GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Respiratory Activity of Bulbospinal Preparations from Newborn Rats Subjected to Periodic Hypercapnia in the Prenatal Period

I. V. Miroshnichenko and V. F. Pyatin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 5, pp. 492-496, May, 2003
Original article submitted October 27, 2002

Periodic exposure of pregnant rats to hypercapnia (10% CO_2) delayed the development of mechanisms underlying respiratory rhythmogenesis in newborn animals during the early postnatal period. *In vitro* studies showed that rhythmic activity of the respiratory center in newborn rats (days 0-3) was 2-3-fold lower than that in intact preparations. Age-related changes of respiratory activity were absent, while the reactions of the respiratory center to CO_2 were suppressed compared to the control.

Key Words: *respiratory rhythmogenesis; prenatal hypercapnia; rats; in vitro*

During postnatal ontogeny of mammals afferent signals from central and peripheral chemoreceptors to the respiratory center modulate functional activity of the respiratory system and contribute to the development of mechanisms underlying respiratory rhythmogenesis [6,10]. These mechanisms develop before the appearance of spontaneous breathing movements in fetuses. Central chemoreceptors play a major role in the regulation of functional activity in the respiratory center. Perfusion of bulbospinal preparations from rat fetal brain with CO_2-free solution blocks spontaneous rhythmic activity of the respiratory center [5]. These data indicate that during perinatal ontogeny changes in the gas medium via chemoreceptors affect physiological characteristics of respiratory rhythmogenesis in the brain.

Plastic rearrangements in the center generating respiratory rhythm at various terms of postnatal ontogeny can result from periodic stimulation of peripheral and central chemoreceptors [8,13]. The exposure of carotid chemoreceptors to periodic hypoxia or burst electrostimulation of the sinus nerve produce persistent changes in lung ventilation [3,11]. Long-term potentiation of activity in the respiratory center is associated with stimulation of serotonin receptors in its structures [7]. In adult rats periodic hypercapnia (PH) causes long-term suppression of respiratory activity, which is realized via α_2-adrenoceptors [2]. The data suggest that repeated exposure of the respiratory center to regulatory factors plays an important role in the development of specific neurotransmitter and generative processes. Here we studied the effect of PH during prenatal ontogeny on the development of mechanisms underlying respiratory rhythmogenesis in newborn animals.

MATERIALS AND METHODS

In vitro experiments were performed on 31 bulbospinal preparations isolated from newborn rats aging 0-1 (BP 0-1) and 2-3 days (BP 2-3). Control animals (n=22, BP 0-1 and BP 2-3, offspring of 3 rats) were not subjected to PH during the prenatal development. The experimental group included 9 BP 0-1 and BP 2-3

Samara State Medical University. ***Address for correspondence:*** miv2000@mailru.com. Miroshnichenko I. V.

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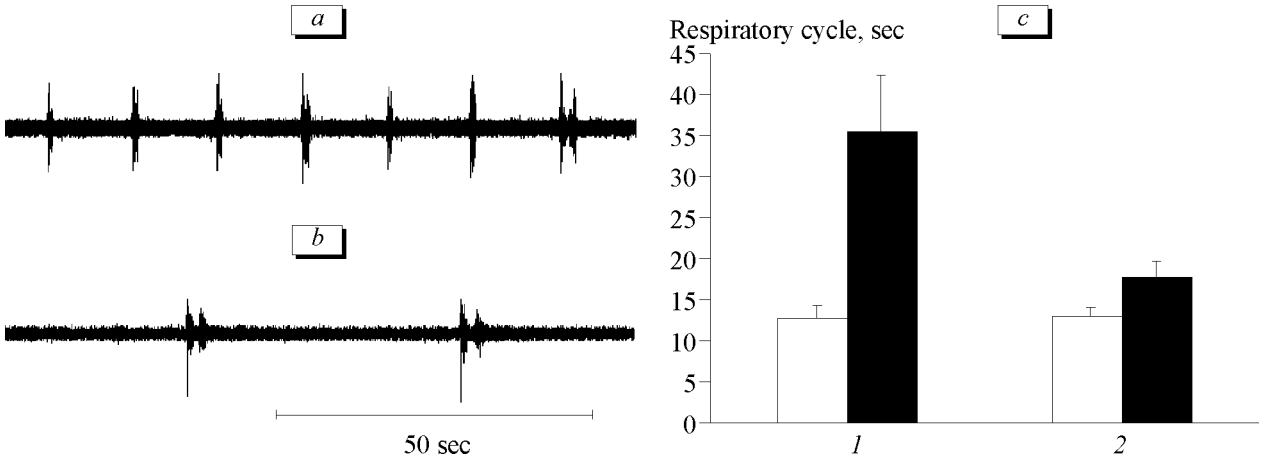

