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Sinoaortic denervation abolishes blood pressure-induced GABA release in the locus coeruleus of conscious rats

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Abstract

Male Sprague–Dawley rats underwent sinoaortic denervation (SAD) or sham operation. We examined changes in the release rates of GABA, glutamate and arginine in the locus coeruleus (LC) elicited by experimental blood pressure increases (i.v. noradrenaline infusion for 3 min, 4 μg kg^{-1} min^{-1}) or decreases (i.v. sodium nitroprusside infusion for 3 min, 150 μg kg^{-1} min^{-1}). The release of the neurotransmitters was monitored by the push–pull superfusion technique. Mean blood pressure did not differ between sham-operated and SAD rats but blood pressure lability was greatly enhanced in SAD rats and accompanied by increased basal release of glutamate in the LC. GABA release was not affected. A rise in blood pressure induced by noradrenaline enhanced GABA release in the LC of sham-operated rats. This effect was abolished by SAD. Glutamate release did not respond to hypertension either in SAD or in sham-operated rats. Nitroprusside led to a fall in blood pressure which was more pronounced and lasted longer in SAD than in sham-operated rats. In SAD rats, glutamate release was enhanced by nitroprusside. The depressor response had no effect on glutamate release in sham-operated rats. GABA release did not respond to this stimulus in either SAD or sham-operated rats. SAD and blood pressure changes did not influence the release rate of arginine. In conclusion, experimental hypertension increases GABAergic activity in the LC by stimulating peripheral baroreceptors. In SAD rats, augmented blood pressure lability seems to be at least partly due to elevated glutamate outflow within the LC.

Keywords: Sinoaortic denervation (SAD); Experimental blood pressure changes; Push–pull superfusion technique; GABA; Glutamate; Locus coeruleus

The pontine nucleus locus coeruleus (LC) is one of the brain areas known to be involved in cardiovascular regulation [6,16], antinociception [17], anxiety [20] and stress response [31]. This nucleus contains the major population of noradrenergic neurons in the brain with widespread projections throughout the central nervous system [5]. In several studies, changes in not only noradrenergic transmission within the LC [4,26,28,33,35] but also in transmission of neurons releasing modulating neurotransmitters like serotonin [4,23,25,29,31], glutamate [31] and GABA [30] have been investigated during experimental cardiovascular changes, and shown that these transmitters participate in central cardiovascular control.

By sinoaortic denervation (SAD), a transection of both the laryngeal nerve and the superior cervical ganglion is carried out, combined with a destruction of baroreceptors located at the internal and external carotid arteries. This procedure results in a nearly total interruption of the peripheral baroreceptor input to central brain structures [11]. Thus, information concerning peripheral blood pressure is no longer transmitted via baroreceptors to central brain structures and neurogenic hypertension is developed [11,14]. Therefore, SAD is a suitable method to explore the effects of peripheral baroreceptor impulses on neurotransmitter release rates in different brain areas contributing to blood pressure control.

To investigate whether cardiovascular changes influence the release rate of excitatory and inhibitory amino acids in the LC, experiments were carried out on conscious sham-operated and SAD rats in which the baroreceptors of carotid arteries were destroyed chemically. Sham-operated rats served as controls. The release of the amino acid arginine, which has no transmitter function, was also recorded for control purposes. The push–pull superfusion technique was used to monitor the changes in the release rate of glutamate and GABA in the LC during the application of pressor (nora-

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drenaline infusion) and depressor (nitroprusside infusion) stimuli.

Attention was paid to minimize pain and discomfort to the animals. Experiments have been conducted in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Male Sprague–Dawley rats, 10 to 12 weeks old (230–300 g), were housed in an environment controlled for temperature, humidity and light (12 h light/12 h dark cycle). Animals had free access to food and water. Under diethyl ether and ketamine (50 mg kg^{-1}; i.p.) anaesthesia, a cervical approach was used to dissect bilaterally the area of carotid bifurcation. Laryngeal nerve and superior cervical ganglion were transected and the common, internal and external carotid arteries were coated with an alcoholic solution of phenol (10%) keeping the vagus nerve intact [11]. Sham-operated rats received similar cervical incisions leaving nerves, vessels and baroreceptors intact.

