Effects of Nitric Oxide on Respiratory Activity in Bulbospinal Preparation From Rat Fetus

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Experiments on bulbospinal preparations isolated from rat fetus (gestation days 18 and 21) showed that exogenous and endogenous NO can stimulate respiratory rhythm generation and change spectral characteristics respiratory discharges.

Key Words: *nitric oxide*; *respiratory rhythm genesis*; *rat fetus*; *in vitro*

The development of neurotransmitter systems involved in respiratory rhythm generation at different stages of respiratory center (RC) ontogeny is an important problem of neurobiology. Of special interest is functional maturation of RC in the prenatal period. However, neurotransmitter mechanisms of respiratory rhythmogenesis in fetuses remain poorly understood.

Recent studies demonstrated that NO-ergic mechanisms are involved in the realization of various functions of CNS, in particular, in the regulation of respiration [2]. Thus, a population of pontomedullary neurons containing NO synthase was revealed by histochemical mapping in newborn rats [1]. *In vivo* and *in vitro* experiments showed that NO-mediated mechanisms participate in the regulation of respiration starting from the first days of life [1,4]. In light of this, the present study was aimed at evaluation of the role of NO in the generation of rhythmic activity of RC during prenatal ontogeny.

MATERIALS AND METHODS

Experiments were carried out on bulbospinal preparations isolated from 18 fetuses obtained from 5 rats on gestation day 18 and 15 fetuses from 3 rats on gestation day 20. The rats were narcotized with nembutal (30 mg/kg, intraperitoneally), fetuses were obtained as described previously [3]. Craniotomy and laminectomy were carried out to expose the brain and the cervical part of the spinal cord. The brain stem was cut at the intercollicular level, cranial and spinal nerves above C_{VI} were transected. The brain was perfused during preparation with cold (7°C) artificial cerebrospinal fluid 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) [10]. The perfusion medium was continuously bubbled with carbogen (5% CO_2+95% O_2). At the end of the isolation procedure, the perfusate was heated to 25°C, and the preparation was transferred into a 3-ml recording chamber. The preparation was perfused with artificial cerebrospinal fluid at a rate of 3 ml/min, the temperature was maintained at 25°C.

NO donor sodium nitroprusside (SNP, 100 μM), NO precursor L-arginine (200 μM), and NO synthase inhibitor NG-nitro-L-arginine-methyl ester hydrochloride (L-NAME, 200 μM) were purchased from RBI.

Electrical signals were recorded in C_{III}-C_{IV} ventral roots via a suction electrode (inner diameter 100 μM), amplified, and fed to a computer. Spectral analysis of neurograms C_{III}-C_{IV} was performed using Fast Furrier Transform with sampling rate of 500 Hz. Power spectra were calculated from 10 consecutive respiratory bursts. The data are presented as means and standard errors analyzed by Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

Two different types of discharges in C_{III}-C_{IV} ventral roots of bulbospinal preparations were recorded: respiratory and non-respiratory [3]. The respiratory dis-

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charges are short bursts (0.356±0.021 sec) generated at a rate of 3-5/min. After medullospinal transection, this type of neural activity in C_{III}-C_{IV} ventral roots disappeared (Fig. 1). Respiratory discharges were observed in 88% of 18-day fetuses and in 93% of 20-day fetuses. Spectral analysis of respiratory activity in bulbospinal preparations from 18-20-day fetuses revealed oscillations in a wide frequency range (from 1 to 150 Hz) with predominance of low-frequency oscillations. There are two distinct peaks in the spectra of respiratory discharges in low-frequency (1-10 Hz) and medium-frequency (10-50 Hz) ranges. The low-frequency-power peak was 2.0-2.5 times higher than the medium-frequency-power. By contrast, the medium-frequency peak dominates in the power spectra of phrenic nerve activity in neonates [6,9].

Five minutes after the start of perfusion with SNP-containing saline, the duration of respiratory cycle in preparations from 18-day fetuses decreased by 56% (Fig. 2, *a*) and the amplitude of respiratory bursts increased by 16%. In addition, the low-frequency peak in the power spectra of respiratory discharge was shifted from 6.08±0.19 to 6.64±0.23 Hz ($p<0.05$). By contrast, in newborn rats, SPN inhibited respiratory rhythm generation [1]. It was assumed that the effect of exogenous NO on RC in bulbospinal preparation from newborn rat is mediated via neuronal structures in the rostral ventrolateral medulla. After elimination of these structures by transection, SNP potentiated generation of respiratory discharges [1]. In our experiments, removal of the rostral ventrolateral medulla did modulate the effect of SNP on respiratory rhythm generation. Hence, NO-mediated regulatory mechanisms in RC of fetal and newborn rats are different.

The effect of L-arginine on preparations from 18-day fetuses was similar to that induced by SNP: shortening of the respiratory cycle (by 31%, $p<0.05$), increase in the amplitude of respiratory bursts (by 16%, $p<0.05$), and a shift of the low-frequency peak of respiratory discharge power spectra towards higher frequencies. Moreover, the low-frequency power increased by 33% ($p<0.05$). Thus, exogenous and endogenous NO produced similar effects on respiratory discharges in preparations from 18-day fetuses.