Fig. 1. Effect of prenatal PH on respiratory rhythmogenesis during early postnatal ontogeny. Electrical activity of ventral roots in C_4 segments of bulbospinal preparations from 0-1-day-old control (*a*) and experimental rat pups (*b*). Age-related changes in the duration of respiratory cycles (*c*) in bulbospinal preparations from control (light bars) and experimental rat pups (dark bars). Here and in Fig. 2: 0-1 (*1*) and 2-3 days (*2*).

from 2 litters subjected to prenatal PH. The rats were placed in a 3-liter chamber and atmospheric air-5% CO_2 mixture was pumped at a flow rate of 3 liters/min. The contents of CO_2 and O_2 in the gas medium were continuously measured with GAU-5 and AK-4 gas analyzers. PH was performed daily from the 8th to 20th day of pregnancy (four 10-min exposures at 5-min intervals).This regimen is optimal for adaptive rearrangement of the mechanisms underlying chemosensitive regulation of the respiratory center in various periods of ontogeny [14].

Bulbospinal preparations from rat pups were obtained as described elsewhere [1]. Electrical activity of ventral roots in C_3-C_4 segments was recorded with a suction electrode (inner diameter 100 μ) and inputted into a personal computer via an alternating current amplifier. Fast Fourier transformation was used to evaluate frequency of the respiratory rhythm. The data are presented as means and standard errors. The results were analyzed by Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

In BP 0-1 from experimental rats the duration of respiratory cycles was 2.8-fold longer than in control preparations (Fig. 1, *a*, *b*). The coefficient of respiratory variability reflecting maturity of the mechanisms of respiratory rhythmogenesis [5] was similar in the control and experimental groups (Table 1). After PH bulbospinal preparations exhibited a peculiar pattern of rhythmic activity (double pulses), which was observed only in 40% control preparations.

We compared respiratory rates of BP 0-1 from control and experiments animals. PH had no effect on the shape (decremental pulses, Fig. 2, *a*) and duration of respiratory pulses, characteristics of low-frequency (0-10 Hz) and medium-frequency peaks (10-50 Hz) in the spectrum of respiratory pulses, and their power ratio (Fig. 2, *b*).

Functional activity of the respiratory center undergoes dynamic changes over the first days of life [15]. In bulbospinal preparations from control rats respira-

TABLE 1. Respiratory Activity of Bulbospinal Preparations from the Brain of Newborn Rats Subjected to PH during Prenatal Ontogeny (*M*±*m*)

Groups of animals		Low-frequency peak, Hz	Medium-frequency peak, Hz	Power ratio between low- and medium-frequency peaks, rel. units	Respiratory cycle, sec	Coefficient of respiratory rate variability, rel. units	Respiratory pulse, sec
Control	0-1 days (*n*=10)	6.18±0.24	21.83±1.26	1.0±0.1	12.8±1.4	0.17±0.03	0.884±0.045
	2-3 days (*n*=12)	6.62±0.29	19.20±0.45	0.50±0.06*	12.9±1.2	0.16±0.02	0.999±0.055
Experiment	0-1 days (*n*=4)	5.97±0.26	19.94±0.75	0.9±0.1	35.4±7.0^{o}	0.21±0.05	0.929±0.082
	2-3 days (*n*=5)	6.54±0.44	18.27±1.12	0.8±0.07*	17.7±1.9^{+x}	0.23±0.03^{x}	0.866±0.042