Two days after SAD or sham operation, a guide cannula was stereotaxically inserted under ketamine (50 mg kg^{-1}; i.p.) and sodium pentobarbital (40 mg kg^{-1}; i.p.) anaesthesia until the tip of the guide cannula was 2 mm above the right LC, as previously described [26]. Briefly, the stereotaxic coordinates were (mm): antero-posterior (AP) 0.8 posterior to interaural line, lateral 1.3 from midline, dorso-ventral (DV) 2.8 above the interaural zero plane [15]. The guide cannula was fixed on the skull with stainless steel screws and dental cement. For recording of arterial blood pressure (Recommed Hellige, Freiburg, Germany) a PE 50 tubing was inserted into the iliac artery with its tip placed in the abdominal aorta. For intravenous infusions of drugs a PE 50 tubing was inserted into the jugular vein. Both catheters were tunnelled subcutaneously and the distal ends were exteriorized at the neck.

At least 5 days after the implantation surgery of the guide cannula, the stylet of the guide cannula was replaced by a push–pull cannula (diameters: outer needle o.d. 0.5 mm, i.d. 0.3 mm; inner needle o.d. 0.2 mm, i.d. 0.1 mm). The inner needle of the push–pull cannula was retracted 0.2 mm within the outer needle. The inner diameter (0.3 mm) of the outer needle confined the superfused area. Artificial cerebrospinal fluid (CSF) used for superfusion contained (mM): NaCl 140, KCl 3.0, $CaCl_2$ 1.3, $MgCl_2$ 1.0, Na_2HPO_4 1.0, glucose 3.0, and was adjusted to pH 7.2 with NaH_2PO_4 1.0. The LC was superfused at a rate of 28 μl min^{-1}; blood pressure and behaviour of the animals were monitored continuously during the experiment.

Superfusion of the LC of the conscious, freely moving animal with CSF started immediately after replacement of the stylet of the guide cannula by the push–pull cannula. After an equilibration period of 80 min, superfusates were collected in time periods of 3 min into tubes kept at −50 °C. The samples were stored at −80 °C until biochemical analysis was carried out. During the experiment, animals were deprived of food and water. At the end of the superfusion experiment, the brain was removed and the localization of the cannula was verified histologically. Experiments with cannula localizations outside the LC were discharged.

Changes in arterial blood pressure were elicited by intravenous infusion of noradrenaline (4 μg kg^{-1} min^{-1}) or sodium nitroprusside (150 μg kg^{-1} min^{-1}). To each animal three to four experimentally induced blood pressure changes were applied randomly. The time intervals between adjacent experiments were at least 60 min.

Amino acids released in the superfusate were determined by HPLC and fluorimetric detection (Merck-Hitachi, Tokyo, Japan) after derivatisation with *o*-phthaldialdehyde (OPA) as previously described [26]. Reproducibility of the derivatisation was controlled by addition of *S*-carboxymethyl-L-cysteine as an internal standard. Water was obtained from Milli-Q system (Millipore, Vienna, Austria), which guaranteed amino acid-free water quality. Results are presented as relative values (means ± S.E.M.). The mean release rates in the three samples preceding superfusion with drugs were taken as one. Data were analyzed statistically by Friedman's test followed by Wilcoxon's signed rank test for paired data. For comparison of basal release rates between sham and SAD rats data were analyzed by Mann–Whitney's *U*-test.

Lability is defined as the coefficient of variation (S.D./mean) of blood pressure values [1]. The standard deviation was calculated for each animal from 60 measurements carried out once per minute over a period of 1 h. The standard deviations from all animals are presented as mean values ± S.E.M. Statistical analysis was carried out after logarithmic transformation. For comparison of lability and basal blood pressure between sham-operated and SAD rats, Student's *t*-test for grouped data was used.

Chronic sinoaortic-denervated rats had a normal daily mean arterial blood pressure (Table 1), although the moment-to-moment variability of blood pressure (lability) was greatly enhanced. After reaching steady state, basal release rate of glutamate in the LC of SAD rats was significantly higher than in control animals (Table 1). On the other hand, basal GABA release rate was similar in sham-operated and SAD rats (Table 1). In sham-operated and SAD rats, i.v. infusion of saline for 3 min did not influence either the blood pressure or the release of

Table 1
Mean arterial blood pressure (BP) and basal release rates of amino acids in the locus coeruleus of sham-operated and SAD rats

Rat	*n*	Lability	BP (mmHg)	Release pmol min^{-1}		
				Arginine	Glutamate	GABA
Sham-operated	12	1.81 ± 0.17	110.5 ± 11.8	1.3 ± 1.6	1.5 ± 1.2	0.19 ± 0.17
SAD	6	9.16 ± 0.83**	106.6 ± 13.7	1.2 ± 1.4	3.9 ± 2.0**	0.31 ± 0.29

Values are presented as mean values ± S.E.M.
** $p < 0.01$ for intergroup comparison (sham-operated vs. SAD).