Control of Respiratory and Hypotensive Response during Hypoxic Chemoreflex by A5 Region Neurons in Rats

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The responses of A5 region neurons, the phrenic nerve, and systemic blood pressure to short-term hypoxia were examined in rats under conditions of spontaneous respiration. Tonic and respiration-modulated neurons increasing their discharge activity during hypoxia were identified. This hypoxia-induced response was more pronounced in the neurons with baseline discharge rate of 0.1-4.5 Hz (electrical activity of neurons increased by 4-5 times) compared to neurons with the baseline activity of 5.4-49.6 Hz (discharge rate increased by 1.4-2.0 times). The latency and duration of activation of all types A5 neurons correlated with the parameters of activation of the phrenic nerve. During hypoxia, activation of A5 neurons corresponded to the period of blood pressure drop (one-third of the reaction time), but not to the period of plateau or recovery phase. Low-, middle, and high-frequency A5 neurons participated in the modulation of hypoxia-provoked respiratory and hypotensive responses. Modulation of the respiratory response by A5 neurons was observed during the entire period of phrenic nerve activation, while modulation of the hypotensive response occurred only during blood pressure decrease.

Key Words: *A5 region; neuron activity; hypoxia; arterial pressure; phrenic nerve activity*

The respiratory and sympathetic responses induced by stimulation of peripheral chemoreceptors with a hypoxic stimulus are mediated by neuronal structures in A5 region of the caudal ventrolateral pons [3]. The hypoxic stimulus activates A5 neurons, while transection of the sinocarotid nerves abolishes this activation [6]. The degree of hypoxia-induced activation of A5 neurons is modulated by NO, because microinjection of NO synthesis blocker L-NAME into A5 region potentiates the respiratory response to hypoxia [3]. The synaptic mechanism underlying the interaction between A5 neurons and neurons of the respiratory center is unknown, although the existence of forward and backward synaptic connections between the pontine caudal ventrolateral subdivisions and neurons of the ventral respiratory group is hypothesized [5,8]. There are no data on the relationships between activity of A5 neurons and the kinetics of respiratory response to hypoxia. It was shown that NO in the A5 region moderates hypoxia-induced hypotension thereby exerting a modulatory influence on the mechanism of hypotensive reaction [3]. However, the relationships between the pattern of NO-modulated neuronal activity in A5 region and the parameters of hypotensive response to hypoxia are unknown.

Here we studied activation of pontine A5 neurons in relation to the pattern of phrenic nerve activation and blood pressure (BP) drop caused by short-term hypoxia.

MATERIALS AND METHODS

The experiments were carried out on pentobarbital-narcotized (40 mg/kg intraperitoneally) laboratory rats (n=16) weighing 200-300 g under conditions of spontaneous respiration. Body temperature was

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stabilized at 37°C. Ventral surface of the brainstem was opened from the midlevel of XII cranial nerves to the caudal pontine subdivision along the line passing 2-3 mm above the origin of VI cranial nerves and 4.0-4.5 mm laterally to the brain midline.

BP was measured in the femoral artery via a catheter connected to a DMI-03 pressure transducer, an ID-2I amplifier, and a digitizer. The signals were fed into PC and stored on a hard disk. Peripheral chemoreceptors were stimulated by a short-term hypoxia (10-sec breathing 100% N_2) [4]. The phrenic nerve was isolated over 4-6 mm, the distal end was cut and placed on bipolar silver electrodes connected to the recording equipment. Electrical activity of the phrenic nerve was also stored in PC. The neurograms were analyzed to determine the rate, duration, and amplitude of phrenic nerve discharges as well as the duration of expiratory phase and the total time of the respiratory cycle [2].

Electrical activity of A5 neurons was recorded extracellularly using microelectrodes filled with 2.5 M KCl (tip diameter <1 μ, resistance 5-10 MΩ). Using a floating micromanipulator, the microelectrode was introduced into the pontine caudal region (1.0 mm lateral to the origin of VI cranial nerves at the depth of 1.0-2.5 mm below the ventral surface of the brain). The neural firing was amplified and stored in PC. The corresponding neurograms were used to determine the discharge rate.