Note. $p<0.05$ compared to: *BP 2-3 in the control group; $^{+}$BP 2-3 in the experimental group; ^{o}BP 0-1 in the control group; ^{x}BP 2-3 in the experimental group.

tory pulses lost their decremental pattern due to increased amplitude of oscillations in the second half of pulses (Table 1, Fig. 2, *a*). The medium-frequency peak of oscillations dominate in the pulse spectrum (Fig. 2, *b*). The power ratio of low- and medium-frequency peaks decreased by 2 times ($p<0.01$).

In bulbospinal preparations from experimental rats rhythmic activity of the respiratory center was lower than in the control (Table 1, Fig. 1, *c*). In these preparations the coefficient of respiratory rate variability surpassed the control. During this period respiratory pulses remained decremental and did not differ from those observed in 0-1-day-old animals. The power ratio of low- and medium-frequency oscillations remained unchanged. These data indicate that PH delayed the development of age-related changes in the mechanism underlying the formation of the rhythm and pattern of respiratory activity.

Rhythmic activity of bulbospinal preparations from the brain of newborn animals is sensitive to pH changes, which reflects the development of central chemosensory mechanisms [12]. Perfusion of bulbospinal preparations from control rats with artificial spinal fluid saturated with 90% O_2 and 10% CO_2 (pH 6.9-7.0) stimulated respiratory rhythmogenesis (Fig. 3, *a*). The duration of respiratory cycles and the amplitude of pulses decreased by 17.1 ($p<0.03$) and 10.0% ($p<0.05$), respectively. These changes were accompanied by a

