Involvement of Rostral Ventromedullar Neuronal Structures in Nitric Oxide Modulation of Central Chemosensitive Drive

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 5, pp. 489-491, May, 2003
Original article submitted October 16, 2002

Surface perfusion of the rostral ventromedullar cerebral subdivisions with artificial cerebrospinal fluid containing exogenous NO donor sodium nitroprusside (0.1 mM) increased the discharge rate of the phrenic nerve and potentiated the response of the respiratory center to hypercapnia in narcotized mature rats. The latter reaction was prevented by blockage of NO-synthase in rostral ventromedullar neural structures with N^{ω}-nitro-L-arginine methyl ester (L-NAME, 0.3 mM). It was hypothesized that rostral ventromedullar neural structures are involved in modulatory action of NO on central chemosensitive drive.

Key Words: *rostral ventromedullar structures; nitric oxide; hypercapnia; rat*

In rats and cats, electrical stimulation of subpial rostral ventromedullar structures (RVMS) stimulates generation of the respiratory rhythm [1,6], while destruction of some RVMS structures (rostral part of ventral respiratory group, subretrofascial area, and retrotrapezoid nuclei) produces hyperventilation and moderates the response of the respiratory center to hypercapnia [4]. Glutamatergic synapses in RVMS involved in integration of the central chemoreceptor signals [3] can participate in these effects. This hypothesis is corroborated by the fact that the blockade of glutamatergic synapses in this cerebral region inhibits central chemosensitivity of respiration. NO modulates transmitter secretion in these synapses [5] and the response of the respiratory center to hypercapnia [2,7]. Our aim was to study the role of NO in the regulation of central chemosensitive drive involving RMVS structures.

MATERIALS AND METHODS

Experiments were carried out on spontaneously breathing albino Wistar male and female rats (n=17) weighing 200-300 g. The animals were intraperitoneally narcotized with sodium etaminal (40 mg/kg). Narcosis was controlled by corneal reflex and respiration rate. Body temperature was measured with a rectal thermometer and maintained with a heater at 37°C.

The response of the respiratory center to hypercapnia was assessed by activity of the phrenic nerve. The amplitude, discharge rate, and duration of respiratory burst were calculated from 10 successive cycles. To this end, the right phrenic nerve was isolated to the length of 4-6 mm and cut. The proximal end was mounted on bipolar silver electrodes and covered with warm mineral oil. Electrical activity was amplified, filtered from 2 Hz to 5 kHz, and fed to computer via a digitizer for storing or monitoring. The ventral surface of the medulla oblongata (from the middle part of nerve XII roots to the emergence of cranial nerves VII-VIII and 4.0-4.5 mm laterally from the midline) was exposed.

The following agents were used: sodium nitroprusside (NO donor, 0.1 mM); L-arginine (NO precursor, 0.1 mM); and L-NAME (NO-synthase blocker, 0.3 mM). Inactive isomers were used as the controls: D-arginine (0.1 mM) and D-NAME (0.3 mM); they were obtained from RBI, Natick, and MA. The chemicals were dissolved in artificial cerebrospinal fluid

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0007-4888/03/1355-0413$25.00

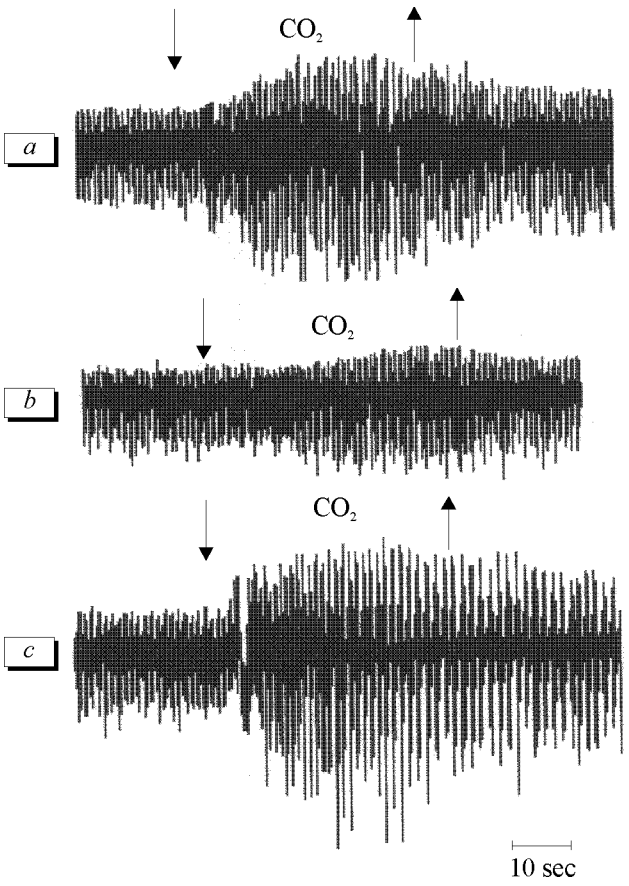

Fig. 1.Chances in electrical activity of the phrenic nerve in response to hypercapcic stimulation after modulation of NO content in the rostral ventromedullary structures (RVMS). *a*) intact rats; *b*) RVMS perfusion with artificial cerebrospinal fluid containing NO-synthase blocker L-NAME (0.3 mM); *c*) RVMS perfusion with exogenous NO donor sodium nitroprusside (0.1 mM). Up and down arrows mark the onset and cessation of hypercapnia, respectively.

containing (in mM): 124.0 NaCl, 5.0 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 26.0 $NaHCO_3$, 1.2 KH_2PO_4, and 30.0 d-glucose. The rate of perfusion was 1 ml/min.

