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Cardiovascular and respiratory responses to microinjection of L-glutamate into the caudal pressor area in conscious and anesthetized rats

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Abstract

The role of the caudal pressor area (CPA) in the maintenance of vasomotor tonus in anesthetized and decerebrate animals has been clearly established. In conscious animals, however, the participation of CPA in the cardiovascular control remains to be fully elucidated. In the present study, unilateral L-glutamate (L-Glu) (10 and/or 20 nmol/70 nl) microinjection into CPA, in conscious male Wistar rats (250-280 g) caused a significant increase in mean arterial blood pressure (MAP; control: 112 ± 1.9 mmHg; after 20 nmol L-Glu: 139 ± 4.5 mmHg, N = 12, P<0.05) and respiratory rate (control: 81 ± 3.5 breaths/min; after 10 nmol L-Glu: 92 ± 3 breaths/min, P<0.05; after 20 nmol L-Glu: 104 ± 5 breaths/min, N = 6, P<0.05). The subsequent anesthesia with urethane caused a significant increase in basal respiratory frequency (conscious: 81 ± 3.5 breaths/min; under urethane: 107 ± 1.3 breaths/min, N = 6, P<0.05). Anesthesia also significantly attenuated L-Glu-evoked pressor (conscious: ΔMAP = +27 mmHg; anesthetized: ΔMAP = +18 mmHg, P<0.05) and respiratory responses. These results suggest that glutamatergic receptors in the CPA are involved in cardiovascular and respiratory modulation in conscious rats.

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Key words
- Caudal pressor area
- Ventrolateral medulla
- Blood pressure
- L-Glutamate
- Respiration

The importance of the ventral medulla in the control of the cardiovascular system has been clearly established in anesthetized animals. Two areas within the ventral medulla were initially identified as involved in this control, namely the rostral ventrolateral medulla (RVLM, which raises blood pressure when stimulated) and the caudal ventrolateral medulla (CVLM, which acts in an opposite way) (1-5). Subsequently, a third bulbar region called “caudal pressor area” (CPA) was identified (6,7). The CPA is situated between the rootlets of the 12th cranial and 1st cervical nerves (i.e., caudal to the CVLM) and its stimulation raises blood pressure, both in anesthetized (7) and decerebrate (8) rats. The responses to CPA stimulation in anesthetized animals are directly or indirectly mediated by RVLM neurons (8,9). Natarajan and Morrison (10) showed that

