PHYSIOLOGY

Nitric Oxide in the A5 Region Modulates Reaction of the Respiratory Center and Blood Pressure to Hypoxia in Rats

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Hypoxia was followed by more pronounced activation of the respiratory center and pronounced hypotensive response after unilateral injection of nitric oxide synthase blocker L-NAME into the A5 region. Microinjection of exogenous nitric oxide donor sodium nitroprusside into the A5 region abolished the effect of L-NAME on hypoxia-induced changes in activity of the respiratory center and blood pressure. Bilateral transection of the vagal and sinocarotid nerves suppressed the response of the respiratory center to hypoxia. However, the hypotensive response to hypoxia in these rats did not differ from that in intact animals. Under conditions of peripheral chemoreceptor deafferentation, the hypotensive response to hypoxia did not differ before and after blockade of nitric oxide synthase in the A5 region. The regulation of respiratory center activity and blood pressure during hypoxia was modulated by A5 neurons with the involvement of nitric oxide.

Key Words: *nitric oxide; A5 region; electrical activity of the diaphragmatic nerve; blood pressure; rat*

Hypoxia causes a variety of changes in the cardiovascular and respiratory system. Hypoxia induces relaxation of smooth muscles in arterioles, which is followed by a decrease in blood pressure (BP) in the systemic circulation. These changes are accompanied by a increase in pulmonary artery pressure and electrical activity of postganglionic sympathetic nerves [10]. BP and respiration during hypoxia are regulated by various neuronal structures in the medulla oblongata. The C1 region and solitary tract nucleus regulate the vascular [5] and respiratory response [9], respectively. These mechanisms are modulated by nitric oxide (NO) [9,10]. A5 neurons of the caudal ventrolateral pons are involved in the regulation of systemic hemodynamics and activity of the respiratory center. Activation is a typical reaction of A5 neurons to hypoxia [7]. Glutamate receptors and α_2-adrenoceptors are localized on membranes of A5 neurons [6]. A5 noradrenergic neurons send their axons to preganglionic sympathetic neurons of the spinal cord [8]. Activation of A5 neurons during glutamate microinjection inhibits activity of the respiratory center and increases systemic BP [6]. Immunocytochemical study showed that NO synthase concentration is high in the caudal ventrolateral pons [4]. These results suggest that NO is involved in the modulation of synaptic transmission in the A5 region during hypoxic regulation of blood flow and respiration. The present work was designed to test this hypothesis.

MATERIALS AND METHODS

Experiments were performed on 14 albino rats weighing 200-300 g. The animals were intraperitoneally narcotized with sodium ethaminal in a dose of 40 mg/kg.

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Body temperature was maintained at 37°C using a heating device. The ventral surface of the brainstem was opened from the level of cervical vertebra C1 to cranial nerve VI (4.0-4.5 mm lateral to the midline). The diaphragmatic nerve was prepared at a distance of 4-6 mm. The peripheral nerve segment was transected and put to bipolar sliver electrodes connected to an amplifier-integrator system [1]. Electrical activity of the diaphragmatic nerve was digitized and recorded. We analyzed the frequency, duration, and amplitude of diaphragmatic nerve discharge, period of the expiratory pause, and total duration of the respiratory cycle [2]. BP in the carotid artery was measured using a catheter filled with heparin and physiological saline in the 1:1000 ratio. The catheter was connected to a hardware module (DMI-03 compact pressure sensor, ID-2I amplifier, and automatic recorder). Hypoxia was produced by inspiration of 100% nitrogen for 10 sec [3].

Microinjections into the A5 region (50 nl) were performed using a glass micropipette with a tip diameter of 20-30 μ. Experiments were performed with an exogenous NO donor sodium nitroprusside (0.1 mmol/liter), endogenous NO donor L-arginine (NO precursor, 0.1 mmol/liter), its inactive form D-arginine (0.1 mmol/liter), NO synthase blocker N_ω-nitro-L-arginine methyl ester hydrochloride (L-NAME, 0.3 mmol/liter), and its inactive form D-NAME (RBI, Natick; 0.3 mmol/liter). These substances were dissolved in the artificial cerebrospinal fluid containing 124.0 mmol/liter NaCl, 5.0 mmol/liter KCl, 2.4 mmol/liter $CaCl_2$, 1.3 mmol/liter $MgSO_4$, 26.0 mmol/liter $NaHCO_3$, 1.2 mmol/liter KH_2PO_4, and 30.0 mmol/liter D-glucose. Inactive D-form or physiological saline served as the control. Then the animals received microinjections of active L-form.

