Role of Neuronal NMDA and non-NMDA Glutamate Receptors in Medial Vestibular Nucleus in the Regulation of Respiratory Rhythmogenesis in Newborn Rats *In Vitro*

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We studied isolated pontobulbospinal preparations from newborn rat brain. In the early postnatal period, the rostral part of the medial vestibular nucleus produces a potent inhibitory effect on neuronal structures of the bulbar respiratory center via the glutamatergic system. Microinjection of L-glutamate (50 mmol/liter) into the rostral part of the vestibular nucleus completely blocks respiratory rhythmogenesis in 0-1-day-old rat pups and reduced the frequency of generation of inspiratory discharges in 2-3-day-old rats from 8.42±0.68 to 2.68±0.32 min^{-1}. It was found that the leading role in the mechanism of glutamatergic modulation of the respiratory rhythmogenesis by neurons of the medial vestibular nucleus is played by NMDA and, to a lesser extent, non-NMDA glutamate receptors.

Key Words: *medial vestibular nucleus; respiratory rhythmogenesis; glutamate receptors; pontobulbospinal preparation; in vitro*

Vestibular afferentation plays an important role in the formation of compensatory responses of somatovisceral systems to changes in spatial position of the head and body and during locomotion and rotation. It was found that vestibular nuclei participate in respiratory regulation: electric stimulation of neuronal structures in these nuclei or vestibular nerves increases or reduces the frequency of respiratory movements [1,4,5,15]. Numerous studies confirm the presence of close anatomical and functional relationships between neurons of the vestibular and respiratory systems. For instance, experiments on adult rats showed that electric stimulation of certain neurons of the vestibular complex modulates the frequency of spike generation by neurons of the ventral respiratory group and pre-Botzinger complex [12]. At the same time, a considerable portion of vestibular nucleus complex neurons had pronounced respiratory modulation [7]. Morphological studies [2,3,6] revealed axonal projections of vestibular nucleus neurons terminating in *n. tractus solitarii*, *n. ambiguous*, and *n. retrofacialis*. However, the neuronal networks mediating the vestibulorespiratory reactions are little studied.

Neuronal structures of the medial vestibular nucleus (MVN) produce the most pronounced modulating effect on respiratory rhythmogenesis. Experiments on adult rats [3] showed that electric stimulation of MVN leads to either increase or decrease in pulmonary ventilation, on the other hand, specific chemical stimulation with glutamate always produces an activating effect on respiration. It is assumed that stimulation of pulmonary ventilation is related to the realization of the effect of glutamate via NMDA receptors of MVN, while neurotransmitter mechanisms mediating inhibition of respiration are little studied. Moreover, little is known about the formation of interaction between the vestibular and respiratory systems in the early ontogeny. Our pilot studies showed that the rostral part of MVN produces a potent inhibitory effect on neuronal structures of the

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bulbar respiratory center via the glutamatergic system during the early postnatal period.

Here we studied the role of NMDA and non-NMDA glutamate receptors of neuronal structures of MVN in the realization of the vestibulorespiratory reactions in isolated pontobulbospinal preparations of newborn rats *in vitro*.

MATERIALS AND METHODS

Experiments were carried out on 26 pontobulbospinal preparations isolated from 0-3-day-old rat pups. The animals were narcotized with ether and cranio- and laminectomy were performed from the dorsal surface. During surgery, the brain was perfused with artificial cerebrospinal fluid (pH 7.3) cooled to 10°C and constantly saturated with carbogen (95% O_2, 5% CO_2). The artificial cerebrospinal fluid contained (in mol/liter): 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 dorsal and ventral spinal roots and V-XII cerebral nerves were cut; the spinal cord was crossed at C_8-Th_1. The pontobulbospinal preparation was removed from the skull and transferred into a 2.5-ml perfusion chamber (24-25°C). The preparation was placed ventral surface to the bottom, the spinal cord was fixed with a clamp.