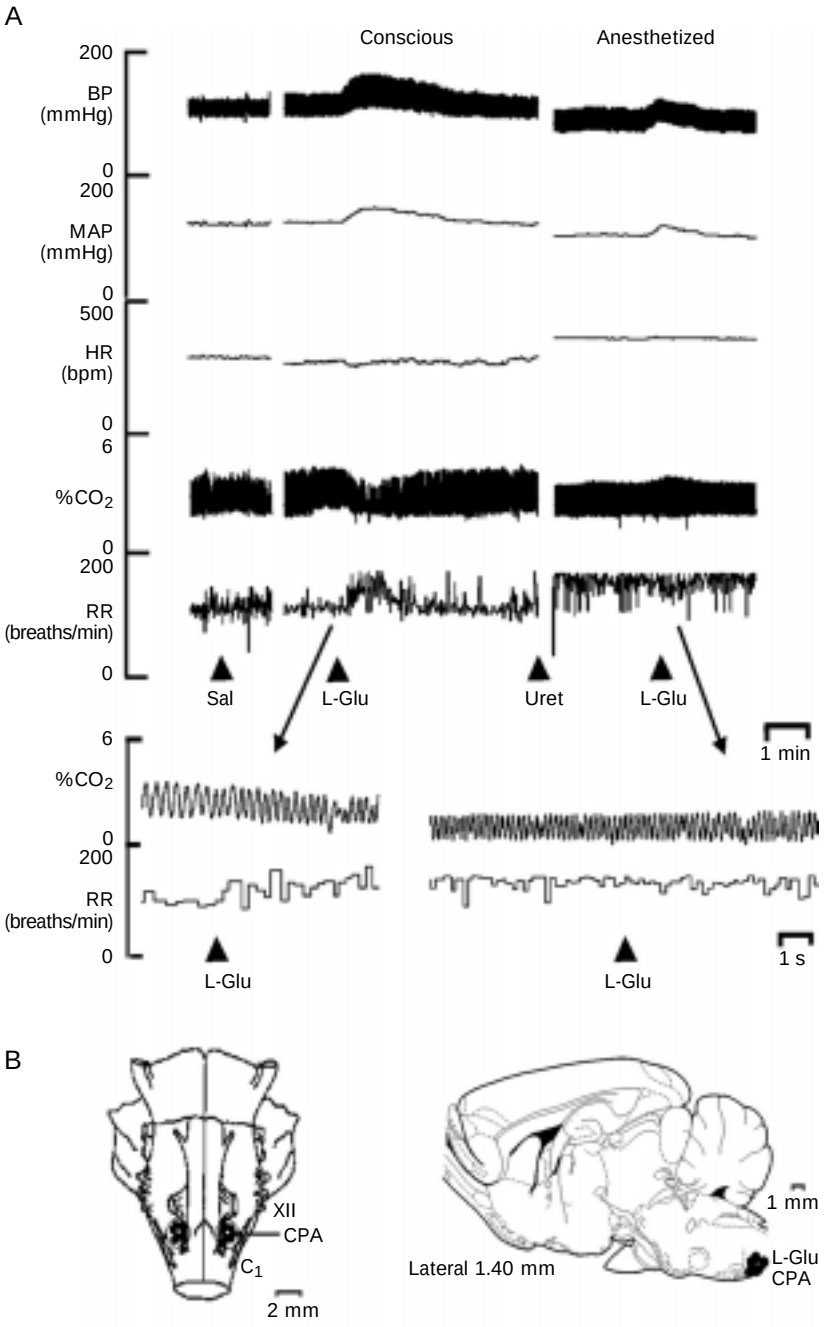

Figure 1. *A*, Traces from one rat comparing the effects of unilateral microinjection of saline (Sal) followed by L-glutamate (L-Glu; 20 nmol/70 nl) into the caudal pressor area (CPA) on blood pressure (BP), mean arterial blood pressure (MAP), heart rate (HR), expired CO_2 concentration (%CO_2) and respiratory rate (RR, breaths/min) before and 30 min after the administration (*iv*) of urethane (Uret, 1.2 g/kg). The arrows point to an expanded time scale (1 min to 1 s) of the recordings of %CO_2 and RR during the microinjections of glutamate. *B* shows the location of the above injection site and also that of four others.

CVLM glutamatergic neurons are an essential link in the functional projections from CPA to RVLM. However, there are few studies about the participation of the ventral medulla in the maintenance of the sympathetic vasomotor tone in conscious animals. In a recent study, it was suggested that not only the RVLM, but also other supraspinal premotor neurons, participate in the maintenance of vasomotor tone in conscious rats (11). The aim of the present study was to evaluate the cardiovascular effects of microinjection of L-glutamate (L-Glu) into the CPA in conscious and anesthetized rats.

Male Wistar rats (250-280 g) were anesthetized with chloral hydrate (0.5 g/kg, *ip*) and mounted on a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). The brainstem was exposed and bilateral stainless guide cannulae were directed to the CPA (1.4 mm lateral to midline, -11.8 mm AP to bregma; incision bar positioned at -11 mm; 27º inclination of the micromanipulator in relation to the vertical plane). After a recovery period of 3 to 4 days, the animals were anesthetized with ether and a heparinized saline-filled catheter (PE50) was inserted into the left femoral artery and vein and tunneled to exit at the back of the neck. A flexible silastic catheter was inserted into the trachea and also tunneled to exit at the back of the neck. Airflow in this catheter occurred only during the recording procedures. Twelve hours later, arterial blood pressure, mean arterial blood pressure (MAP), heart rate (HR), tidal CO_2 and respiratory rate (RR, derived from %CO_2 variation during the respiratory cycle) were recorded (Biopac MP 100) and stored on a PC hard disk for later processing.

After recording basal values, L-Glu (10 and 20 nmol/70 nl) or vehicle (0.9% NaCl/70 nl) with pontamine sky-blue (1%) was microinjected unilaterally within the CPA through a micropipette placed inside a guide cannula 6-8 mm shorter than the micropipette. Positioning of the micropipette was achieved by slowly lowering it into the guide

cannula until the tip of the micropipette touched the pia mater on the ventral medullary surface. Thirty minutes after microinjections the animals were anesthetized with urethane (1.2 g/kg) via the femoral vein cannula. Thirty minutes later the microinjection was repeated. During anesthesia, rectal temperature was maintained between 36-37ºC with a homeothermic heat blanket (Harvard Apparatus Co., South Natick, MA, USA). At the end of each experiment the animal was transcardially perfused with 200 ml 0.9% saline solution and fixed in 10% formaldehyde saline. The brain was removed and microinjection sites were identified and plotted on a standard diagram. The data are reported as means ± SEM. The paired *t*-test was used for statistical comparisons of the means and the criterion for statistical significance was $P<0.05$.