In 12% preparations from 18-day fetuses, neuronal activity recorded in C_{III}-C_{IV} ventral root consisted of non-respiratory discharges. We assume that RC in these cases was inhibited with a narcotic drug injected to pregnant rat before surgery [3]. Perfusion of these preparations with saline containing SNP or L-arginine for 5-9 min led to appearance of respiratory bursts in ventral root activity (Fig. 2, *b*). Moreover, our data suggest that exogenous and endogenous NO not only stimulated respiratory activity in bulbospinal preparations, but also suppressed non-respiratory discharges (Fig. 2, *c*). In the case when both types of discharges were recorded in C_{III}-C_{IV} ventral roots, the non-respiratory discharges were gradually inhibited in the presence of NO (Fig. 2, *c*). These findings suggest that NO modulates not only respiratory rhythmogenesis in RC, but also other neuronal mechanisms.

Inhibition on endogenous NO synthesis by L-NAME produced a 2.2-fold decrease in rhythmic discharges in bulbospinal preparations from 18-day fetuses at 20 min of perfusion, without significant changes in other

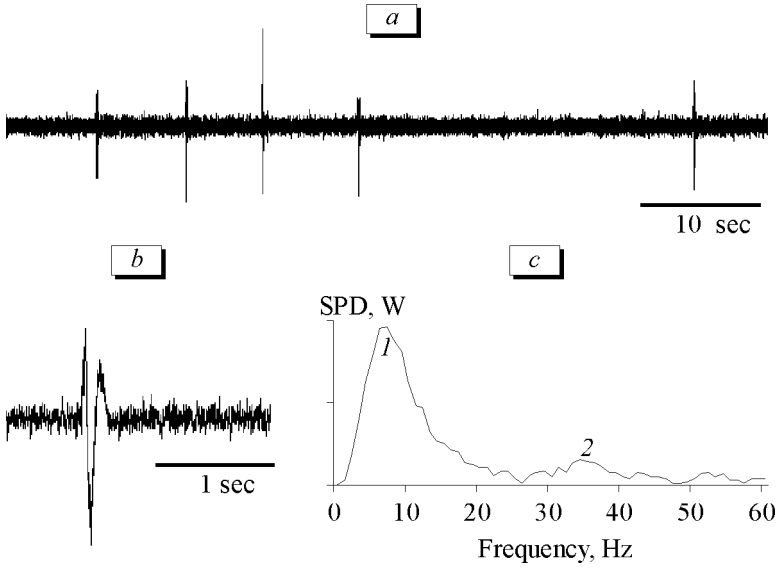

Fig. 1. Neural activity in ventral roots C_{III}-C_{IV} of the bulbospinal preparations from 18-day fetuses. *a*) neurogram of respiratory activity, *b*) neurogram of a single respiratory burst, *c*) power spectrum of a single respiratory burst containing a low-frequency (*1*) and medium-frequency (*2*) peaks. SPD: spectral power density.

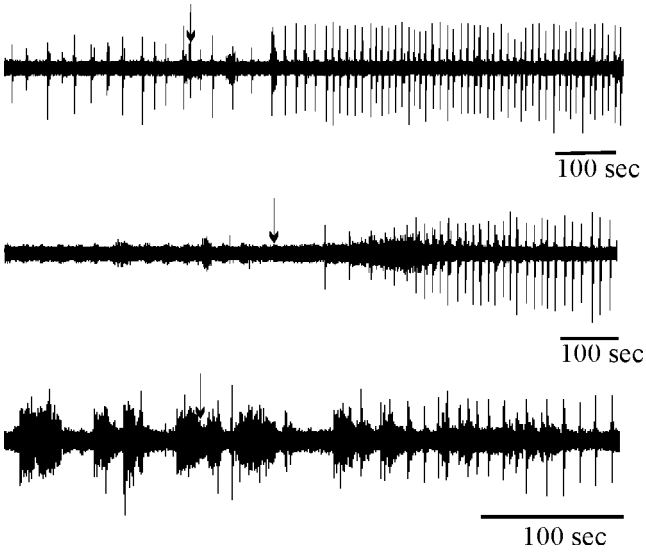

Fig. 2. Effect of sodium nitroprusside (SNP, 100 μM) on electrical activity of bulbospinal preparation from rat fetus. *a*) changes in respiratory neural activity, *b*) induction of respiratory discharges, *c*) inhibition of non-respiratory and activation of respiratory discharges. Addition of SNP is indicated by arrows.

respiratory parameters. Pronounced suppres-sion of respiratory activity induced by inhibition of NO synthase in brain structures of 18-day fetuses indicates that NO-mediated mechanisms play an important role in RC function during the prenatal period.