Hypercapnia was produced by breathing 7% CO_2+ 93% O_2 gas mixture. The concentration of CO_2 in the mixture was controlled using a GAU-5 gas analyzer.

The data were processed statistically using Student's *t* test.

RESULTS

Perfusion of RVMS with artificial cerebrospinal fluid containing exogenous NO donor sodium nitroprusside produced maximum changes in electrical activity of the phrenic nerve after 2-3 min, which then persisted for the entire period of chemical stimulation. The discharge rate of respiratory firing increased from 35.7± 1.7 to 40.5±1.9 min^{-1}, while the discharge amplitude decreased by 10.8±4.6% in comparison with the baseline. The latency of the respiratory response to perfusion of RVMS with L-NAME was 10 min. The discharge rate in the phrenic nerve decreased from 37.9± 2.7 to 32±2.6 min^{-1} without significant changes in the amplitude and duration of respiratory burst. Perfusion of RVMS with L-arginine (endogenous NO donor), its inactive isomer D-arginine, and D-NAME produced no effect on the baseline activity of the phrenic nerve.

The response of the respiratory center to hypercapnia was examined in intact rats (control group) and in rats subjected to RVMS perfusion with artificial cerebrospinal fluid for 10 min. This time was chosen after preliminary tests showing that stable responses of phrenic firing pattern were formed during this time. Perfusion of RVMS with exogenous NO donor potentiated the hypercapnia response of the respiratory center: the amplitude of phrenic nerve bursts increased by 58.1±21.6% (Fig. 1, *a*) and the discharge rate increased by 10.1±1.3 min^{-1} (Fig. 2, *1*). Sodium nitroprusside increased these parameters by 83.3±16.8% (Fig. 1, *c*) and 16.6±1.8 min^{-1}, respectively (Fig. 2, *2*). By contrast, L-arginine (Fig. 2, *3*) and its isomer D-arginine (Fig. 2, *4*) produced no significant effect on the response of respiratory center to hypercapnia.

Other experiments showed that NO-synthase blockade in RVMS with L-NAME decreased the response of the respiratory center to hypercapnia. In the control group hypercapnia increased the amplitude of electrical activity of the phrenic nerve and the rate of respiratory discharges by 60.8±17.8% and 9.8±0.9 min^{-1}, respectively, while the same stimulation during perfusion of RVMS with L-NAME increased the corresponding parameters only by 25.3±9.2% and 2.0±0.8 min^{-1} (Fig. 2, *5*). There were no significant changes in the response of the phrenia nerve to hypercapnia

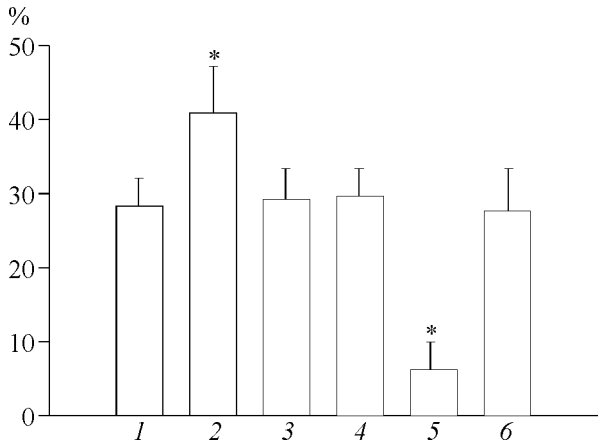

Fig. 2. Dynamics of phrenic nerve firing rate in response to hypercapnia stimulation during modulation of NO level in RVMS. *1*) intact rats; *2*) 10-min perfusion of RVMS with artificial cerebrospinal fluid containing sodium nitroprusside (0.1 mM); *3*) L-arginine (0.1 mM); *4*) D-arginine (0.1 mM); *5*) L-NAME (0.3 mM); and D-NAME (0.3 mM). *$p<0.05$ compared to intact rats.

before and after perfusion of RVMS with artificial cerebrospinal fluid containing D-NAME (Fig. 2, *6*).

This study showed that the increase in NO content in RVMS potentiates, while NO-synthase blockade moderates the response of the respiratory center to hypercapnia, which indicates involvement of exogenous NO in modulation of central chemosensitive drive in RVMS neurons. These neurons can be located in the rostral subdivision of the ventral respiratory group, subretrofascial area, and retrotrapezoid nuclei, and they can be involved in the integration of signals from central chemoreceptors [4]. Taking into consideration NO potency to modulate neurotransmitter secretion in glutamatergic synapses [5] and our findings, we can hypothesize that NO plays a similar role in synapses of neural clusters in RVMS, which control chemosensitivity of the respiratory center.

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