Functioning of the respiratory center was evaluated by summary electrical activity of diaphragmatic motoneurons recorded in ventral roots of C_4-C_5 spinal cord segments using a chlorine-silver suction electrode. Electric signals via an alternating current amplifier (DL-302N-14; Neurobiolab Company) were inputed into an analog-to-digital converter (L-Card E14-440, FBM Engineering) of personal computer and recorded in wave format using PowerGraph software (version 3.3 Professional). The total duration of the respiratory cycle (sec) and duration (sec) and amplitude (rel. units) of the respiratory burst were calculated from the neurogram. Spectral analysis of respiratory discharges was performed using a fast Fourier transform algorithm with discretization rate of 500 Hz. The mean frequency and amplitude of low-frequency (1-10 Hz) and medium-frequency (11-50 Hz) power peaks were calculated from spectrograms of inspiratory signals.

The following substances were used: L-glutamate (50 mmol/liter, Sigma Chemicals), selective NMDA-receptor antagonist ketamine hydrochloride (4 mmol/liter, RBI, Natick), and selective non-NMDA-receptor antagonist GAMS (γ-D-glutamylaminomethylsulphonic acid, 4 mmol/liter, RBI, Natick). The test solutions were injected into the rostral part of MVN via a glass microcannula (tip diameter 20 μ) using a nanoliter injector in a volume of 40 nl (900-1100 μ rostral from the obex, 200-400 μ lateral from the medial line, and 100-200 μ deep from the dorsal surface of the brain). In control series, artificial cerebrospinal fluid (40 nl) was injected into the same brain area.

The data are presented as mean and error of the mean. Statistical analysis was performed by Student *t* test using standard SigmaStat 2.0 software (Jandel Scientific). The changes of the mean values for the test parameters were significant at $p<0.05$.

RESULTS

Microinjection of L-glutamate into the rostral part of MVN reduced the frequency of generation of inspiratory bursts in C_4-C_5 from 8.42±0.68 to 2.68±0.32 min^{-1} ($p<0.001$). In most cases, injection of glutamate into this part of MVN led to complete blockade of respiratory rhythmogenesis. The latency of these reactions did not exceed 5-10 sec, inspiratory activity in ventral C_4-C_5 spinal roots appeared after 4/1 min on average (in some experiments, depression of the respiratory rhythm lasted more than 20 min). Some age-related peculiarities of the observed reactions should be noted. Compete blockade of the respiratory rhythmogenesis in 95% cases appeared in 0-1-day-old rat pups (Fig. 1, *a*), whereas in 2-3-day-old pups activation of glutamate receptors in MVN just decreased the frequency of inspiratory bursts (Fig. 1, *b*).

On the contrary, under conditions of NMDA receptor blockade in the rostral part of MVN by selective antagonist ketamine hydrochloride we observed an increase in the frequency of generation of inspiratory discharges recorded in C_4-C_5 by 2.5 times compared to the initial value (Fig. 2, *a*). This was determined by shortening of both the respiratory cycle by 67.4±2.7% and duration of the respiratory burst by 36.2±2.5%. Analysis of spectral characteristics of inspiratory bursts revealed no significant changes in low- and medium-frequency power peaks, but the amplitude of the low-frequency peak decreased by 32.8±1.9% (Table 1).

The blockade of non-NMDA receptors in the rostral part of MVN after microinjection of GAMS induced less pronounced increase in the frequency of generation of inspiratory bursts (by 52.3±4.1%), compared to blockade of NMDA receptors (Fig. 2, *b*). The total duration of the respiratory cycle and inspiratory burst decreased by 37.1±2.6 and 20.3±3.4%, respectively; this was accompanied by a decrease in respiratory discharge amplitude. The amplitude of low- and medium-frequency power peaks in the frequency spectrum of inspiratory bursts also decreased (Table 1).

