Effects of Microinjections of Glutamate and Glutamate Receptor Antagonists into A5 Zone on Generation of Respiratory Rhythm in Ponto-Bulbospinal Preparations from Newborn Rats *In Vitro*

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Activation of glutamate receptors in A5 neurons enhanced their tonic inhibitory influence on the respiratory rhythm generator in isolated newborn rat ponto-bulbospinal preparations. The stimulating effect of glutamate on A5 neurons is determined by its effect mainly on non-NMDA receptors and less so on NMDA receptors.

Key Words: *A5; glutamate; ponto-bulbospinal preparations; respiratory rhythmogenesis; in vitro*

Spontaneously active noradrenergic A5 neurons produce a tonic inhibitory effect on the respiratory rhythm generator in newborn mouse and rat brain preparations *in vitro* [5,9]. Norepinephrine released from their axon terminals [5] decreases burst activity of rhythm generating neurons of the respiratory center via α_2-adrenoreceptors [2,9]. Spontaneous activity of A5 neurons is retained even after blockade of synaptic transmission [10]. It is hypothesized that endogenous stimulatory amino acids participate in the regulation of the basal level of spontaneous activity of central neurons [4] through receptors of different types. For example, basal activity of medullar lateral tegmental field neurons in adult cats and inspiratory neurons of the pre-Botzinger complex in newborn rats *in vitro* is determined by the effect of endogenous glutamate on non-NMDA receptors [3,8,13]. Synchronization of oscillations of spontaneous activity of neocortical neurons in newborn rats is associated with stimulation of NMDA-, non-NMDA-, and metabotropic glutamate receptors (GR) [6,7]. The possibility of glutamate control of basal activity of A5 neurons and the role of glutamatergic reception in the regulation of the tonic inhibitory effect of A5 neurons on the respiratory rhythm generator in newborn animals remain not studied.

We studied the effect of microinjections of glutamate and GR antagonists into A5 zone on the respiratory rhythm generation in ponto-bulbospinal preparations (PBSP) from newborn rats *in vitro*.

MATERIALS AND METHODS

In vitro experiments were carried out on 25 isolated PBSP from newborn (0-4 days) pigmented outbred rats. The preparations placed into a thermostat (23-24°C) flow chamber were perfused with artificial cerebrospinal fluid containing (in mmol/liter): 124 NaCl, 5 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 26 $NaHCO_3$, 1.2 KH_2PO_4, and 30 d-glucose and saturated with gas mixture (95% O_2 and 5% CO_2; pH 7.3-7.4). The rate of perfusion was 3 ml/min [1].

Microinjections (40-60 nl) of L-glutamate (50 mmol/liter, Sigma Chemicals), ketamine hydrochloride (4 mmol/liter, selective NMDA receptor

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antagonist; RBI), and γ-D-glutamylaminomethylsulfonic acid (4 mmol/liter, GAMS, non-NMDA receptor antagonist; RBI) into the A5 zone were made. When the parameters of respiratory rhythm generation returned to the initial level, the treatment was repeated. The coordinates for microinjections were as follows: 100-300 μ medially from facial nerve root, 100-400 μ deep from the ventral surface of the pons [10]. Controls were injected with the same volume of 0.9% NaCl or artificial cerebrospinal fluid into the same sites. For histological control, sites of microinjections of glutamate, ketamine hydrochloride, and GAMS (in the PBSP) were fixed in 10% formalin and transverse coronary sections (10-20 μ) were stained with thionine after Nissl. The site of microinjection was detected by the micropipette track. Microphotographs of transverse sections of the pons of newborn rats [10] and atlas of adult rat brain [12] were used for comparison.

Electrical activity of the respiratory center was recorded from the ventral roots (C3-C5) with a suction electrode and then transferred to a PC through a bioamplifier and an analog-digital converter. Analysis of neurograms included evaluation of the duration of the respiratory cycle and duration and amplitude of inspiratory bursts.

The data were statistically processed using Student's *t* test for the means. The differences were considered significant at $p<0.05$.

RESULTS

Stimulation of A5 neurons in PBSP from newborn rats caused by microinjection of L-glutamate decreased the frequency of inspiratory discharge generation recorded in C3-C5 by 37.2±2.4% (Fig. 1, *a*), which was due to prolongation of the respiratory cycle and was paralleled by an increase in the parameter variability (Table 1). The reaction manifested directly after the microinjection and lasted for 0.5-2.0 min.

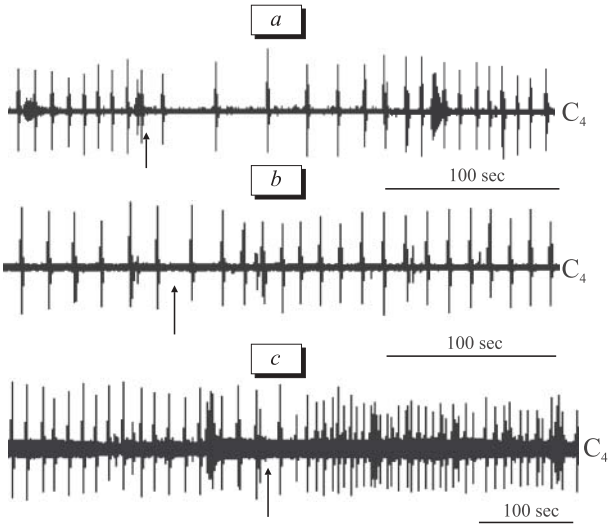

Fig. 1. Effects of microinjections of glutamate and GR antagonists into A5 zone on the generation of spontaneous inspiratory discharges in the newborn rat PBSP respiratory center *in vitro*. *a*) suppression of respiratory rhythmogenesis caused by microinjection of L-glutamate; *b*) stimulation of respiratory rhythmogenesis caused by microinjection of ketamine hydrochloride; *c*) stimulation of respiratory rhythmogenesis induced by GAMS acid microinjection. Arrows show the moments of microinjections.