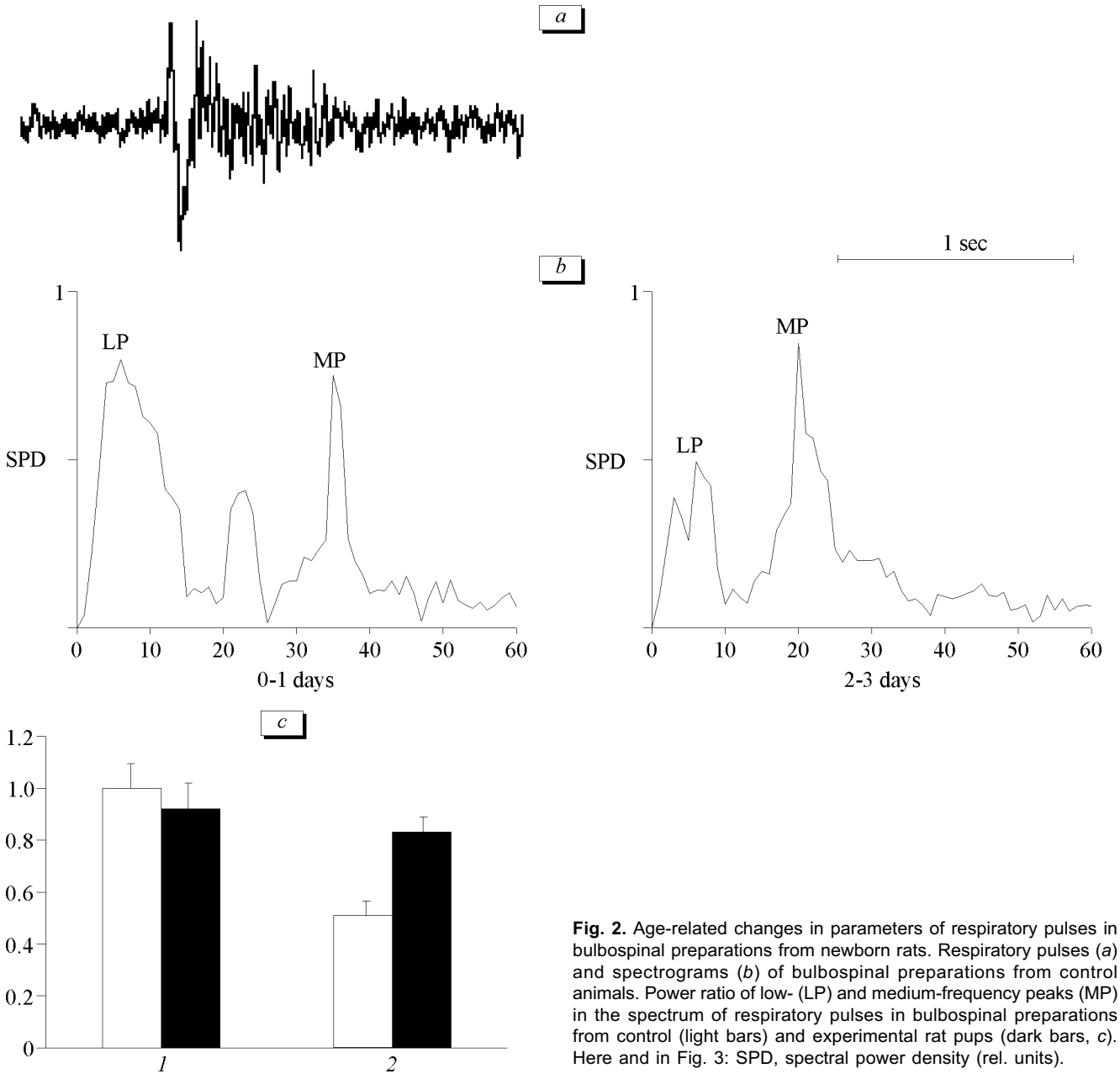

Fig. 2. Age-related changes in parameters of respiratory pulses in bulbospinal preparations from newborn rats. Respiratory pulses (*a*) and spectrograms (*b*) of bulbospinal preparations from control animals. Power ratio of low- (LP) and medium-frequency peaks (MP) in the spectrum of respiratory pulses in bulbospinal preparations from control (light bars) and experimental rat pups (dark bars, *c*). Here and in Fig. 3: SPD, spectral power density (rel. units).