The data were presented as mean and standard deviation, the significance of differences were analyzed using Student's t test at $p<0.05$.

RESULTS

We examined activity of 36 neurons in pontine A5 region responding to short-term hypoxia by increased discharge rate. In previous experiments, noradrenergic cells were identified in the examined region, most of which responded to stimulation of the peripheral chemoreceptors during hypoxia [6]. We analyzed only A5 neurons increasing baseline activity in response to stimulation of the peripheral chemoreceptors during short-term hypoxia. The microelectrode study showed that these neurons had different patterns of baseline activity, and they responded in a similar way to the hypoxic stimulus (Table 1). In addition, there was one neuron with baseline discharge rate of 19.4 Hz and the hypoxia-induced response of 26.8 Hz. Two neurons had an extremely high baseline discharge rate of 49.6 Hz and the hypoxia-induced response of 71.0 Hz. Irrespective of the type of neurons, their activation was characterized by similar latency and duration of hypoxic response (Table 1, Figs. 1-3). Probably, this peculiarity indicates a single source of afferent signals to A5 region: peripheral chemoreceptors. The hypoxic response was greater in the low-frequency neurons, which increased their discharge rate by 4-5 times, then in the middle-frequency and the high-frequency neurons (n=2), which enhanced their activity by on average 1.4 times.

Latency and duration of hypoxia-induced activation of A5 neurons correlated with the latency of potentiation of phrenic nerve activity (7.1±0.3 sec) and with duration of its response (16.8±0.7 sec). The discharge rate of the phrenic nerve increased during short-term hypoxia from 37.1±0.7 to 64.5±1.8 min^{-1}. The duration of activation of low- and middle-frequency tonic A5 neurons surpassed the duration of stimulation of respiratory activity by 0.9-1.0 sec on average and virtually coincided with that of the respiratory-modulated cells. Based on the data [1,3], we can hypothesize that integrative neurons of the A5 region limits the increase in respiratory response during hypoxia and contribute to the posthypoxic recovery of baseline activity of the phrenic nerve.

The time relationships between A5 neuron response and the respiratory reaction differed from that in case of hypotensive reaction induced by short-term hypoxia. During hypoxia, the latency of activation of neurons of various types (Table 1) was shorter than that of hypotensive reaction by on average 5-6 sec, being equal to 14.6±0.9 sec. The hypotensive reaction to hypoxic stimulus lasted for 34.1±3.3 sec, which 2-fold surpassed the duration

TABLE 1. Responses Short-Term Hypoxia of Basic Types A5 Neurons ($M±m$)

Neuron type		Mean discharge rate, Hz	Parameters of neuron response		
			latency, sec	duration of reaction, sec	mean baseline discharge rate, Hz
Tonic	low-frequency (n=23)	2.17±0.44	8.15±0.86	17.76±1.56	10.19±1.06*
	middle-frequency (n=7)	8.51±1.75	7.85±1.01	17.85±1.18	16.42±2.80*
Respiration-modulated (n=4)		3.01±1.75	10.25±2.13	17.00±1.47	16.95±5.16*

Note. *$p<0.05$ compared to the baseline.

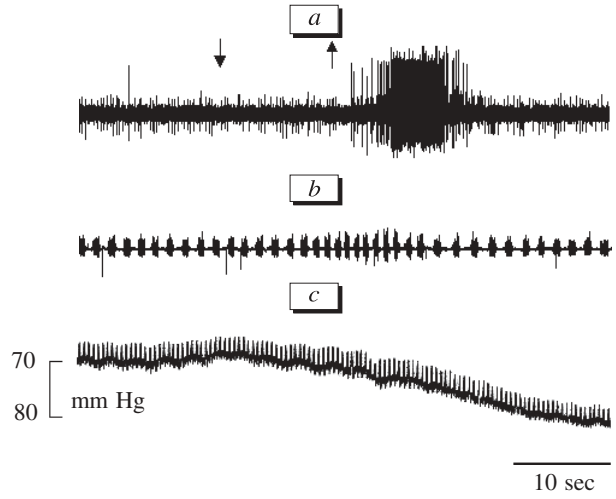

Fig. 1. Effect of a short-term hypoxia on activity of low-frequency A5 neuron (*a*), phrenic nerve firing (*b*), and systemic BP (*c*). Here and in Figs. 2, 3: the downward and upward arrows indicate onset and offset of N_2 breathing, respectively.