By contrast, blockade of NMDA receptors in A5 neurons after ketamine hydrochloride microinjection increased frequency of inspiratory discharge generation recorded in C3-C5 by 34.5±2.5% (Fig. 1, *b*), which was caused by shortening of the respiratory cycle and was paralleled by reduction of its variability (Table 1). The reaction was observed 20-40 sec after injection and persisted for 2-5 min.

Blockade of non-NMDA receptors in A5 neurons after GAMS microinjection increased the frequency of inspiratory charges recorded in C3-C5 by 80.3±12.3% (Fig. 1, *c*), which was caused by shortening of the respiratory cycle and was also paralleled by reduction of parameter variability (Table 1). The effect was observed 10-20 sec after

TABLE 1. Effects of Microinjections into A5 Zone on Respiratory Activity Parameters in Newborn Rat PBSP (*M*±*m*)

Parameter	L-glutamate (*n*=25)		Ketamine hydrochloride (*n*=23)		GAMS acid (*n*=15)	
	control	experiment	control	experiment	control	experiment
Duration of respiratory cycle, sec	10.1±0.3	16.5±0.8**	13.0±0.4	9.7±0.3**	15.2±0.9	7.6±1.5**
Coefficient of variability of respiratory cycle duration	0.149±0.014	0.209±0.020*	0.160±0.022	0.093±0.011*	0.194±0.019	0.108±0.006*
Duration of inspiratory discharge, sec	0.958±0.026	1.037±0.032	0.973±0.021	0.993±0.053	0.718±0.027	0.674±0.038
Amplitude of inspiratory burst, %	100.0	103.2±1.4	100.0	94.9±1.6*	100.0	88.8±4.4*

Note. *$p<0.05$; **$p<0.00001$ compared to the control.

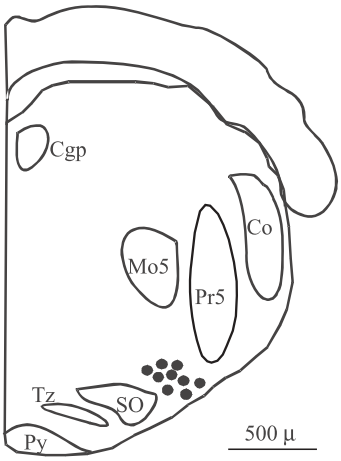

Fig. 2. Pons section of a newborn rat; sites of microinjections of L-glutamate, ketamine hydrochloride, and GAMS acid are shown as black circles. Mo5: trigeminal motor nucleus; Pr5: principal sensory trigeminal nucleus; SO: superior olive; Tz: trapezoid nucleus; Py: pyramidal tract; Co: cochlear nucleus; Cgp: central gray matter of pons.

injection and persisted for 1-3 min. The increase in the respiratory rhythm frequency caused by blockade of non-NMDA receptors of A5 neurons with GAMS was 2.5-fold more pronounced than the effect of ketamine hydrochloride in an equimolar dose ($p<0.01$).

The micropipette track in transverse sections of the pons ($n=10$) stretched 100-400 μ into the depth from the ventral surface and ended between the principal sensory trigeminal nucleus and superior olive complex (Fig. 2), which corresponded to A5 zone in newborn and adult rats [10,12].

Hence, activation of GR in spontaneously active A5 neurons potentiated their inhibitory effect on the respiratory rhythm generator. This effect is linked with activation of both NMDA and non-NMDA receptors of A5 neurons, but non-NMDA receptors make the predominant contribution to this process. Hence, A5 neurons, similarly as the central pacemaker neurons of other location, generate spontaneous activity with the leading role played by non-NMDA receptors [3,8,13]. Inhibition of the respiratory rhythmogenesis during stimulation of GR in A5 neurons can be caused by stimulation of norepinephrine release from axon terminals [5] and its interaction with α_2-adrenoreceptors on respiratory pacemaker neurons [2,9].

Along with *in vitro* reduction of the frequency of respiratory rhythm in newborn animals, stimulation of A5 neurons with glutamate increased variability of the respiratory cycle duration, while GR blockade led to its shortening. It was previously shown that postnatal development of the respiratory center was associated with stabilization of the respiratory rhythm [1]. This can be due to a decrease in the number of noradrenergic A5 neurons [11] or to reduction of the role of the glutamatergic mechanism of A5 neurons in the modulation of respiratory activity in newborn animals.

Hence, activation of GR in A5 neurons of isolated PBSP from newborn (0-4 days) rats stimulates their tonic inhibitory effect on the respiratory rhythm generator. The leading role in the mechanism of glutamatergic modulation of the respiratory rhythmogenesis via A5 neurons belongs to non-NMDA receptors and less so to NMDA receptors. The glutamatergic mechanism of A5 neurons in newborn rats *in vitro* modulates the frequency and variability of the respiratory rhythm.

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