The data are presented as means and standard errors. The significance of differences was estimated by Student's *t* test ($p<0.05$).

RESULTS

Electrical activity of the diaphragmatic nerve and BP were measured in intact animals under hypoxic conditions and after microinjection of the solution containing sodium nitroprusside, L-arginine, or L-NAME into the A5 region (n=14). Hypoxia was followed by an increase in the frequency and amplitude of diaphragmatic nerve discharge by 73.8±1.9 and 20.1±0.5%, respectively (Fig. 1, *a*). Hypoxia also shortened the duration of nerve discharge, period of the expiratory pause, and total time of the respiratory cycle by 10.6±0.3, 55.6±1.5, and 42.2±0.9%, respectively ($p<0.05$). The hypotensive response to hypoxia persisted for 32.9±3.0 sec. BP decreased by 23.6±1.6 mm Hg (Fig. 3, *a*). Microinjection of the test substances into the A5 region did not modulate baseline activity of the respiratory center and level of BP. The respiratory center response and changes in BP produced by hypoxia were studied in animals receiving microinjection of L-NAME into the A5 region (n=9). Blockade of NO synthesis in the A5 region modulated changes in respiratory center activity and BP produced by hypoxia (Fig. 2). The duration of hypoxia-induced hypotension increased to 57.3±3.7 sec (Fig. 3, *b*), while BP decreased by 39.7±1.7 mm Hg ($p<0.05$). Microinjection of exogenous NO donor sodium nitroprusside abolished the strong hypotensive response to hypoxia observed under the influence of L-NAME on A5 neurons. Microinjection of physiological saline or inactive isomer D-NAME had no effect on hypoxia-induced changes in activity of the respiratory center and level of BP.

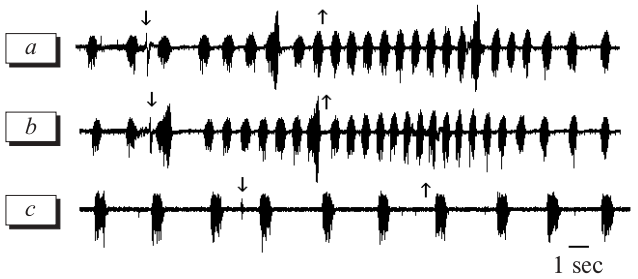

Fig. 1. Effect of hypoxia on electrical activity of the diaphragmatic nerve: intact animals (*a*); microinjection of L-NAME (50 nl, 0.03 mmol/liter) into the A5 region (*b*); bilateral transection of the sinocarotid and vagal nerves (*c*). Here and in Fig. 3: down arrow, start of hypoxia; up arrow, end of hypoxia.

Hypoxia was followed by greater activation of the respiratory center after blockade of NO synthase in the A5 region (compared to the hypoxia group, Table 1, Fig. 1, *b*). After microinjection of L-NAME into the

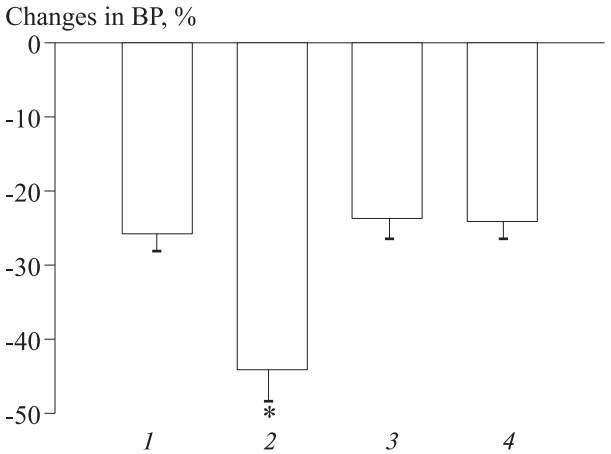

Fig. 2. Hypoxia-induced changes in blood pressure (% of the baseline level) in intact animals (*1*) and after blockade of NO synthase in the A5 region (*2*), bilateral transection of the sinocarotid and vagal nerves (*3*), and blockade of NO synthase and transection of the sinocarotid and vagal nerves (*4*). *$p<0.05$ compared to intact animals.

