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Stimulation of locus coeruleus increases arterial pressure in rabbits

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KEY WORDS locus coeruleus; glutamates; GABA; morphine; sympathetic nervous system; blood pressure

AIM: To study the effect of electric and chemical stimulation of locus coeruleus (LC) on arterial pressure and renal sympathetic nerve discharge activity (RSA). **METHODS:** Electric stimulation of LC and microinjection of *L*-glutamate (*L*-Glu), morphine, and GABA into the LC of rabbits were made. The LC was destructed electrolytically. Arterial pressure and RSA were recorded. **RESULTS:** Both electric stimulation (150 μA, 50 Hz) of the LC and microinjection of *L*-Glu (0.5 μmol) into unilateral LC elicited increases in arterial pressure (13.5 ± 0.3 *vs* 19.5 ± 0.8 kPa, $P < 0.01$ and 13.8 ± 0.4 *vs* 17.5 ± 0.8 kPa, $P < 0.01$, respectively) and RSA (by 107 ± 14 %, $P < 0.01$, and 88 ± 21 %, $P < 0.01$, respectively). Microinjection of morphine or GABA did not induce any significant changes in the above two parameters. Electrolytic lesion of the LC eliminated the pressor response induced by microinjection of *L*-Glu. **CONCLUSION:** Excitation of LC has a pressor effect in rabbits, but LC is not a crucial nucleus in tonic regulation of blood pressure.

The locus coeruleus (LC), a collection of tightly packed catecholamine cells in the brain, has extensive efferent projections to many parts of the central nervous system[1]. Evidences implicate its global brain functions such as emotion and vigilance, and cardiovascular regulation[2]. Changes in blood volume, blood pressure (BP), or direct stimulation of baroreceptor afferent fibers affected the firing rate of neurons within LC[3-5]. The release of neurotransmitters in LC was also affected by cardiovascular manipulations[6,7]. While electric stimulation of LC increased BP in rats, cats, and rabbits, chemical stimulation of LC has presented different results from different laboratories in rats and cats[11-14]. No report appeared investigating the pressure effects of chemical stimulation of the LC in rabbits. There are some differences in structure and efferent projections of the LC between rats and rabbits[1]. Therefore, it is worthwhile to investigate the cardiovascular effect of chemical stimulation, as compared with electric stimulation, of the LC in rabbits.

MATERIALS AND METHODS

Rabbits ($n = 55$) of either sex weighing 2.0 - 3.0 kg were anesthetized with sodium pentobarbital 40 mg · kg^{-1} iv and maintained on positive pressure artificial respiration by tracheal cannulation. The head of rabbit was mounted on an SN-3 stereotaxic frame (Narashige, Japan). The dorsal surface of brain stem was exposed by removal of portions of the occipital bone and cerebellum. Rabbits were paralyzed with gallamine triethiodide 4 mg·kg^{-1} iv, initial dose. Rectal temperature was maintained at 39.0 ± 0.5 ℃ using infrared lamp.

Arterial blood pressure was recorded on a RJG-4002 recorder (Nihon Kohden, Japan) via an FY-2 pressure preamplifier and a CYS transducer (Chengdu Instrument & Apparatus Factory) connected to a catheter in femoral artery. One third of systolic pressure plus two thirds of diastolic pressure was calculated as mean arterial blood pressure (MAP). Left renal sympathetic nerve was isolated and prepared. The cut central ends of the nerves were placed on bipolar Teflon-coated platinum electrodes. Nerve signal was amplified, integrated, and recorded (RJG-4002) on VC-10 series (Nihon Kohden, Japan).

Electric stimulation (10 - 500 μA, 5 - 50 Hz, 20 s) on the LC was delivered through a coaxial bipolar stainless steel electrode (diameters of outer and inner electrode were 400 μm and 100 μm, respectively) with the tip separation of 250 μm. Electrolytic lesion of the LC was made by a coated monopolar electrode (200 μm in diameter) with the tip exposed 400 μm. DC current of 3 mA was passed through the inserted monopolar electrode for 30 s with the brain electrode as the anode. Drugs were injected into the LC through a concentric stainless steel cannula with an inner micropipette of 80 μm in diameter. *L*-Glu (sodium glutamate 1.0 mol·L^{-1}, pH 7.5 - 8.0, Sigma), GABA (50 g·L^{-1}, pH 5.0, Sigma), morphine chloride (20 g·L^{-1}, Sigma), or NaCl 1.5 mol·L^{-1} was injected into the LC

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at the speed of 1.0 $\mu L \cdot min^{-1}$ with intervals of 60 min. The LC was located by using the obex as surface landmark. According to the atlas of the rhombencephalon of rabbit[10], the stimulation electrode and the injection cannula were placed stereotaxically in the LC at A 9.5 – 10.5 mm, RL 1.8 – 2.0 mm, H 0 – 0.5 mm. At the end of experiment, stimulation and microinjection site was injected pontamine sky blue 1.0 $\mu L \cdot min^{-1}$. The brain stem was then frozen and sectioned (40 μm thick) with a cryostat. The site was projected onto the stereotaxic planes of the same atlas.

