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Influences of locus coeruleus lesions and reserpine treatment on opioid physical dependence in rats[1]

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AIM: To study the role of locus coeruleus (LC) noradrenergic neurons in the opioid dependence. **METHODS**: Chemical lesion of LC was produced by 6-hydroxydopamine (4 μg in 1 μL). Reserpine (Res) was used to deplete central noradrenaline. Composite scores of naloxone (4 mg·kg^{-1}, ip) precipitated abstinence syndromes were calculated and the magnitude of weight loss was determined in chronic morphine (Mor)- or dihydroetorphine (DHE)-treated rats. **RESULTS**: Lesions of LC made Mor, but not DHE, abstinence syndrome more serious. Multiple doses of Res (0.5 mg·kg^{-1}·d^{-1} × 3 d) increased scores of Mor and DHE abstinence syndrome, made worse the loss of body weight in chronic Mor-, but not DHE-, treated rats. A single dose of Res (0.5 mg·kg^{-1}) speeded up the development of physical dependence on Mor. **CONCLUSION**: The LC noradrenergic neurons play a role in suppression of some withdrawal symptoms.

KEY WORDS locus coeruleus; reserpine; morphine; dihydroetorphine; narcotic dependence; substance withdrawal syndrome

The largest noradrenergic nucleus in brain is locus coeruleus (LC), which possesses a high density of opiate receptors[1]. In either anesthetized or awake rats, local or systemic administration of opioids results in the inhibition of LC neuronal spontaneous firing rates[2]. In opioid-dependent rats administration of opiate receptor antagonist results in an increase in LC firing rates[3]. This withdrawal-induced activation of the LC might play a role in the manifestations of the opiate abstinence syndrome in both animals and humans[4].

Dihydroetorphine (DHE) is a new narcotic analgesic with very high potency and low physical dependence, and also effective in the detoxification of opioid addiction[5—11]. So it can be used as a tool in the study of the mechanisms of analgesia and dependence of opioids.

In the present work physical dependence on morphine (Mor) and DHE in LC lesioned and reserpine (Res) treated rats were compared to inquire into the role of the LC in opioid dependence.

MATERIALS AND METHODS

Drugs DHE and naloxone (Nal) were synthesized by our institute, batch number 860315 and 910201, purity 98.2 % and 99.06 %, $[\alpha]_D^{22}$ −67.0° and −176.4°, respectively. Mor was product of Qinghai Pharmaceutical Factory, № 910907. Res was product of Tianjing Renmin Pharmaceutical Factory, № 880913. 6-Hydroxydopamine was purchased from Fluka AG Co.

Lesioning of LC Wistar rats of either sex (n = 93) weighing initially 228 ± s 19 g were anestbetized with complex anesthetics (chloral hydrate 4.25 g, $MgSO_4$ 2.12 g, pentobarbital 866 mg, ethanol 14.25 mL, propylene glycol 33.80 mL, water 51.95 mL) 2 mL·kg^{-1} ip. 6-Hydroxydopamine 4 μg in 1 μL of saline containing 0.02 % ascorbic acid was injected into each LC (1.1 mm posterior to the lambdoidal suture, 1.1 mm lateral to the median raphe and 7.5 mm under the skull surface) at a speed of 0.2 μL·min^{-1}.

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The syringe needle was kept *in situ* for 5 min after injection. Sham rats received the same operation and inserting the needle as lesioned rats but no injection of any solution. Dependence experiments were begun 2—3 wk later.

After the experiments the brain was perfused with saline through the ascending aorta to wash the blood out, followed by perfusion with 4 % polyformaldehyde phosphate buffer (pH 7.2, 250 mL) for fixation. Additional fixation was carried out in the same solution for 4 h after the brain was taken out. Then the brain was transferred into a 15 % sucrose phosphate buffer (pH 7.2) overnight. Frozen brain sections of 40 μm thick were made, stained with neutral red or cresyl violet, and examined under light microscope (×10) to identify the lesioned sites. Valid data were gotten from the rats those more than half of LC neurons had been destroyed.