In preparations from 20-day fetuses, exogenous and endogenous NO produced different effects on respiratory motoneurons. The effects of L-arginine on electrical activity of bulbospinal preparations were similar in 20- and 18-day fetuses, while the effects of SNP in these groups were different. In 20-day fetal preparations, SNP had no effect on the low-frequency peak in the power spectra of respiratory discharges, but shifted the medium-frequency peak towards lower frequencies (from 24.66±2.28 to 20.78±1.70 Hz, $p<0.05$). The changes in respiratory cycle period and amplitude of discharges induced by SNP were similar to those observed in 18-day fetal preparations. Inhibition of NO synthase activity in bulbospinal preparations from 20-day fetuses with L-NAME not only reduced the rate of respiratory rhythm by 62% ($p<0.001$), but also prolonged the respiratory bursts by 13% ($p<0.05$). It was accompanied by a shift of the medium-frequency spectral power peak towards higher frequencies from 24.38±1.65 to 28.82±1.32 Hz ($p<0.05$, Fig. 3). Thus, in 18-day fetuses, endogenous NO affected only low-frequency oscillations, while, in 20-day fetuses, it produced changes in both low- and medium-frequency oscillations of respiratory discharges.

Thus, NO-mediated mechanisms play an important role in the respiratory rhythm genesis and the formation of inspiratory neural activity patterns during prenatal development of RC. The rise of NO level in fetal brain structures associated with RC, results in a powerful enhancement of respiratory discharges *in vitro* in bulbospinal preparations. It is considered that in the prenatal period, the respiratory rhythm is generated by a pacemaker mechanism, in which the interactions between the respiratory neurons are mediated by excitatory amino acid transmitters [4,8]. There is evidence that NO is as a retrograde messenger in glutamatergic processes in the brain, in particular, during hypoxic hyperventilation [7]. One can conclude that glutamatergic mechanism can be responsible for NO-induced potentiation of respiratory discharges in fe-

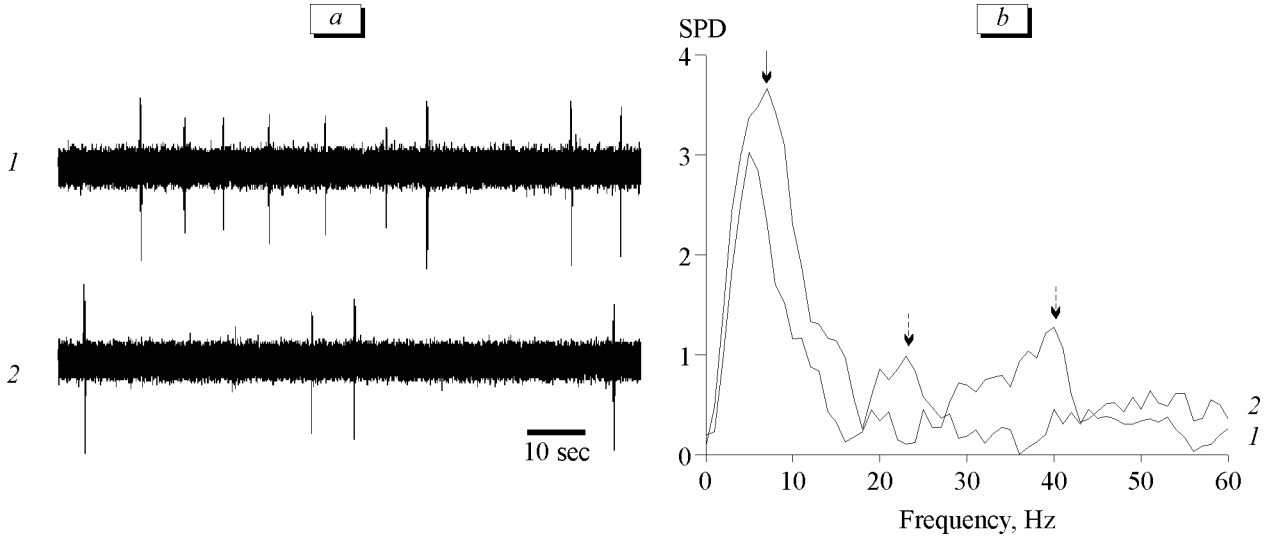

Fig. 3. Effect of L-NAME (200 μM) on electrical activity of bulbospinal preparation from 20-day rat fetuses. *a*) decrease in the frequency of respiratory discharges after 20-min perfusion with L-NAME, *b*) spectrogram of respiratory discharges, *1*) baseline; *2*) after 20-min perfusion with L-NAME. SPD: spectral power density.

tuses. The role of NO in the regulation of respiratory rhythm genesis in fetuses is more certain than in neonates. Recent *in vitro* studies on bulbospinal preparations revealed both the inhibitory and excitatory mechanisms of respiratory rhythm control mediated by NO, which are localized in different medullary structures [1]. The inhibitory effect of NO on respiratory activity in newborn rat preparations is associated with neuronal structures located in the rostral ventrolateral medulla. Our findings indicate that these structures are not involved in NO-mediated control of respiratory rhythm in fetuses.

Thus, exogenous and endogenous NO considerably potentiates RC function in fetuses. Effects of exogenous NO on spectral parameters of respiratory discharges change dramatically during the prenatal ontogeny.

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