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Integration of the Proprioceptive and Central Inspiratory Inhibitory Afferent Inputs by Pontine Noradrenergic A5 Neurons in Rats

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Inhibitory afferent inputs to pontine A5 noradrenergic neurons (A5 NN) are not known, except partial baroreceptor input. In spontaneously breathing pentobarbital-anesthetized rats, we registered 35 A5 NN that were activated by hypoxia (100% N_2, 10 sec) by more than 5 times in comparison with the background. Cooling of retrotrapezoid nucleus (15°C, 6 sec) completely blocked the motor inspiratory output and A5 NN discharge frequency increased (23/23) by more than 7 times in comparison with the background values. The beginning of A5 NN activation coincided with cessation of inspiratory activity. Short-term passive stretching of the shin muscles (1 sec, 100 g) caused BP drop and complete inhibition of A5 NN (12/12) activated by hypoxia. Inhibitory afferent inputs from proprioceptors and central inspiratory neurons that can limit A5 NN activity were demonstrated.

Key Words: *A5 noradrenergic neurons; proprioceptors; retrotrapezoid nucleus; hypoxia*

A5 noradrenergic neurons (A5 NN) are localized in the ventrocaudal pons [12,13] and are projected almost exclusively to the neurons of the lateral horns of the spinal cord [8]. A5 NN are involved in the regulation of respiration [1,4], blood circulation [3,7], sympathetic activity [13], nociception [5,7]. Most A5 NN are activated during hypoxic and nociceptive stimulation [4,5] and only slightly more than half of A5 NN is inhibited by increased BP [10]. At the same time, only the baroreceptor input is known from the inhibitory afferent inputs to the A5 NN. Activation of A5 NN in the post-respiratory and expiratory phases was revealed. However, it remains unclear which inputs (excitatory from postinspiratory and expiratory neurons or inhibitory from inspiratory neurons) modulate A5 NN activity. At the brainstem level, the somatosensory and baroreceptor afferent inputs are integrated in the nucleus of the solitary tract. This interaction consists in inhibition of baroreceptor neurons by somatosensory interneurons in this nucleus [9]. We hypothesized that somatosensory afferentation could be integrated by A5 NN.

We studied the effect of the proposed inhibitory inputs from inspiratory neurons and proprioceptors on A5 NN activity.

MATERIALS AND METHODS

The experiments were performed on 12 Wistar male rats weighing 260-320 g. The animals were anaesthetized with sodium pentobarbital (initial dose 40 mg/kg intraperitoneally and, if necessary, supplemented with 4 mg/kg intravenously), the trachea was intubated below the larynx. Animals breathed spontaneously with atmospheric air. The body temperature was measured rectally and maintained at 37°C with servoheater. The level of anesthesia was sufficient to eliminate pain reflexes. Right Achilles tendon together with a part of the calcaneal bone was isolated. The right lower limb was fixed using clamps attached to the tibial bone. Phrenic nerve was isolated and pontine ventral surface was opened. The approach was described in details earlier [2,6].

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Electrical activity of A5 neurons was measured extracellularly using glass microelectrodes filled with 2.5 M KCl (electrode resistance 5-10 MΩ). Phrenic nerve discharges (PND) were recorded via bipolar metal electrodes. Extracellular potentials and PND were amplified with a DL 314N-725 amplifier (Neurobiolab Company). PND amplitude was estimated from their integrated activity. BP was measured in the left femoral artery via a catheter connected to a small pressure transducer and a polygraph. The signals were processed and stored using an L-Card E14-440 analog-to-digital converter (FBM Engineering) and PowerGraph 3.3 software.

