stimulation of LC selectively inhibited the evoked nociceptive responses of 16 WDR neurons to 26±20 % (range 0—50.0 %, n=16) of control level.

Intrathecal injection of Yoh (0.2 %, 5—10 μl, mean 7±2 μl) markedly blocked the LC-induced inhibition of FR. The inhibition was altered from 27±12 % to 84±20 % (range 41.6 % — 1.8 % and 120.3 % — 68.2 %, respectively) of control, without fluctuation of BP in 15 rats. A typical example was shown in Fig 1. The effect of Yoh lasted 15—130 min. In contrast, Yoh failed to reduce the inhibitory effect of LC on evoked nociceptive responses of 10 dorsal horn neurons tested in 6 rats (Fig 2). In addition, the A-afferent volley induced responses of both FR and WDR neurons were not affected by LC and Yoh.

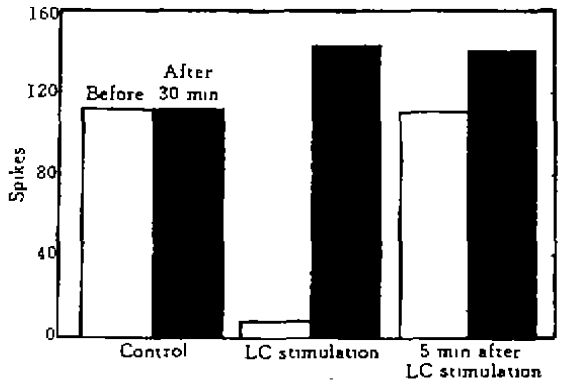

Fig 1. Blockade of LC-induced inhibition of nociceptive reflex by Yoh in one experiment. Histogram representing firing recorded from posterior biceps semitendinosus muscle to excitation of unmyelinated afferents by electric stimulation (1 ms, 100 V, 3 pulses, 100 Hz, at 5 min interval) of the ipsilateral hindpaw. Yoh (0.2 %, 5 μl) was applied to the dorsal region surface of the spinal cord at L_{3-4}. White and black columns represent spikes of EMG before and after Yoh, respectively. EMG: electromyogram; LC stimulation: electric stimulation of locus coeruleus (50 μA, 0.2 ms, 100 Hz, for 500 ms).

In 2 rats, the effects of Yoh on LC-induced inhibition of FR and responses of the dorsal horn neurons were simultaneously tested. Intrathecal injection of Yoh blocked the

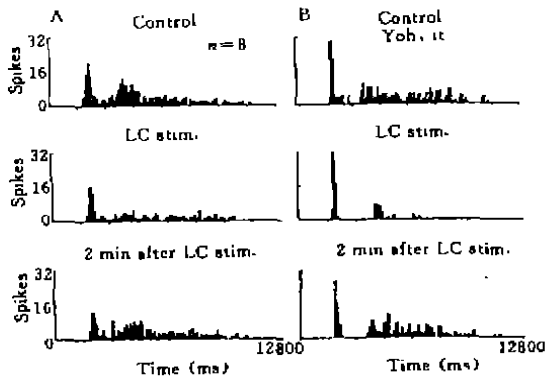

Fig 2. LC-induced inhibition of C responses ($n=8$) of a spinal WDR neuron by peripheral electric stimulation (2 ms, 100 V, 3 pulses, at 5 min interval). A, Control series; B, Tested series; Topical application of Yoh (0.2 %, 10 μl) to the dorsal surface of the spinal cord at L_{3-4}. Upper panels: Control responses; Middle panels: Stimulation of LC (0.2 ms, 50 μA, 100 Hz for 500 ms); Bottom panels: 2 min after LC stimulation.

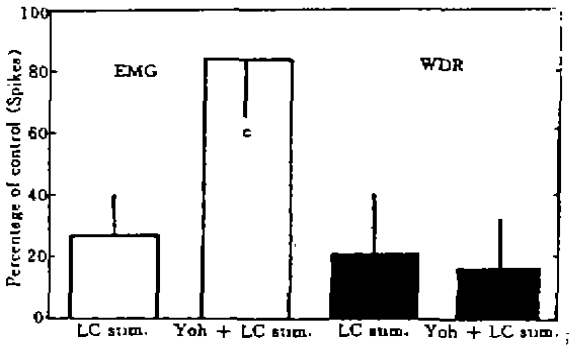

Fig 3. Differential effects of Yoh on LC-induced inhibition of nociceptive reflexes of PBST muscle and C responses of WDR neurons by peripheral electric stimulation (1 ms, 100 V, 3 pulses, 100 Hz, at 5 min interval). Topical application of Yoh (0.2 %, 5—10 μl) significantly reduced LC-induced inhibition of nociceptive reflex without affecting that of C responses of WDR neurons. The white and black columns represent EMG ($n=11$) and C responses of neurons ($n=9$), respectively. $^{c}P<0.01$ *vs* LC stimulating group of EMG.

inhibitory effect of LC on FR in both rats. However, Yoh did not change the LC-induced inhibition of C responses of 5 dorsal horn neurons in the same rats (Fig 3).

DISCUSSION

Consistent with our previous observations[3,5], the present results showed that α_2-adrenoceptor antagonists failed to block the LC-induced inhibition of nociceptive responses of dorsal horn neurons. Interestingly, the present study also supported the view that α_2-adrenoceptors played an important role in LC-induced inhibition of nociceptive FR[4]. In view of the present recordings of FR and firing of the dorsal horn neurons under the same conditions and even in the same animals, it was shown that there were differential effects of α_2-adrenoceptors on LC-induced spinal antinociception. The previous results, which seemed to be conflicting, may be attributable to the activation of different neuronal pathways in LC-induced spinal antinociception.

Norepinephrine-containing neurons projected primarily to the intermediate zone, lamina X, and the ventral horn of the spinal cord[8]. Since lamina X neurons conduct the spinal nociceptive signals[9,10] and also send their axons to ventral horn, it would be possible that a pathway (LC-lamina X-ventral horn) may be involved in LC-induced inhibition of FR. Should it be the case, it would also be possible that Yoh reduced the LC-induced inhibition of both the firing of lamina X neurons and the FR by noxious stimulation. Our recent study strongly supported this likelihood[11]. Another possible explanation may be that LC-induced inhibition of nociceptive reflex result from an inhibitory action on motorneurons or ventral horn interneurons. However, some evidences did not seem to support this assumption[12,13].

The kind of transmitters that mediate the LC-induced inhibition of nociceptive responses

of the dorsal horn neurons remains undetermined. In the light of the co-localization of neuropeptide Y (NPY), serotonin or galanin and norepinephrine in LC neurons[14,15], whether coerulospinally projecting NPY-, 5-HT-, and galanin-containing cells are contributing factors in LC-induced spinal antinociception via the dorsal horn pathway merits further study.

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育亨宾阻断蓝斑对伤害性屈反射的抑制而不影响其对背角神经元 C 反应的抑制

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摘要 在18只麻醉大鼠上刺激蓝斑可以抑制强电流刺激后肢引起的后二头半腱肌的屈反射和16个背角神经元的 C 反应，在脊髓腰3—4节段表面滴注 Yoh (0.2 %，5—10 μl)明显减弱蓝斑对反射的抑制而不影响其对 C 反应(n=10)的抑制。结果提示，α_2受体参与蓝斑对伤害性反射的抑制，而蓝斑对背角神元 C 反应的抑制可能由其它递质介导。

关键词 肾上腺素受体；痛测定；肌电描记术；蓝斑；去甲肾上腺素；脊髓；育亨宾