Data were expressed as $\bar{x} \pm s$. RSA and the highest MAP changes within 60 s after microinjection or during electric stimulation were compared with their control by paired t test.

RESULTS

Effects of electric stimulation of LC on MAP and RSA Electric stimulation of the LC elicited increases in MAP and RSA (Tab 1).

The rise in BP evoked by electric stimulation of the LC appeared gradually, reached a peak and gradually returned to the baseline upon cessation of the stimulus. The increase of RSA appeared 1 – 2 s earlier than the rise in BP. Following a quiescent period upon cessation of the stimulus, the RSA slowly returned towards its control level. The pressor response and increase in RSA increased with the increasing in stimulus intensity (50 Hz, 20 s, the threshold intensity was 30 μA) and stimulus frequency (150 μA, 20 s, the threshold frequency was 20 Hz).

Effects of chemical stimulation of LC on MAP and RSA Unilateral microinjection of *L*-Glu into the LC induced increases in MAP and RSA (Tab 1). The latency of the pressor response was 6 – 10 s followed by a 5 – 15-min hypertension. The increase of RSA also appeared 3 – 4 s earlier than pressor response, but the RSA returned to its baseline earlier than the BP. The rise in MAP and RSA increase to microinjection of *L*-Glu into the LC was dose-related. The smallest doses to elicit increases in MAP and RSA were 0.2 and 0.1 μmol, respectively (Fig 1).

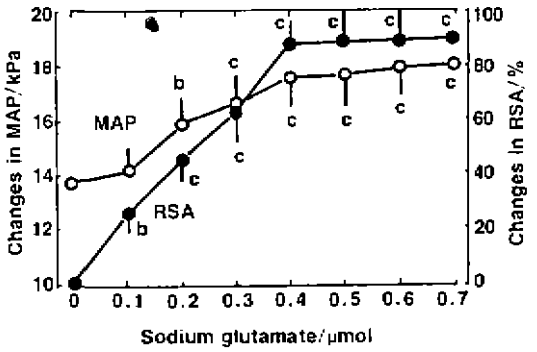

Fig 1. Pressor response and increase of renal sympathetic activity (RSA) after sodium glutamate injection into LC.
$n = 10$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Unilateral microinjection of GABA, morphine, or saline into the LC failed to elicit any significant alteration in MAP and RSA (Tab 1). Nevertheless, bilateral injection of GABA or morphine into the LC rendered the BP unstable.

Effects of LC lesions on responses to stimulation of LC The LC was electrolytically destructed via a DC anodal current (3 mA, 30 s). The BP became unstable, but did not show any significant unidirectional rise or decrease after destruction of the LC. Electric stimulation of the destruction site with 50 Hz and 150 μA, or 20 Hz and 200 μA, both of which evoked increases in MAP and RSA on LC intact rabbits, failed to elicit significant changes in MAP and RSA. But electric stimulation of the destructed LC site with 500 μA and 50 Hz elicited increases in MAP and RSA (Tab 2), although these

Tab 1. Effects of electric and chemical stimulations of the LC on mean arterial pressure (MAP) and renal sympathetic activity (RSA). $^aP > 0.05$, $^cP < 0.01$ *vs* control.

Treatment	Rabbits	MAP/kPa		(RSA change-control)/(RSA control)/%
		Control	Change	
Electric stimulation (150 μA, 50 Hz, 20 s)	18	13.5 ± 0.3	19.5 ± 0.8^c	107 ± 13^c
Sodium glutamate (0.5 μmol)	42	13.8 ± 0.4	17.5 ± 0.7^c	88 ± 21^c
GABA (25 μg)	10	13.6 ± 0.3	12.9 ± 1.8^a	10 ± 2^a
Morphine chloride (10 μg)	7	13.3 ± 0.4	12.0 ± 2.4^a	8 ± 2^a
NaCl (0.75 μmol)	5	13.5 ± 0.4	13.5 ± 0.1^a	5 ± 1^a

Tab 2. Effects of LC lesion on responses of mean arterial pressure (MAP) and renal sympathetic activity (RSA) to LC stimulation. $n = 15$. $^aP > 0.05$, $^cP < 0.01$ *vs* baseline.