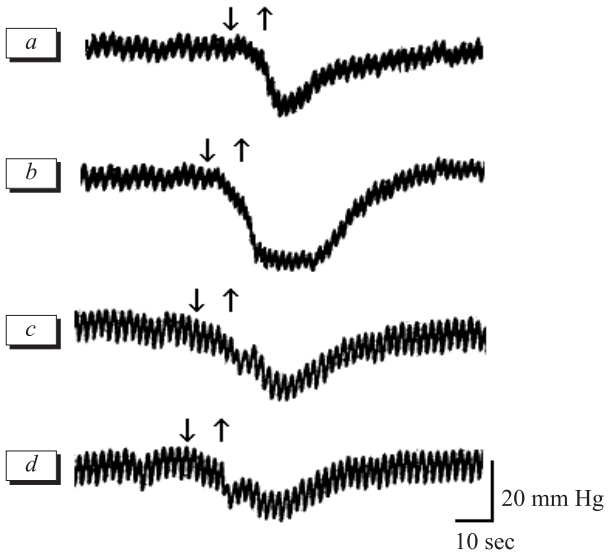

Fig. 3. Effect of hypoxia on blood pressure: intact animals (*a*); microinjection of L-NAME (50 nl, 0.03 mmol/liter) into the A5 region (*b*); bilateral transection of the sinocarotid and vagal nerves (*c*); bilateral transection of the sinocarotid and vagal nerves and microinjection of L-NAME into the A5 region (*d*).

A5 region, the frequency of diaphragmatic nerve discharge in response to hypoxia increased by 105.1± 2.6%, total time of the respiratory cycle decreased by 50.9±1.1%, and interspike expiratory pause was shortened by 69.5±1.7% ($p<0.05$). It should be emphasized that the decrease in the duration and increase in the amplitude of diaphragmatic nerve discharge in treated rats did not differ from those in intact animals (Table 1). The respiratory effect of hypoxia was abolished after microinjection of the solution with sodium nitroprusside into the A5 region.

Deafferentation of peripheral chemoreceptors by bilateral transection of the sinocarotid and vagal nerves (n=6) abolished the respiratory center response to hypoxia (Fig. 1, *c*). However, the hypotensive response in treated rats did not differ from that in intact animals (Fig. 3, *c*). Under conditions of peripheral chemoreceptor deafferentation, the hypotensive responses to hypoxia before and after blockade of NO synthase in the A5 region were similar (Fig. 3, *d*).

Stimulation of peripheral chemoreceptors with the hypoxic factor is followed by activation of NO synthesis in A5 neurons. Previous studies revealed a direct relationship between the solitary tract nucleus and A5 region [8]. It can be hypothesized that activation of neuronal terminals projecting from the solitary tract nucleus to A5 region initiates NO synthesis in postsynaptic A5 neurons. NO synthesis potentiates synaptic transmission in synapses (probably glutamatergic synapses) of A5 neurons, which increases their activity during hypoxia [7].

After activation of NO synthesis, A5 neurons modulate the respiratory response to hypoxia by suppressing activity of the respiratory center. The effect of A5 structures on activity of the respiratory center can be realized via the Betzinger complex, which contains axonal projections of A5 neurons. In the solitary tract nucleus (level of the medulla oblongata) NO increases the respiratory center response to hypoxia [9]. NO modulates the regulation of respiration during hypoxia by affecting the solitary tract nucleus and structures of the A5 region. The modulatory effect of NO on A5 neurons manifested in decreased hypotensive response to hypoxia. Microinjection of L-NAME into the A5 region increases the degree and duration of the hypotensive response to hypoxia under conditions of NO synthase blockade. NO increases the hypotensive response to hypoxia, which is realized via the rostral ventrolateral medulla (C1 region) [10]. Our results and published data indicate that apart from the C1 region of the brainstem, NO in A5 neurons modulates BP regulation under hypoxic conditions.

These data show that NO serves as a modulator of the relay function in A5 neurons involved in the regulation of respiratory center activity and BP in experimental animals during hypoxia.

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TABLE 1. Electrical Activity of the Diaphragmatic Nerve during Hypoxia in Intact Animals and under Conditions of NO Synthase Blockade in the A5 Region ($M\pm m$)

Experimental conditions	Electrical activity of the diaphragmatic nerve				
	T_t, sec	T_i, sec	T_e, sec	DA, rel. units	DF, 1/min
Baseline level	1.61±0.03	0.47±0.01	1.15±0.02	16.6±0.3	37.1±0.7
Intact animals	0.93±0.02*	0.42±0.01*	0.50±0.02*	20.1±0.5*	64.5±1.8*
NO synthase blockade	0.79±0.02*+	0.43±0.01*	0.35±0.01*+	20.1±0.4*	76.1±2.3*+

Note. T_t, total time of the respiratory cycle; T_i, duration of diaphragmatic nerve discharge; T_e, duration of the interspike expiratory pause; DA, maximum discharge amplitude; DF, discharge frequency. $p<0.05$ *compared to the baseline level; +compared to intact animals.

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