PHYSIOLOGY

Role of NMDA- and non-NMDA Subtypes of Glutamate Receptors in A5 Neuronal Structures in the Regulation of Respiration and Circulation during Thermal Nociceptive Stimulation in Rats

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In narcotized albino rats, thermal nociceptive stimulation elevated systemic blood pressure and increased the frequency of respiratory rhythm generation. Unilateral microinjection of ketamine hydrochloride, a selective blocker for NMDA receptors, into A5 region did not change the baseline parameters of multineuronal activity in the phrenic nerve and systemic blood pressure. Under conditions of NMDA-receptor blockade, thermal nociceptive stimulation evoked more pronounced respiratory response (in comparison to that observed before ketamine treatment), but induced smaller blood pressure rise. Unilateral microinjection of GAMS, a selective blocker for non-NMDA receptors, into A5 region did not modify the examined baseline parameters and the nociceptive response. It is concluded that during thermal nociceptive stimulation, activity of the respiratory center and blood pressure in rats are controlled by neuronal structures in A5 region via NMDA subtype of glutamate receptors.

Key Words: *A5 area; glutamate receptors; thermal nociceptive stimulus; phrenic nerve; blood pressure*

In mammals, neuronal structures in A5 region are involved in the regulation of various functions, including respiration, blood circulation, and nociception [4,8,9,11,12,15]. Glutamate receptors (GR) probably play a key role in these regulatory influences. Microinjection of L-glutamate in A5 region elevates systemic blood pressure (SBP) and simultaneously inhibits activity of the respiratory center via prolongation of the expiratory phase [7]. Activation of A5 region after L-glutamate microinjection inhibits thermal nociceptive reflex assessed by the tail flick response. Microiontophoresis revealed GR on membranes of noradrenergic A5 neurons [10].

We previously established the role of thermal nociceptive stimulation in the regulation of respiration and blood circulation, which involves the multireceptive A5 noradrenergic neurons [5]. These neurons are activated by hypoxic and thermal nociceptive stimuli and by inhibition of the central respiratory generator. Since noradrenergic A5 neurons are monosynaptically connected with spinal preganglionic sympathetic neurons and with neurons in caudal portion of the ventral respiratory group [14], it can be hypothesized that during thermal noci-

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ceptive stimulation the respiratory effect and SBP reactions involves glutamatergic A5 neurons.

Our aim was to study the role of NMDA- and non-NMDA glutamatergic A5 neurons in the regulation of respiration and SBP during thermal nociceptive stimulation.

MATERIALS AND METHODS

The study was carried out on laboratory albino rats (n=14) weighing 200-300 g. The rats were narcotized intraperitoneally with sodium etaminal (40 mg/kg). Body temperature was maintained at 37°C with a heater. Surgical approach to ventral surface of the brainstem was described elsewhere [1,2].

SBP was measured in the femoral artery via a catheter filled with physiological saline and heparin in proportion of 1000:1. The catheter was connected to a DMI-03 pressure transducer, an ID-2I amplifier, and a polygraph.

The phrenic nerve was isolated at the length of 4-6 mm and cut. The proximal stump was mounted on bipolar silver electrodes coupled to an AC amplifier. The signal was filtered within the range of 0.01-10.00 kHz. Amplified multineuronal activity of the phrenic nerve (MAPN) was recorded on a computer using an L-Card E14-440 digital converter (FBM Engineering) and PowerGraph software. The analyzed parameters were phrenic nerve firing duration, the length of respiratory pause between the bursts, and the total time of the respiratory circle. The amplitude of discharges was assessed by integrated activity of the phrenic nerve.

The thermal nociceptive stimuli were applied to the rat tail in the routine tail flick test. To this end, the tip of the tail (approximately 1/3 total length) was put into a temperature-stabilized cell filled with water (52°C). The thermal nociceptive stimulus evoked the nociceptive reflex (tail flick), thereafter the tail was placed into a temperature-stabilized air cell to restore skin temperature to 37°C [13].

Microinjections (50 nl, 1 min) into A5 area were made via a glass micropipette with a tip diameter of 20-30 μ using a nanoliter microinjector. The following agents dissolved in distilled water were used in the study: ketamine hydrochloride, a selective antagonist of NMDA glutamate receptors (4 mM, RBI) and GAMS (gamma-D-glutamylaminomethyl sulfonic acid, 4 mM, RBI), a selective antagonist for non-NMDA glutamate receptors. The interval between the injections was no less than 1 h.

