Role of NO-ergic Mechanism in Regulation of Rhythmogenesis in Respiratory Center in Bulbospinal Preparation from Newborn Rat

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In vitro experiments on bulbospinal preparations isolated from newborn rats (postnatal days 0-4) demonstrated that exogenous and endogenous NO can modulate respiratory rhythm generation and change various spectral parameters of respiratory discharges.

Key Words: *nitric oxide*; *respiratory rhythm genesis*; *newborn rat*

It was recently established that nitric oxide (NO) is involved in central mechanisms of respiratory control. Selective inhibitor of neuronal NO synthase (NOS) diminished the respiratory response to hypoxic stimulation in nonanesthetized adult rats [1]. Similar inhibition of the respiratory response to hypoxia was observed in freely moving rats after microinjection of NOS inhibitor into the nucleus tractus solitarius, whereas microinjection of NO-donor into the same region enhanced the hypoxic response [7]. It was assumed that NO acts as a retrograde messenger in glutamatergic positive feedback system, which is involved in the regulation of the respiratory response to hypoxia [7]. NO can also plays a role in plasticity of the respiratory control in newborn rats [2]. However, the role of NO in the mechanisms of respiratory rhythm genesis in newborns is little studied. Our aim was to study the effects of exogenous and endogenous NO on the generation of respiratory discharges and spectral parameters of respiratory burst activity in bulbospinal preparations (BSP) isolated from newborn rats.

MATERIALS AND METHODS

Experiments were carried out on BSP (n=35) isolated from newborn rats (postnatal day 0-4). The rats were anesthetized with ether, craniotomy and laminectomy were carried out to expose the brain and cervical part of the spinal cord. The brain stem was cut at the intercollicular level, and the bulbospinal part of the brain and C_{III}-C_{IV} ventral roots were isolated. The preparations were constantly perfused with cold (7°C) artificial cerebrospinal fluid [10] 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 (pH 7.3-7.4), continuously bubbled with carbogen (5% CO_2+95% O_2). After the isolation procedure was completed, the perfusate was gradually heated to 24-25°C, and the preparation was transferred into a thermocontrolled flow chamber (3 ml volume) perfused with artificial cerebrospinal fluid (24-25°C, 2-3 ml/min perfusion rate).

Electrical activity in C_{III}-C_{IV} ventral roots was recorded using a suction electrode (inner diameter 100-150 μ), amplified, and fed to a computer.

NO donor sodium nitroprusside (SNP), NO precursor L-arginine (200 and 300 μM), and NO synthase inhibitor N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME, 200 μM, RBI) were used. All substances were diluted in artificial cerebrospinal fluid.

Analysis of neurograms included determination of the respiratory activity period and the duration and amplitude of respiratory bursts (RB). The frequency components of RB were evaluated by spectral analysis. The spectral power density was calculated using 1024-point fast Furrier transform (500 Hz sampling rate). Spectral parameters were calculated from 10 consecutive RB. The data are presented as means and standard er-

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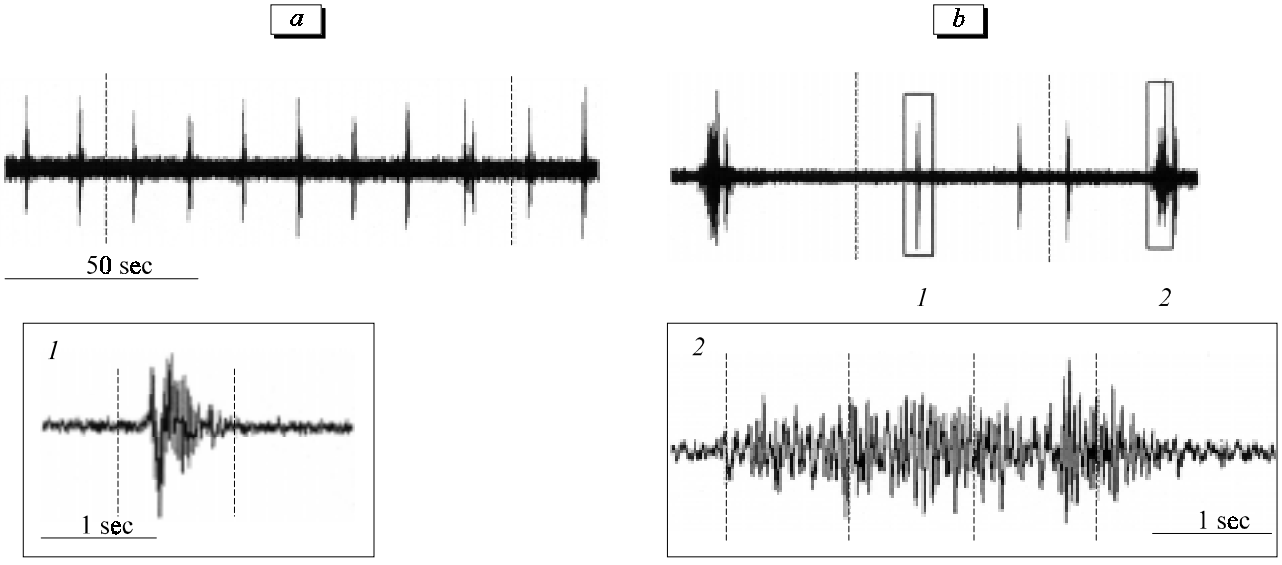