Dependence experiment Mor and DHE were injected sc tid for 3 d with a total dose of 155 $mg \cdot kg^{-1}$ and 18.6 $\mu g \cdot kg^{-1}$ (31 times their ED_{50} for analgesia), respectively. The daily doses of Mor (30 $mg \cdot kg^{-1}$ on d 1) and DHE (3.6 $\mu g \cdot kg^{-1}$ on d 1) were progressively increased by 3 ED_{50}. Nal 4 $mg \cdot kg^{-1}$ was injected ip 3—4 h after the last injection of opioids on d 4. Opiate abstinence syndromes (jumping, wet dog shakes, writhing, irritability, stereotyped head bobbing, sweeping tail movements, yawning, teeth chatter, chewing, lacrimation, ptosis, salivation, diarrhea, piloerection) were analyzed in 15 min epochs and composite scores were calculated[4]. The magnitude of weight loss was also determined before and 1 h after Nal precipitation.

RESULTS

Influences of LC lesions In chronic Mor-treated group, the scores of withdrawal syndromes and weight loss in LC lesioned rats were higher than those in the sham rats. In chronic DHE-treated group, there were no significant differences between lesioned and sham rats, neither were so in the saline control group (Tab 1).

Influences of Res treatment

1 Multiple doses Chronic Mor or DHE treated rats were divided into 2 subgroups. One subgroup was injected ip Res 0.5 mg $\cdot kg^{-1}$ just before the sc injection of opioids in the morning on d 1, d 2, and d 3. Results showed that in Mor, DHE, and saline groups, the scores of withdrawal syndromes in Res treated rats were higher than those in rats without Res injections, while only in Mor group the weight loss in Res-treated rats was more marked than that in rats without Res injections (Tab 1). These reaults demonstrated that Res treatment aggravated the Mor and DHE abstinence syndrome.

2 Single dose Subgroups were divided as described above. Mor or DHE was injected tid only for 1 d, with a total dose of 9 times their respective ED_{50} for analgesia. Res 0.5 $mg \cdot kg^{-1}$ was ip just before the first dose of opioids. Nal 4 $mg \cdot kg^{-1}$ was injected ip 4 h after the last dose of opioids. The results showed single dose of Res made the Mor abstinence syndrome more obvious and their severity approximated that in rats medicated for 3 d. Single dose of Res did not influence the abstinence syndrome from DHE and saline (Tab 1).

DISCUSSION

Preliminary studies demonstrated that brain LC neurons are hyperactive during the opiate withdrawal. If these noradrenergic responses indicate the LC is necessary for the syndrome, then lesions of the nucleus should theoretically decrease the behaviors associated with Mor abstinence. However the present studies showed that lesions of LC made Mor abstinence syndrome more serious, which do not support this hypothesis. The possibility is that different brain areas innervated by LC response differently during the opiate abstinence, and some play a role in manifestation of the abstinence syndrome while some play

Tab 1. ***Effects of locus coeruleus lesions and reserpine (Res) treatment on scores of withdrawal symptoms and weight loss after naloxone precipitating abstinence from morphine (Mor) and dihydroetorphine (DHE) in rats.*** $\bar{x}\pm s$. $^{a}P>0.05$, $^{b}P<0.05$, *vs sham or normal.*