Our findings suggest that activation of the glutamatergic system of the rostral part of MVN during the early ontogeny produces a potent inhibitory effect on respiratory rhythmogenesis. Changes in the

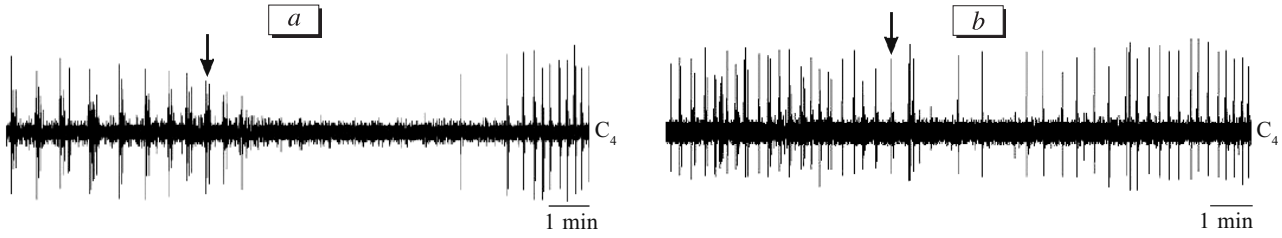

Fig. 1. Effect of L-glutamate microinjection into rostral part of MVN on generation of the respiratory rhythm in the pontobulbospinal preparation from newborn rats *in vitro*. *a*) blockade of respiratory rhythmogenesis in 0-1-day-old rat pups, *b*) decreased frequency of generation of inspiratory bursts in 2-3-day-old rat pups. Arrow shows the moment of microinjection.

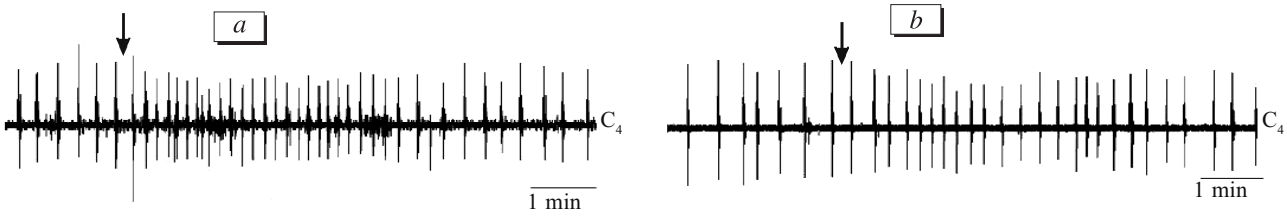

Fig. 2. Effect of microinjection of glutamate receptor antagonists into rostral part of MVN on generation of the respiratory rhythm in the pontobulbospinal preparation from newborn rats *in vitro*. *a*) microinjection of ketamine hydrochloride; *b*) microinjection of GAMS. Arrows show the moments of microinjections.

spectral characteristics of respiratory discharges after microinjection of agonists and antagonists of glutamate receptors in MVN suggest that MVN modulates activity of not only respiratory rhythm generator, but also generator of the inspiratory pattern. It was shown that the inhibitory effect of the rostral part of MVN on structures of the bulbar respiratory center in newborn rats involves NMDA and to a lesser extent non-NMDA glutamate receptors.

Published data suggest that MVN is a heterogeneous structure and MVN neurons exhibits spontaneous activity [8,12], which attests to their possible tonic inhibitory effect on respiratory rhythm generator. The neurotransmitter mechanisms underlying the inhibitory vestibulorespiratory reactions are still not deciphered. It was demonstrated that glutamatergic stimulation of MVN in adult rats enhances lung ventilation [13], while electric stimulation of this structure can reduce it. These

TABLE 1. Effect of Microinjections of Glutamate Receptors Antagonists into Rostral Part of MVN on Parameters of Respiratory Activity of Pontobulbospinal Preparation from Newborn Rat Brain (*M*±*m*)