Fig. 1. Electrical activity in C_{III}-C_{IV} ventral roots of the bulbospinal preparation from a newborn rat. *a*) neurogram with respiratory bursts; *b*) neurogram containing respiratory (*1*) and non-respiratory bursts (*2*).

rors and analyzed by Student's *t* test. The differences between the means were significant at $p<0.05$.

RESULTS

Two types of discharges (respiratory and non-respiratory) were recorded in C_{III}-C_{IV} ventral roots of BSP. RB were generated by bulbar neurons and consisted of short bursts lasting on average 1.094±0.048 sec. These discharges were generated at the rate of 3-6/min (Fig. 1), the period of respiratory activity was 16.80± 1.13 sec. Non-respiratory bursts lasted for 3-25 sec and were paused with intervals of 3-15 min. After separation of the medulla oblongata and spinal cord the respiratory discharges disappeared, while the non-respiratory (spinal) discharges were preserved. When analyzing electrical activity of BSP, only RB in C_{III}-C_{IV} ventral roots were recorded. Spectral analysis of discharge oscillations forming RB in nerves innervating different respiratory muscles is now often used for evaluation of the effects of various stimuli on inspiratory activity of the respiratory center.

Spectral analysis showed that the frequency of RB oscillations in BSP varied from 1 to 150 Hz. The peak of RB power spectral density corresponded to 12-45 Hz (Fig. 2). We found no published data on spectral parameters of respiratory activity in BSP of newborn rats. However, inspiratory nerve discharges in narcotized newborn animals [6,8,9] and in BSP of newborn kittens [4] are characterized by a dominant peak in power spectra in the frequency range of 12-45 Hz. In our experiments, spectral analysis of the first and the second halves of RB revealed a low-frequency peak (3-8 Hz) in the initial part of RB. Thus, power spectra of respiratory discharges generated by respiratory center in BSP are characterized by the presence of low (3-8 Hz) and the middle-frequency (12-45 Hz) peaks of RB oscillations.

Addition of SNP into the perfusate enhanced respiratory activity in BSP. This effect developed 3 min after the start of perfusion and peaked on the 5th minute (Fig. 3). The period of respiratory activity increased from 16.5±1.1 to 33.1±4.6 sec ($p<0.01$) and the amplitude of respiratory discharge increased by 18.4±5.9% ($p<0.01$) compared to the control values. In the presence of SNP, the amplitude of the middle-frequency peak in RB power spectrum increased by 29.7±2.1% ($p<0.01$).

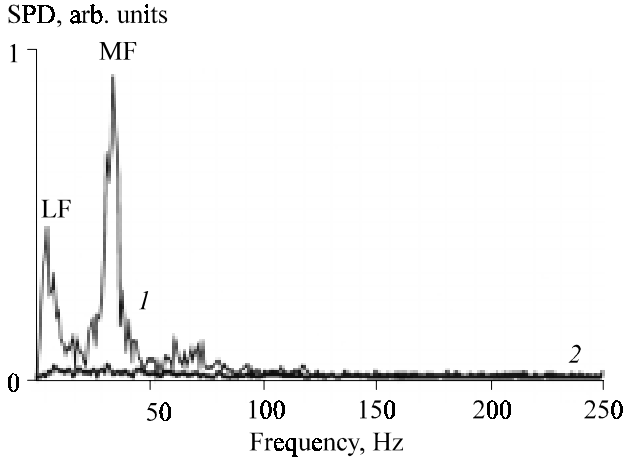

Fig. 2. Power spectra of neural activity in C_{IV} ventral root of the bulbospinal preparation from newborn rat (postnatal day 3). *1*) fragment of respiratory burst, *2*) interburst period. Spectral power density (SPD); low-frequency peak (LF); middle-frequency peak (MF).