Peripheral chemoreceptors were stimulated by short-term hypoxia (breathing with 100% N_2 for 10 sec). For blockade of the inspiratory motor output, the ventral projection of the retrotrapezoid nucleus (RTN) was bilaterally cooled with a thermode (15°C, 6 sec): 1.5-3.5 mm rostral from nerve XII upper root and 1.2-2.2 mm lateral from the midline. To stretch the gastrocnemius and soleus muscle, the right Achilles tendon was tied with a silk thread to a force transducer and a block with 100-g weight standing on the platform. The platform was lowered and raised for 1 sec. Glass microelectrodes were positioned stereotaxically in the pontine A5 region according to the rat brain atlas [15]. The cells were assigned to A5 NN, if they fit the following criteria [12,13]: discharge frequency <5 Hz, duration of action potentials> 0.6 msec, strong activation by hypoxic stimulus and location of the recorded cell coordinates in the pontine A5 region. A5 NN were tested for RTN cooling or hypoxia-preactivated A5 NN were tested for muscles stretch. Each next stimulus was applied after a ~10-min pause, when activity of A5 NN, PND, and BP returned to the baseline values.

In case of normal data distribution (Kolmogorov—Smirnov test), paired *t*-test was used for comparison of PND, BP, and A5 NN activity parameters before and after stimulation. To compare the latency (LP) and the durations of BP, PND, and A5 NN responses, *t* test for independent variables was used. In other cases, the Wilcoxon test and Mann—Whitney tests were used. Changes in the mean values of each parameter were considered significant at $p<0.05$.