Treatment	MAP/kPa		RSA (changed RSA/basline RSA), %	
	Pre-lesion	Post-lesion	Pre-lesion	Post-lesion
Electric stimulation (150 μA, 50 Hz, 20 s)	19.5 ± 0.5[c]	14.3 ± 0.7[a]	211 ± 21[c]	115 ± 17[a]
Electric stimulation (500 μA, 50 Hz, 20 s)	21.3 ± 1.6[c]	18.0 ± 0.9[c]	283 ± 35[c]	161 ± 21[c]
Sodium glutamate (0.5 μmol)	17.8 ± 0.7[c]	13.8 ± 0.3[a]	184 ± 18[c]	112 ± 10[a]

responses were much smaller than the responses induced on the LC intact rabbits. Microinjection of *L*-Glu into the destructed site failed to produce any changes in MAP and RSA.

DISCUSSION

L-Glu microinjection into LC elicited increases in MAP showing a dose related response, which suggested that activation of LC neurons had a pressor effect in rabbits. The responses induced by injection of *L*-Glu into LC were specific, since injection of saline had no detectable hemodynamic changes, and lesion of LC abolished the *L*-Glu induced effects also. Electric stimulation of LC induced a stronger pressor response than that of *L*-Glu stimulation, suggesting that activation of fibers in LC had a pressor effect too. This conclusion was similar to that reached on conscious and anesthetized rats[11,12]. As to our study, it is the first time to investigate the pressure effects of chemical stimulation of the LC neurons on pentobarbital-anesthetized rabbit. Results that stimulation of the LC elicited increase of RSA and the RSA response appeared earlier than the pressor response indicated that the pressor response to the LC stimulation at least partly resulted from the increase of RSA. However, a few laboratories[13,14] presented contradictory conclusion in rats and cats to our observations. On chloralose-anesthetized rats or cats, they found that *L*-Glu activation of LC elicited a depressor response but electric stimulation of LC induced a pressor response. It was difficult to explain these seemly discrepant results. Considering the differences in neuronal compositions and efferent projections in the nervous system of the LC among animal species, and that different anesthetics could cause the difference in experiment results, further deliberately designed experiments are needed to clarify whether these seemly discrepant conclusions were resulted from difference in animal species and/or difference in anesthetics.

Electrolytic destruction of the LC or microinjection of GABA or morphine, both of which were proved as neuroinhibitory agents to LC neurons[1], did not produce significant alteration in MAP except having BP more changeable. These showed that LC, unlike the rostral ventrolateral medulla, has no tonic effect on cardiovascular activity. However, LC did play an important role in the maintenance of the stability of BP. The results that a higher intensity electric stimulation of the destructed LC induced pressor response suggested actually that electric stimulation of structures around the LC could induce increase in BP. It is necessary to be cautious when using electric stimulation to study the role of discrete brain nuclei in physiological processes.

In conclusion, the present study indicated that either electric or chemical activation of the LC in rabbits produced increases of blood pressure and renal sympathetic activity.

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兔蓝斑兴奋引起动脉血压升高

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关键词 蓝斑; 谷氨酸; γ-氨基丁酸; 吗啡; 交感神经系统; 血压

目的: 研究电刺激和化学刺激兔蓝斑(LC)对动脉血压(AP)和肾交感神经传出活动(RSA)的影响. **方法**: 电刺激 LC, LC 微量注射 *L*-Glu、盐酸吗啡、GABA、电解毁损 LC, 记录 AP 和 RSA. **结果**: 电刺激 LC 和 LC 注射 *L*-Glu 均引起 AP 升高(分别为 13.5±0.3 *vs* 19.5±0.8 kPa 和 13.8±0.4 *vs* 17.5±0.8 kPa)和 RSA 增加. LC 注射吗啡、GABA 对 AP 和 RSA 无明显影响. 电解毁损 LC 后电刺激 LC 区、LC 区注射 *L*-Glu 对 AP 和 RSA 无明显影响. **结论**: 兔 LC 兴奋引起 AP 升高和 RSA 增加, 但 LC 不是 AP 和 RSA 的紧张性中枢.