In conscious rats, unilateral L-Glu microinjections into the CPA caused dose-dependent increases in MAP, variable responses in HR and an increase in RR. In urethane (1.2 g/kg, *iv*)-anesthetized rats these responses to L-Glu microinjection into the CPA, although present, were markedly attenuated (Figure 1). As previously mentioned, L-Glu (20 nmol/70 nl) microinjection into the CPA in conscious rats caused a significant increase in MAP (control: 112 ± 1.9 mmHg; after L-Glu: 139 ± 4.5 mmHg, N = 12, $P<0.05$). These responses were significantly reduced (conscious: ΔMAP = +27 mmHg; anesthetized: ΔMAP = +18 mmHg, $P<0.01$) in anesthetized animals (control: 98 ± 3.6 mmHg; after L-Glu: 116 ± 3.2 mmHg, N = 8) (Figure 2A). Unilateral L-Glu microinjections also produced dose-dependent increases in RR (control: 81 ± 3.5 breaths/min; after 10 nmol L-Glu: 92 ± 3 breaths/min, $P<0.05$; after 20 nmol L-Glu: 104 ± 5 breaths/min, $P<0.05$, N = 6). The subsequent anesthesia with urethane significantly increased the RR (conscious: 81 ± 3.5 breaths/min; under urethane: 107 ± 1.3 breaths/min, N = 6, $P<0.05$), but L-Glu-dependent respiratory responses after anesthesia were attenuated (conscious: ΔRR = +22 ± 4 breaths/min; anesthetized: ΔRR = +2 ± 1 breaths/min, $P<0.01$) (Figure 2B).

The first report concerning the existence of a pressor area situated in the caudal portion of the ventral surface of the medulla

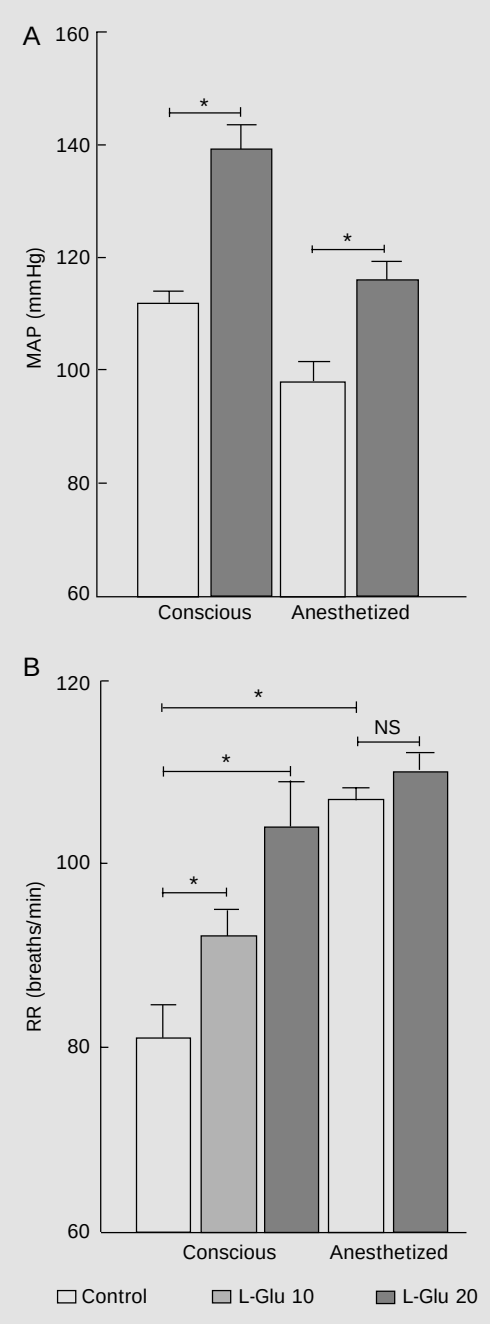

Figure 2. Effects of unilateral microinjections of glutamic acid (L-Glu, 10 and/or 20 nmol/70 nl) into the caudal pressor area on mean arterial blood pressure (A) and respiratory rate (B) in conscious and anesthetized rats. MAP = mean arterial pressure; RR = respiratory rate; NS = not significant. *$P<0.05$ compared to control rats (paired *t*-test).

suggested that the activity of this area was dependent on the level of anesthesia (6). With the subsequent development of new techniques for accessing the ventral surface of the medulla, it was shown that this region, thereafter called CPA, plays a role in the central cardiovascular control both in decerebrate and anesthetized animals (6,8,10). There is evidence that neurons from the CPA act via the RVLM, which in turn excites the preganglionic sympathetic neurons (5,9). In conscious animals, the vasomotor tonus is influenced by the activity of higher centers, whose effects upon the CPA are not fully understood (11). The present results showing a strong pressor effect after chemical stimulation of the CPA neurons in conscious rats, and an attenuation of these responses under general anesthesia, suggest that the CPA is more functionally active in conscious animals. However, since we did not measure the basal activity of CPA neurons, other possibilities cannot be ruled out, including the influence of urethane on other neuronal sites. With regard to the possible respiratory modulation exerted by neurons located in the CPA, it has been shown in anesthetized rats (12) that L-Glu microinjection elicits increases in RR, together with the pressor responses. Here we show that the same pattern can be obtained in conscious rats, and we suggest that this increase in RR is possibly due to connections with premotor neurons of the phrenic nerve, as previously shown (13,14).

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