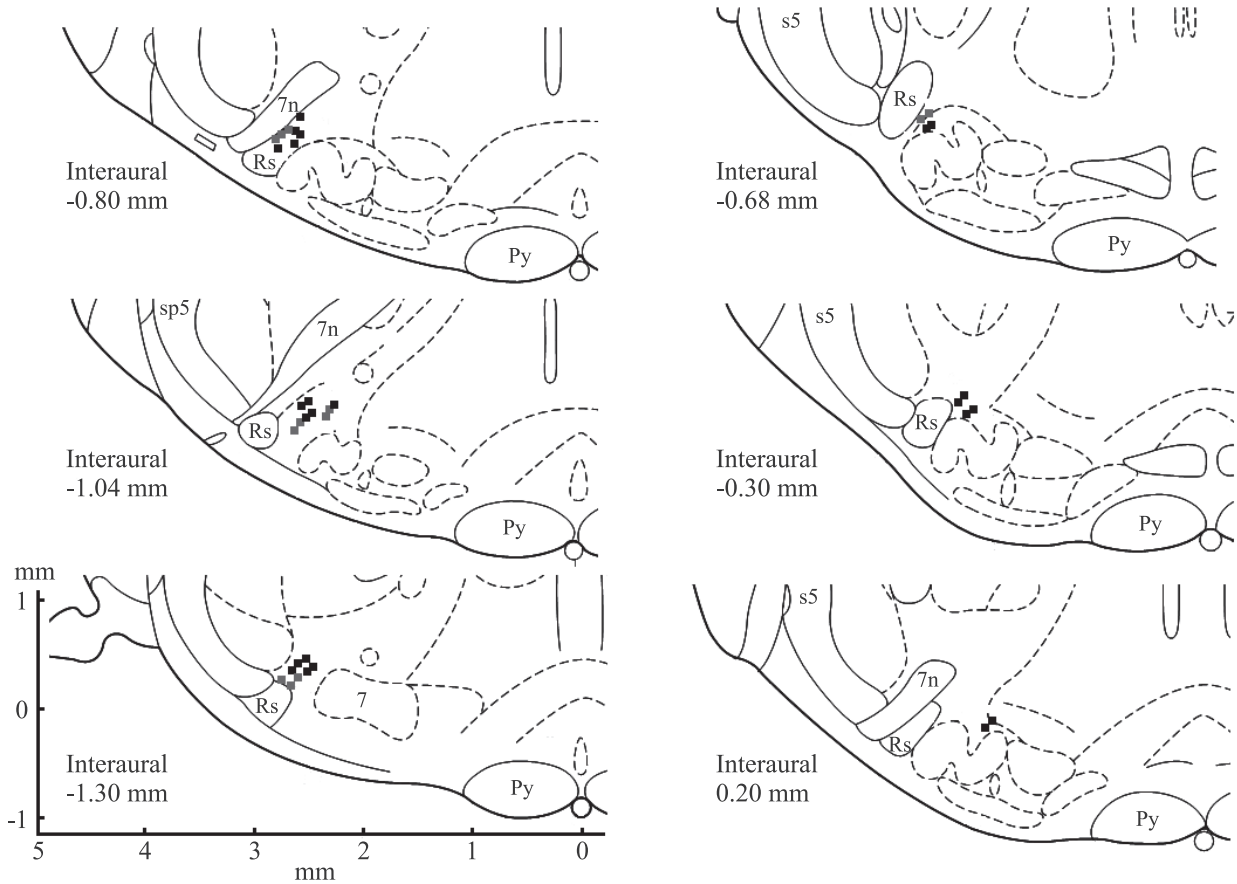

Fig. 1. Localization of registered A5 NN. Pontine cross sections were taken from the atlas of rat brain. Black squares are neurons tested for cooling of the supposed main central chemoreceptors of RTN (*n*=23), gray squares are neurons tested for short-term passive stretching of leg muscles (*n*=12). Py, pyramidal tract; 7, nucleus of the facial nerve; Rs, rubrospinal tract; 7n, root of the facial nerve; sp5, spinal cord of the trigeminal nerve; s5, sensitive spine of the trigeminal nerve.

RESULTS

In the pontine A5 region, we recorded 35 A5 NN with low regular discharge frequency (from 0.1 to 5 Hz), which were activated during hypoxia from 2.1±0.2 to 10.3±0.4 Hz (Fig. 1). Hypoxic stimulation increased the frequency and amplitude of PND. LP of A5 NN and PND reactions to hypoxia were 8.2±0.3 and 8.3±0.4 sec, respectively. Hypoxic stimulus also caused hypotension with LP 14.7±0.5 sec. During hypoxia, BP decreased from 107.8±0.5 to 78.3±0.7 mm Hg (in all cases $p<0.001$).

Cooling of RTN caused complete blockade of the motor inspiratory output (apnea without PND and without tension of inspiratory or expiratory muscles). LP from the beginning of cooling to apnea was 3.5±0.2 sec. During RTN cooling, 23 A5 NN responses were recorded; the discharge frequency increased from 1.8±0.4 to 13.3±0.4 Hz ($p<0.001$; Wilcoxon's test). LP of A5 NN reaction was 3.4±0.2 sec, which was significantly less than during hypoxia ($p<0.001$; Mann—Whitney *U* test). In all cases, the onset of the activation reaction of A5 NN coincided with the inspiratory motor output depression and respiratory arrest (Fig. 2). BP decreased from 106.5±0.6 to 91.0±2.5 mm Hg ($p<0.001$; Wilcoxon's test). LP of the hypotensive reaction was 6.4±0.2 sec. After the end of RTN cooling, BP, activity of A5 NN, and PND returned to baseline in 1-2 min.

Short-term passive muscle stretching caused an increase in the frequency of generation of PND in several respiratory cycles due to a decrease in expiration time and BP drop from 106.3±0.5 to 88.3±0.6 mm Hg ($p<0.001$). Twelve A5 NN previously activated by hypoxia were tested under conditions of passive muscle stretching. Somatic afferent stimulation caused a decrease in the discharge frequency in A5 NN under hypoxic conditions from 9.8±0.5 to 0.6±0.3 Hz ($p<0.01$; Wilcoxon's test). LP of A5 NN, PND, and AD reactions for passive muscle stretching were <1 sec (Fig. 3).