Parameters of respiratory activity	Experimental conditions			
	Baseline	Blockade of NMDA glutamate receptors	Baseline	Blockade of non-NMDA glutamate receptors
Low-frequency (LF) peak, Hz	3.01±0.20	2.76±0.14	2.76±0.41	3.50±0.53
Amplitude of LF peak, rel. units	5.23±0.78	3.46±0.53**	4.80±1.01	2.84±0.47*
Medium-frequency (MF) peak, Hz	20.97±0.82	16.90±1.82	10.53±3.09	9.63±2.82
Amplitude of MF peak, rel. units	1.10±0.27	0.66±0.15	1.85±0.58	1.01±0.30*
Total duration of the respiratory cycle, sec	20.03±2.85	5.83±0.38***	14.10±2.19	8.67±0.96*
Duration of inspiratory burst, sec	0.74±0.04	0.45±0.02***	0.86±0.11	0.68±0.09**
Amplitude of inspiratory burst, rel. units	94.93±6.14	66.75±4.83***	91.62±14.78	67.75±9.15*
Frequency of burst generation, min^{-1}	4.12±1.26	10.50±0.80*	5.31±1.29	8.19±1.30*

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ significant differences from the baseline value.

two reactions can be explained by activation of different types of neurons (glutamatergic and GABA-ergic) during electric stimulation of MVN and involvement of various neurotransmitter systems into realization of the vestibulorespiratory reactions [10,14].

Moreover, neurons of the respiratory center receiving afferentation from the vestubular neuronal network can initiate both inspiration and expiration. For instance, activity of 80% bulbospinal expiratory neurons and 50% inspiratory neurons was recorded during electric stimulation of the vestibular nerve near the ventral respiratory group [11].

Thus, our pilot studies showed that the rostral part of MVN produces a potent inhibitory effect on neuronal structures of the bulbar respiratory center via the glutamatergic system in the early postnatal period. The leading role in the mechanism of glutamatergic modulation of the respiratory rhythmogenesis by MVN neurons is played by NMDA and, to a lesser extent, non-NMDA glutamate receptors.

REFERENCES

1. J. P. Hernandez, F. Xu, and D. T. Frazier, *J. Appl. Physiol*., **97**, No. 3, 835-842 (2004).
2. B. J. Jian, A. W. Acernese, J. Lorenzo, *et al., Brain Res*., **1044**, No. 2, 241-250 (2005).
3. C. Matesz, T. Bacskai, E. Nagy, *et al., Brain Res*. *Bull*., **57**, Nos. 3-4, 313-315 (2002).
4. M. Miyashita, T. Suzuki-Inatomi, and N. Hirai, *J. Vestib. Res*., **13**, Nos. 2-3, 57-64 (2003).
5. R. L. Mori, A. E. Bergsman, M. J. Holmes, and B. J. Yates, *Brain Res*., **902**, No. 1, 82-91 (2001).
6. J. D. Porter and C. D. Balaban, *J. Vestib. Res.* **7**, No. 1, 63-76 (1997).
7. H. Reinke and J. M. Wild, *J. Comp. Neurol.,* **391**, No. 2, 147-163 (1998).
8. M. Shao, J. C. Hirsh, C. Giaume, K. D. Peusner, *Neuroscience*, **127**, No. 1, 81-90 (2004).
9. T. Takazawa, Y. Saito, K. Tsuzuki, and S. Ozawa, *J. Neurophysiol*., **92**, No. 5, 3106-3120 (2004).
10. S. Takeshita, M. Sasa, K. Ishihara, *et al., Brain Res*., **840**, Nos. 1-2, 99-105 (1999).
11. J. M. Wenninger, L. G. Pan, P. Martino, *et al., J. Appl. Physiol*., **91**, No. 2, 777-788 (2001).
12. S. F. Woodring and B. J. Yates, *Am. J. Physiol*., **273**, No. 6, Pt. 2, R1946-R1956 (1997).
13. F. Xu, J. Zhuang, T. R. Zhou, *et al., J. Physiol.*, **544**, No. 1, 211-223 (2002).
14. T. Yamanaka, M. Sasa, and T. Matsunaga, *Brain Res*., **762**, Nos. 1-2, 243-246 (1997).
15. B. J. Yates and A. D. Miller, *J. Vestib. Res.* **8**, No. 1, 17-25 (1998).