The results were analyzed using ANOVA, Dunnett, and Tukey tests. The changes in the means were significant at p<0.05.

RESULTS

When applied before GR antagonists, the thermal nociceptive stimulus increased the discharge rate in the phrenic nerve without significant variation of the burst duration and amplitude. The duration of the respiratory circle decreased due to shortening of the expiratory phase from 0.92±0.02 to 0.68±0.01 sec (p<0.05, Fig. 1). The mean latency of the respiratory reaction was 2.3±0.2 sec, and the increase in duration of the respiratory activity was 13.4±1.1 sec (Fig. 2, *a*, *b*). The same series of experiments showed that thermal nociceptive stimulation provoked hypertensive response in intact rats: SBP increased by 22.5±0.8 mm Hg. The latency and duration of this reaction were 4.3±0.3 and 26.8±1.1 sec, respectively (Fig. 2, *a*).

Microinjection of ketamine hydrochloride, a selective antagonist of NMDA GR (n=8) had no effect on baseline MAPN. However, the thermal nociceptive stimulus induced more pronounced increase in MAPN under conditions of blockade NMDA GR in A5 region than in the intact rats (Fig. 1). The discharge rate in the phrenic nerve increased, the total time of respiratory cycle decreased, and the expiratory pause between the bursts shortened (Table 1). At the same time, the duration of discharges and their amplitude did not significantly differ from those in intact rats. There were no significant differences in the latency and duration of the respiratory reaction evoked by thermal nociceptive stimulus before and after blockade of NMDA GR in A5 region with ketamine.

Microinjection of ketamine hydrochloride into A5 region had no effect on baseline SBP, but it

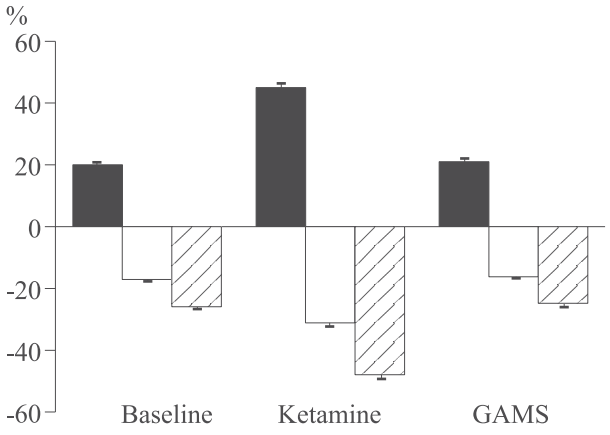

Fig. 1. Modulation of MAPN response to thermal nociceptive stimulation with ketamine hydrochloride or GAMS injected into A5 region. Closed, dashed, and open bars show the discharge rate in the phrenic nerve, total duration of respiratory circle, and duration of expiratory pause between the bursts, respectively. *p<0.05 compared to baseline values.