More than 10 years ago, neuron that gradually increased activity by ~2.5 Hz with increasing CO_2 content in the expired gas by 1% were detected on the ventral surface of medulla oblongata in the region of RTN (up to 250 μ in depth) [14]. In all previous experiments, neurons with much weaker dynamics (by one order of magnitude) of the reaction to CO_2 were found in different brain areas. The existence of the principal central chemoreceptors that activate the respiratory center upon changes in end-tidal CO_2 concentration by few tenths of percent was hypothesized. Then, coordinates of this region from the ventral surface of the brain were determined by using immunohistochemistry methods (we used these coordinates for cooling in our study). We have found that under conditions of pentobarbital anesthesia, cooling of RTN led to complete blockade of the motor inspira-

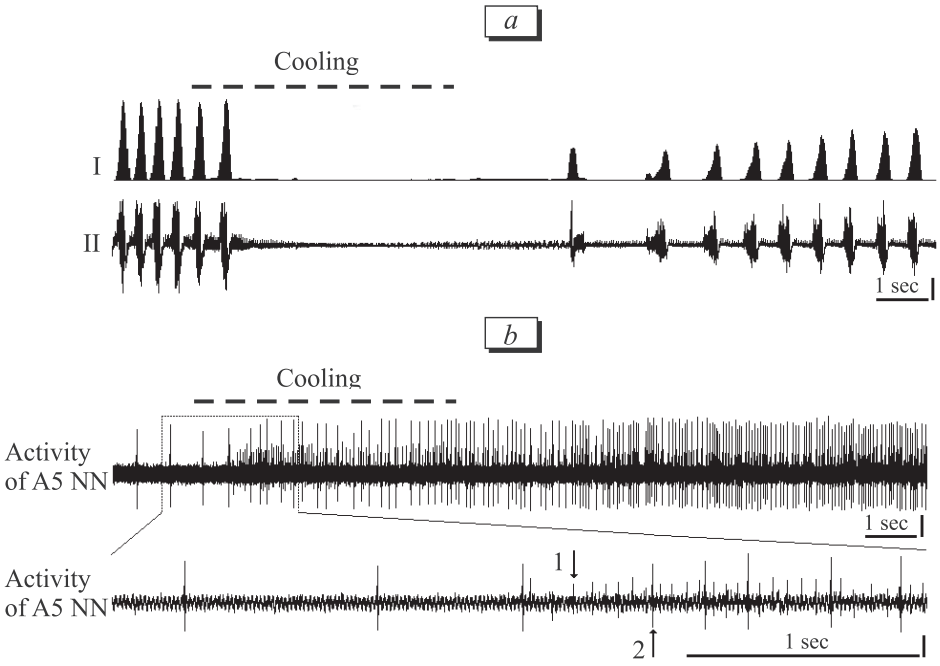

Fig. 2. Effect of cooling of the supposed main central chemoreceptors of the RTN on activity of A5 NN and PND. *a*) Complete blockade of the inspiratory motor output during cooling (15°C, 6 sec) of RTN structures. I, integrated PND, II, native PND. *b*) Increase in the frequency of discharges of A5 NN (2) during a cold apnea. The appearance of activity in another A5 NN (1) at the time of respiratory arrest. Dotted lines: duration of cooling. Vertical lines: 50 μV.