Treatment	n	Saline	Mor	DHE
			Scores of symptoms	
		10 mL·kg^{-1}, sc	155 mg·kg^{-1}, sc	18.6 μg·kg^{-1}, sc
Intact	9—13	1.12±0.84	12.69±3.33	4.11±2.03
Sham	9—13	1.35±1.32	12.46±3.84	4.00±2.55
Lesion	9—13	1.33±0.87^{a}	15.69±3.35^{b}	5.57±1.94^{a}
Normal	4—6	2.50±1.29	11.33±4.01	4.50±1.30
Res 0.5 mg·kg^{-1}·d^{-1}×3 d	5	5.40±2.61^{b}	15.40±1.14^{b}	7.00±2.12^{b}
		3 mL·kg^{-1}, sc	45 mg·kg^{-1}, sc	5.4 μg·kg^{-1}, sc
Normal	4—5	0.50±0.58	6.40±2.70	3.00±1.41
Res 0.5 mg·kg^{-1}	4—5	1.75±1.71^{a}	12.17±2.40^{b}	5.60±3.05^{a}
			Loss of body weight (g)	
		10 mL·kg^{-1}, sc	155 mg·kg^{-1}, sc	18.6 μg·kg^{-1}, sc
Intact	9—13	2.06±1.08	8.81±3.18	3.50±1.90
Sham	9—13	2.50±1.58	7.92±3.53	4.89±1.87
Lesion	9—13	2.11±1.60^{a}	11.84±3.62^{b}	4.65±2.64^{a}
Normal	4—6	2.62±1.11	7.92±4.05	4.04±1.53
Res 0.5 mg·kg^{-1}·d^{-1}×3 d	5	4.40±2.68^{a}	12.80±1.60^{b}	3.94±2.04^{a}
		3 mL·kg^{-1}, sc	45 mg·kg^{-1}, sc	5.4 μg·kg^{-1}, sc
Normal	4—5	1.75±0.96	5.64±3.46	4.84±1.08
Res 0.5 mg·kg^{-1}	4—5	1.68±0.85^{a}	9.82±2.24^{b}	3.40±1.88^{a}

a role in suppression of the syndrome. In this way, lesions of LC may influence differently on different areas containing the terminals of LC noradrenergic neurons. Results of this paper indicate that the suppressive action of LC is dominant during the opiate abstinence. It has been reported that lesions of dorsal noradrenergic bundle which originates mainly from the LC, had no effect on Mor abstinence syndrome[12]. The different results between lesions of dorsal bundle and LC suggest that other pathways from LC may be responsible for suppression of the opiate abstinence syndrome.

The same experiment carried out in Res treated rats further confirmed the above results. Res treatment could not only make Mor and DHE abstinence syndrome much more serious but also speed up the development of dependence on Mor. That means the effect of Res on the opioids abstinence syndrome is more potent than that of LC lesions. It might because Res could deplete NA levels in other noradrenergic neurons besides the LC, which also suggests that there are other noradrenergic neurons having the same effects as the LC on the opioids abstinence syndrome. NA content in LC decreased by 34 % after 24-h Res treatment, while the mRNA level and activity of tyrosine hydroxylase in the LC neurons increased[13]. The same changes also happened in chronically Mor treated rats[14]. Such similar changes can explain partly the phenomenon that saline control rats exhibited light Nal-precipitated withdrawal symptoms when treated repeatedly with Res, indicating that exhaustion of NA in the CNS may imitate opiate dependence state.

It is concluded from the present studies that LC noradrenergic neurons play a role in suppression of some withdrawal symptoms and are benificial to the stabilization of the CNS.

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大鼠损毁蓝斑和利血平处理对阿片类药物身体依赖性的影响

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目的：探讨蓝斑去甲肾上腺素能(LC-NA)神经元在阿片类药物依赖中的作用. **方法**：用6-羟基多巴胺4 g·L^{-1}和利血平(Res)造成蓝斑损毁和中枢 NA 耗竭. 慢性用吗啡(Mor)或二氢埃托啡(DHE)后，ip 纳洛酮4 mg·kg^{-1}催促戒断，对戒断症状综合评分并记录体重丧失. **结果**：损毁蓝斑加重 Mor 用药鼠戒断症状，但不明显影响 DHE 用药鼠戒断症状. 多剂量 Res (0.5 mg·kg^{-1}·d^{-1}×3 d)升高 Mor 和 DHE 用药鼠戒断症状记分；加重 Mor 而非 DHE 用药鼠体重丧失. 单剂量 Res 0.5 mg·kg^{-1}加速 Mor 身体依赖的形成. **结论**：LC-NA 神经元对抑制某些阿片类戒断症状起作用.

关键词 蓝斑；利血平；吗啡；二氢埃托啡；麻醉剂依赖性；物质撤除综合征