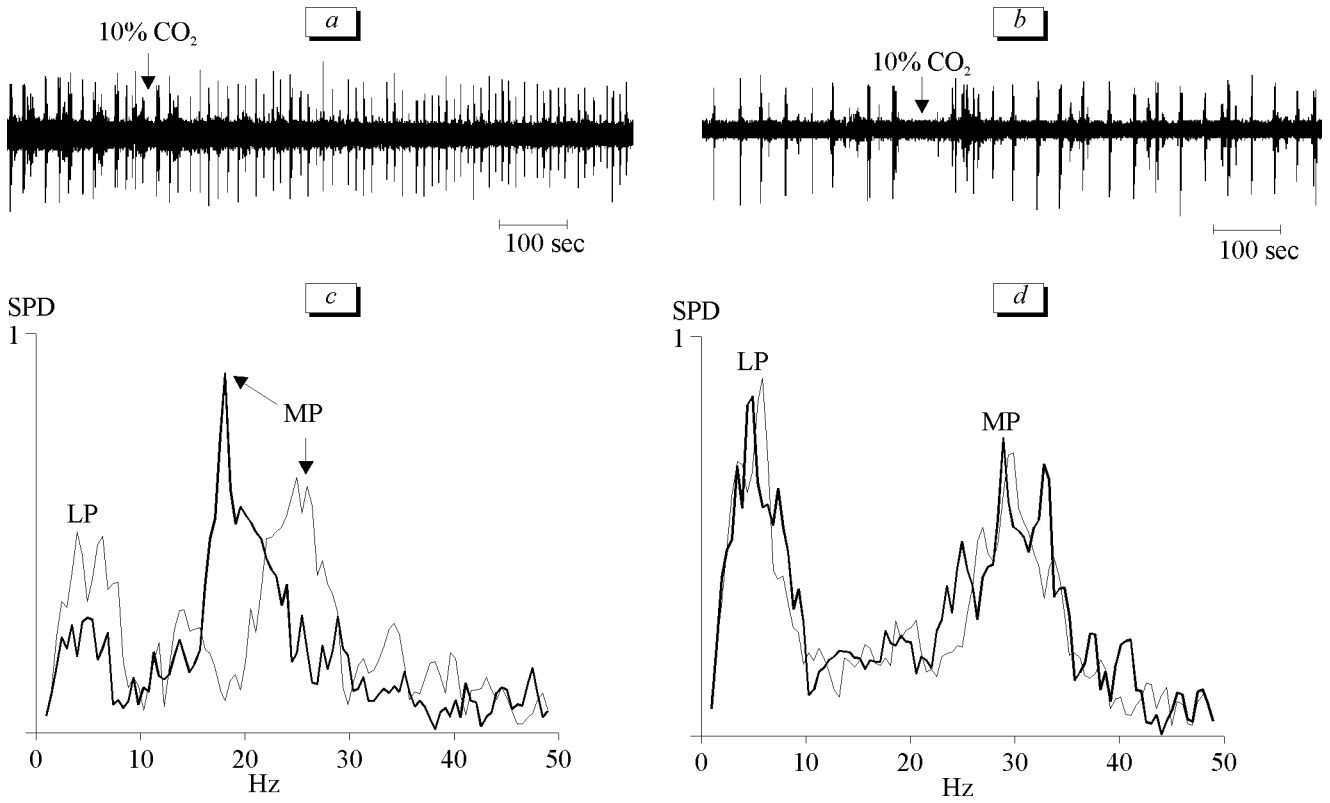

Fig. 3. CO_2-induced response of the respiratory center in bulbospinal preparations from rat pups subjected to PH. Electrical activity of C_4 and spectrograms of respiratory pulses during perfusion of bulbospinal preparations from control (*a*, *c*) and experimental animals (*b*, *d*) with the artificial spinal fluid saturated with 90% O_2 and 10% CO_2. Arrow: start of treatment. Fragments *c* and *d*: thin line, before treatment; thick line, 10 min after treatment with CO_2.

decrease in the duration of respiratory pulses by 14.6% ($p<0.05$). In the spectrum of respiratory pulses the medium-frequency power peak was shifted from 19.5± 0.7 to 17.4±0.6 Hz ($p<0.04$) and the power was redistributed from low-frequency to medium-frequency peaks. The power ratio of these peaks decreased from 1.05±0.10 to 0.73±0.08 ($p<0.001$, Fig. 3, *b*).

In experimental rats CO_2 decreased only the amplitude of respiratory pulses by 8.2% ($p<0.03$). The respiratory rate and frequency spectrum remained unchanged. These results indicate that PH reduces chemosensitivity of central chemoreceptors to CO_2, which is probably associated with the increase in the sensitivity threshold for this compound. Previous studies showed that after 2-week acclimatization of ducks to hypercapnia activity of intrapulmonary chemoreceptors decreases due to the increase in the sensitivity threshold for CO_2. It can be hypothesized that PH decreases functional activity of central chemoreceptors. These changes probably determine delayed maturation of respiratory centers during early postnatal ontogeny. Denervation of arterial chemoreceptors in newborn animals delays the formation of rhythmic breathing [10]. Transgenic mice with neurotrophin BDNF deficiency are characterized by slow and arrhythmic breathing, which is related to impaired development of sensory neurons in the ganglion petrosal and suppression of afferent stimulation from the carotid sinus to the respiratory center [6].

Our results indicate that prenatal PH delays the development of mechanisms underlying respiratory rhythmogenesis during early postnatal ontogeny of the respiratory system. These changes probably result from functional inactivation of the central chemosensitive mechanism.

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