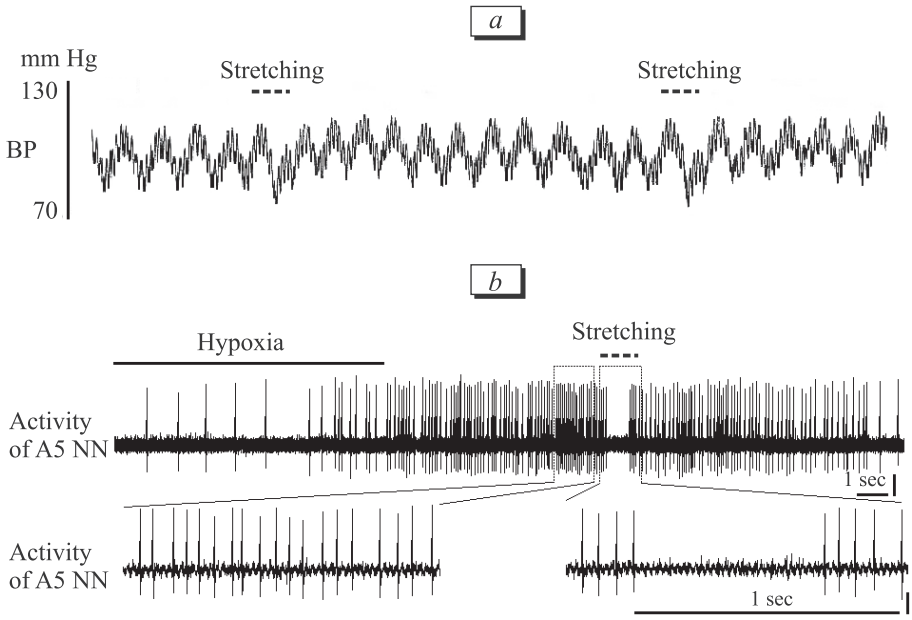

Fig. 3. Effect of somatic afferent stimulation on BP and activity of pontine A5 NN. *a*) Two consecutive episodes of BP lowering during short-term passive stretching (1 sec, 100 g) of the leg muscles; *b*) complete inhibition of hypoxia-activated A5 NN by passive stretching of the muscles. Duration of hypoxia is 10 sec. Dotted line: duration of stretching. Vertical line: 50 μV.

tory output (apnea without PND and without tension of inspiratory or expiratory muscles). Hence, activity of inspiratory and expiratory neurons is absent during apnea. This observation is important, because cooling of the rostral part of the ventral respiratory group causes apnea with tension of expiratory muscles and tonic activation of expiratory neurons. In this case, one cannot make unambiguous conclusions about the activating effect of expiratory neurons or inhibitory effect of inspiratory neurons on A5 NN by their activation during cooling. We found that the frequency of A5 NN discharges increased simultaneously with the onset of apnea and before the decrease of BP. LP of A5 NN reactions during hypoxic stimulation was several times greater than in case of cold apnea. This proves that initial activation of A5 NN is associated with termination of inhibitory influence from the inspiratory neurons of the respiratory center, and not with the influence of the baroreceptors and peripheral chemoreceptors.

Tension of skeletal muscles during electrical stimulation or physical exercises causes BP rise (exercise pressor reflex). It is shown that activation of the fine myelinated group III and not myelinated group IV muscle afferents [11]. There are two components of the reflex: mechanical (effect of muscle length change on receptive afferent fields) and metabolic (effect of substances released in the contracting skeletal muscle on receptors). In our experiments, we used passive stretching of the muscles, which has little effect on their metabolism. In addition, we used a relatively low level of stretching (in other experiments, force ≥5000 H was used) to minimize stimulation of nociceptors. In addition, muscle spindles are located along the muscle fiber, which determines their maximum sensitivity to stretching, unlike mechanoreceptors that are not oriented relative the myocytes. We concluded that somatic stimuli used in our study (short-term stretching for 1 sec, weight 100 g or a force of up to 1000 H) first activated thick myelinated afferent fibers of Ia and II group in muscle spindles. That was the main reason for BP decrease and inhibition of A5 NN. This phenomenon (we called it as stretch depressor reflex) can play an important role in limiting of BP increase and effect of exercise pressor reflex during intensive physical strain.

The experimentally confirmed properties of A5 NN are their sensitivity to hypoxia and pain [3,6]. However, just over half of A5 NN are activated by BP drop and inhibited by BP rise [10]. The absence of the baroreceptor afferent loop of regulation in some A5 NN can provoke their hyperactivity (*e.g.*, in chronic hypoxia or pain). At the same time, the inhibitory afferent inputs to A5 NN, except the partial baroreceptor, are not known. There are assumptions that hyperfunction of A5 NN can be the cause of the essential hypertension [10].

In our study, inhibitory inputs to A5 NN from proprioceptors and inspiratory neurons of the respiratory center, which can limit activity of A5 NN, are demonstrated. This observation suggests that breathing exercises aimed at inspiratory activity increase, as well

as passive stretching of the muscles, will cause BP drop, which can be used for medical purposes.

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