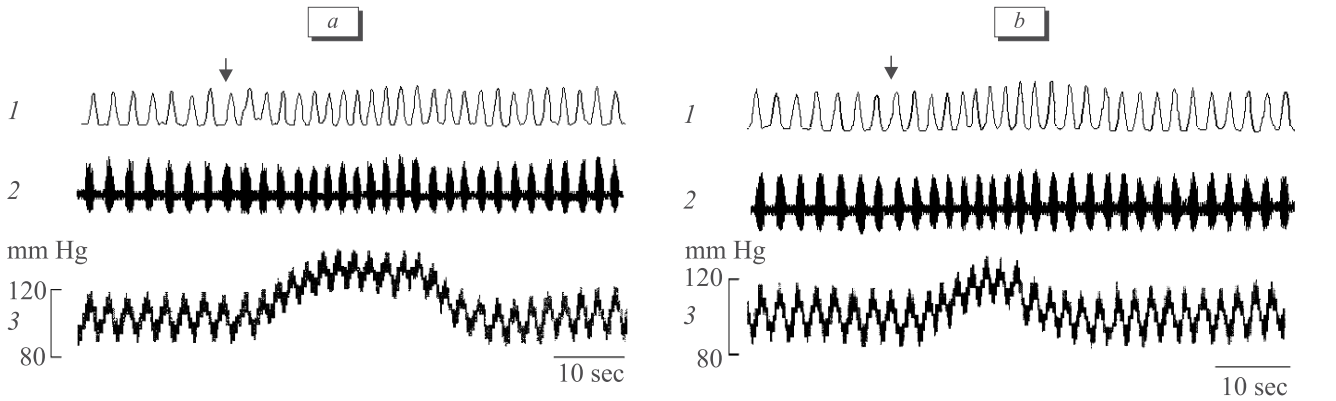

Fig. 2. Modulatory effect of ketamine hydrochloride (a selective blocker for NMDA GR) injected into A5 region on MAPN and SBP responses to thermal nociceptive stimulation: *a*) intact rats; *b*) experimental rats. *1*) integral activity in the phrenic nerve; *2*) MAPN; *3*) SBP. Arrow marks the start of thermal nociceptive stimulation.

modulated SBP response to thermal nociceptive stimulus. Specifically, the duration of hypertensive response decreased to 16.0±1.3 sec, while the increment in SBP was only 11.7±1.2 mm Hg, which was significantly smaller ($p<0.05$) than that in intact rats. Ketamine produced no significant effect on the latency of hypertensive response to thermal nociceptive stimulus, which was 4.1±0.4 sec in control and experimental rats (Fig. 2, *b*).

Blockade of non-NMDA GR after microinjection of GAMS-containing solution into theA5 region ($n=6$) did not affect the baseline parameters of MAPN and SBP. Moreover, it did not significantly modify changes in these parameters induced by thermal nociceptive stimulation (Table 1, Fig. 1).

Pontine A5 neurons are either directly involved into nociceptive regulation or can modulate nociception at the spinal level [6,14]. Blockade of NMDA GR in this region significantly moderated SBP response and enhanced MAPN response to thermal nociceptive stimulation. These data attest to involvement of A5 GR into regulation of respiration and circulation via the synaptic mechanism. Noradrenergic A5 neurons exert their regulatory action on respiration and circulation via monosynaptic connections with the spinal preganglionic sympathetic neurons and neurons in the caudal portion of the ventral respiration group [14]. Stimulation of thermal nociceptors initiate burst-like activity of multireceptive A5 neurons, which integrate not only the nociceptive signals, but also the afferent inputs from peripheral chemoreceptors and from neurons of the respiratory center [5]. We hypothesize that being the basic efferent neuron population in sympathetic SBP and respiratory center regulation at the brainstem level, these multireceptive A5 neurons regulate circulation and respiration, and NMDA GR are involved into this function. Probably, NO can play a modulatory role in the regulation of activation of multireceptive A5 neurons during nociceptive stimulation. This hypothesis is based on the fact that pontine multireceptive A5 neurons regulate peripheral chemoreflex via stimulation of NO synthesis. In particular, after blockade of NO-synthase by microinjection of L-NAME into A5 region in rats, hypoxia produces more pronounced respiratory and hypotensive reactions in comparison with those evoked before the blockade [3]. Thus, NMDA GR are involved in the synaptic mechanism of the regulation of SBP and respiration during thermal nociceptive stimulation.

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TABLE 1. Effect of Blockade of NMDA- and Non-NMDA Subtypes of GR in A5 Region on Changes in MAPN Parameters in Response to Thermal Nociceptive Stimulation ($M\pm m$)

Experimental conditions	1	2	3	4	5
Baseline	1.54±0.05	0.62±0.02	0.92±0.02	16.6±0.4	39.3±1.3
Control	1.28±0.04*	0.60±0.03	0.68±0.01*	16.8±0.3	47.2±1.4*
Blockade of NMDA GR	1.08±0.03*+	0.59±0.02	0.49±0.01*+	17.1±0.4	55.9±1.5*+
Blockade of non-NMDA GR	1.24±0.03*	0.62±0.03	0.35±0.01*	16.7±0.3	46.1±1.2*

Note. *1*) total duration of respiratory cycle (sec); *2*) burst duration (sec); *3*) length of expiratory pause between the bursts (sec); *4*) maximum discharge amplitude (relative units); *5*) discharge rate (min^{-1}). $p<0.05$ compared to *baseline and +intact rats.

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