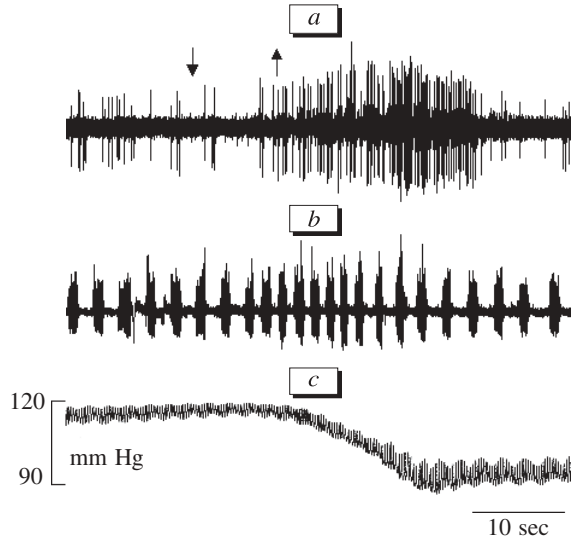

Fig. 2. Effect of short-term hypoxia on activity of a middle-frequency A5 neuron (*a*), discharges in phrenic nerve (*b*), and systemic BP (*c*).

of activation of A5 neurons of the examined types. In addition, hypoxia-induced activation of these neurons corresponded to the period of BP drop (one-third of the entire duration of the reaction), but not to the period of plateau or recovery of BP to the initial value. The short-term hypoxia decreased BP by 21.3±1.6 mm Hg compared to the baseline level (Figs. 1-3). It can be hypothesized that A5 neurons of various types that increase their discharge rate during peripheral chemical stimulation are involved in the central mechanism, which limits the degree of BP drop during hypoxia [1,3]. This central mechanism is effected via the monosynaptic connections of the noradrenergic neurons in

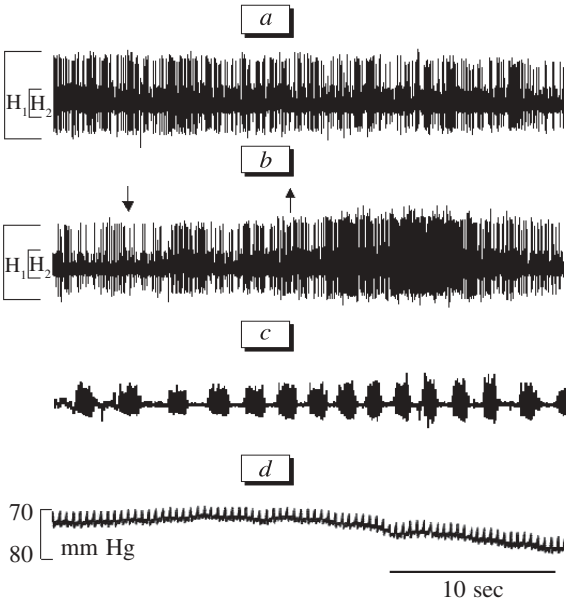

Fig. 3. The baseline activity of high-frequency A5 neurons (*a*) and the effect of short-term hypoxia on their firing (*b*), firing in the phrenic nerve (*c*), and systemic BP (*d*). Discharges of two A5 neurons (N1 and N2) were recorded simultaneously.

A5 region with the preganglionic sympathetic neurons [8]. A5 neurons modulate the hypotensive response during the hypoxic peripheral chemoreflex together with C1 neurons in the rostral ventrolateral subdivisions of the medulla oblongata [9] involved in baroreflex [7]. Thus, low-, middle-, and high-frequency pontine A5 neurons are involved in modulation of respiratory and hypotensive responses during hypoxic chemoreflex. The modulation of respiratory response is effected by these neurons during the entire period of phrenic nerve activation, while modulation of hypotensive response takes place only during drop of systemic BP.

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