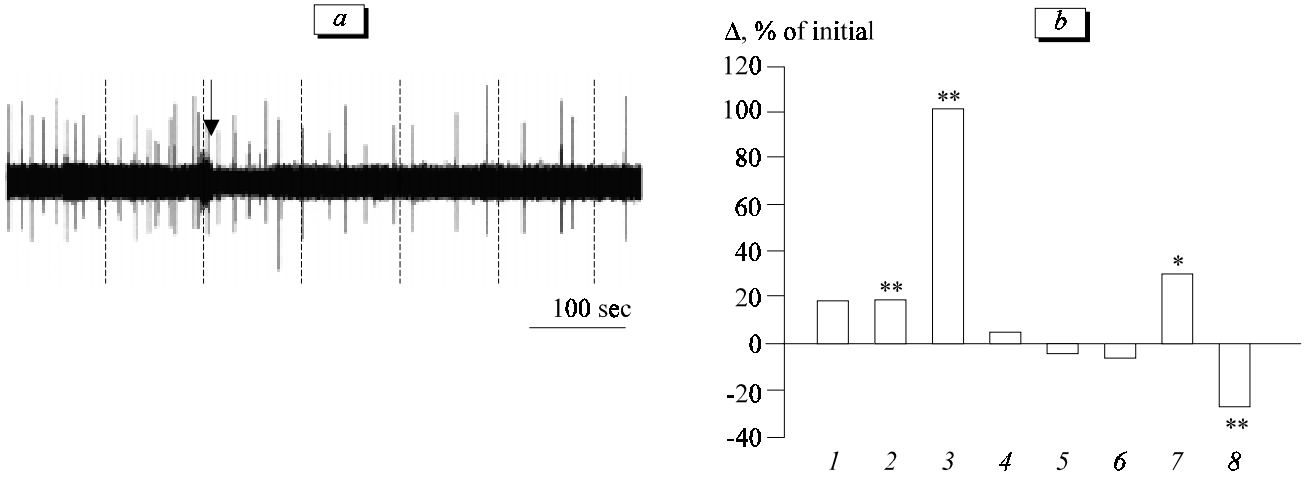

Fig. 3. Effect of sodium nitroprusside (SNP, 100 μM) on respiratory rhythm generation in bulbospinal preparation from newborn rats. *a*) Neurogram recorded in C_{IV} ventral root. Time of SNP introduction is indicated by an arrow; *b*) changes in respiratory activity of bulbospinal preparation after 5-min perfusion. *1*) Duration of respiratory bust, *2*) amplitude of respiratory bust, *3*) period of respiratory cycle, *4*) frequency of low-frequency peak, *5*) frequency of middle-frequency peak, *6*) amplitude of low-frequency peak, *7*) amplitude of middle-frequency peak, *8*) low-frequency peak/middle-frequency peak amplitude ratio (*6*/*7*).

L-arginine introduced into the perfusate also decreased RB generation rate. Starting from the 10th minute after addition of L-arginine, the respiratory period increased from 13.1±1.2 to 16.1±1.2 sec ($p<0.05$), the low-frequency peak shifted from 5.43±0.34 to 6.26± 0.35 Hz ($p<0.05$). After 20 min, the amplitude of RB decreased by 14.2±3.2% ($p<0.002$) compared to the baseline value (Table 1), the middle-frequency peak shifted from 18.40±0.81 to 16.1±0.6 Hz ($p<0.05$), and the amplitude of low-frequency peak decreased by 18.1±5.7% ($p<0.01$). It is known that NO produces a cytotoxic effect on neurons in CNS [5]. In our experiments the neurotoxic effect was produced by L-arginine in a dose of 300 μM (endogenous NO): 15 min after the start of perfusion respiratory activity of BSP was inhibited in 70% cases.

L-NAME did not change the frequency and amplitude of RB and their spectral characteristics, but by the 20th minute significantly reduced RB duration from 1.283±0.163 to 1.045±0.153 sec ($p<0.002$).

Our findings suggest that NO affects the respiratory rhythmogenesis in BSP of newborn rats: exogenous and endogenous NO decreases RB generation frequency and modulates spectral characteristics of RB discharges. Exogenous NO changes the power of middle-frequency oscillations in RB, while activation of endogenous NO synthesis shifts the low-frequency peak towards higher frequencies. The effect of NO on RB spectral parameters depends mainly on the source of NO [3]. This was confirmed by the fact that L-arginine in high doses irreversibly inhibits respiratory activity, while SNP produced no such effects.

In our experiments, inhibition of NOS in the respiratory center of BSP from newborn rats did not change the rate of RB generation. At the same time, inhibition of NO production reduced RB duration. Taking into account long latency of this effects, we assumed that L-NAME acts on deep respiratory neurons of BSP located far from its surface.

Thus, power spectra of RB discharges in BSP from newborn rats contains two regular peaks corresponding to low- and middle-frequency oscillations. Both exogenous and endogenous NO can modulate the respiratory generator and modify spectral characteristics of respiratory discharges.

TABLE 1. Effects of L-Arginine on Respiratory Rhythmic Activity in Bulbospinal Preparation (*M*±*m*)

Parameters		Baseline values	Stimulation time	
			10 min	20 min
Duration, sec	respiratory cycle	13.06±1.23	16.12±1.16**	15.34±0.76**
	RB	0.939±0.049	0.876±0.059	0.875±0.070
Frequency, Hz	LF peak	18.35±0.81	17.27±0.63	16.34±0.59**
	MF peak	5.43±0.34	6.26±0.35**	6.23±0.31*

Note. *$p<0.01$, **$p<0.05$ compared to the baseline.

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