PHYSIOLOGY

Effect of Bilateral Destruction of the Subretrofacial Area on Central Inspiratory Activity of the Respiratory Center and on the Respiratory Response to Hypercapnia

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Local bilateral destruction with 1 M glutamic acid of neuronal structures located between ventral surface of the medulla oblongata and the retrofacial nucleus (subretrofacial area) in rats increases the amplitude of pulses from the phrenic nerve and lowers respiratory rate. Hypercapnia does not affect the amplitude of phrenic nerve pulses but increases respiratory rate. It is suggested that the amplitude of central respiratory activity is regulated by the central chemoreceptors with participation of neuronal structures located in the subretrofacial area.

Key Words: *central inspiratory activity; subretrofacial area; hypercapnia*

Signals from the central chemoreceptors play a crucial role in generating the inspiratory activity of the respiratory center [6]. Neuronal structures in rostral parts of the ventral bulbar surface (VBS) participate in the integration of various afferent signals addressed to the respiratory center, including signals from central chemoreceptors [3,4,7]. Stimulation of these structures in a bulbospinal preparation from neonatal rats evokes rhythmic discharges in the phrenic nerve [5]. Previously, we showed that respiratory reactions are most pronounced upon electrical stimulation of zones located between the VBS and retrofacial nucleus [2]; however, physiological mechanisms of these reactions remained unclear. The objective of this study was to evaluate the role of rostral structures of the VBS in the responses of the respiratory center to hypercapnia.

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MATERIALS AND METHODS

The study was conducted on 20 randomly bred albino rats under Nembutal anesthesia (40 mg/kg). The VBS was exposed in a region extending from the C_1 to the level of exit of cranial nerves VII-VIII and located 4.0-4.5 mm lateral to the midline of the brain. Neuronal structures of the VBS were stimulated with bipolar tungsten electrodes (distance between the electrodes 50 μ) using square pulses (100 Hz, 1 msec, 0.5-20 μA) delivered by an ELS-2 electrostimulator. Glutamic acid (1 M solution) was applied to the VBS bilaterally using a glass electrode with a tip diameter of 1 mm^2. Hypercapnia was produced for 1 min with a gaseous mixture containing 7% CO_2 in O_2. CO_2 was measured with a GAU-5 gas analyzer. The phrenic nerve activity (PNA) was determined with bipolar silver electrodes connected to an amplifying and integrated unit [1]. The medulla oblongata was removed for examination after each test.

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The results were statistically analyzed by Student's t test, taking 5% as the minimal level of significance.

RESULTS

The greatest changes in PNA were caused by electrostimulation of the area located 3.5-4.5 mm rostral to the middle of nerve XII roots, 2.5-3.5 mm lateral to the brain midline, and up to 500 μ deep into the VBS. This area, which occurs between the VBS and the retrofacial nucleus of the medulla oblongata, has been referred to as subretrofacial (SRF). Histological examinations of the sites whose electrostimulation was effective showed that the SRF area is represented by neurons of three types: spindle-shaped, oval, and cells-nuclei. Electrostimulation of the SRF area shortened the respiratory cycle by 13.1±1.4% as a result of prolonged inspiratory and active expiratory phases, the duration of the postinspiratory phase remaining unchanged. Electrostimulation of this area shortened by 14.7±1.5% the period ($+dt$) during which the phrenic nerve discharges reach the maximum and by 18.7±1.7% the period of the maximum plateau ($+dt_{max}$). At the same time, the amplitudes of the PNA pattern increased: by 18.3±1.6% for the initial discharge (PNA_{ini}), 13.5±1.2% for the plateau amplitude (PNA_{max}), and 13.8±1.4% for the postinspiratory discharge amplitude (PNA_{maxPI}).

Application of glutamic acid (1 M) onto the SRF area caused a short-term respiratory standstill (for 6.5±2.5 sec), which was followed by spontaneous restoration of respiratory center activity; by the end of the first minute of glutamic acid application the PNA parameters exceeded their baseline values. The duration of the inspiratory phase increased by 17.2±4.2%, predominantly as a result of an increase in $+dt_{max}$ (by 43.5±11.0%). The time taken by the amplitude of PNA activity to increase to its maximum did not change significantly. The initial amplitude of PNA increased by 39.2±10.7% and its maximum amplitude by 47.5±14.2%. The expiratory phase and respiratory cycle duration increased by 41.7±29.9% and 29.2±18.9%, respectively ($p<0.05$).

During subsequent 10 min of glutamic acid application, the PNA parameters reached the maximum: the respiratory cycle length exceeded its baseline level by 82.2±21.3%, the inspiratory phase length by 42.2±9.0%, $+dt_{max}$ by 61.0±22.4%, the expiratory

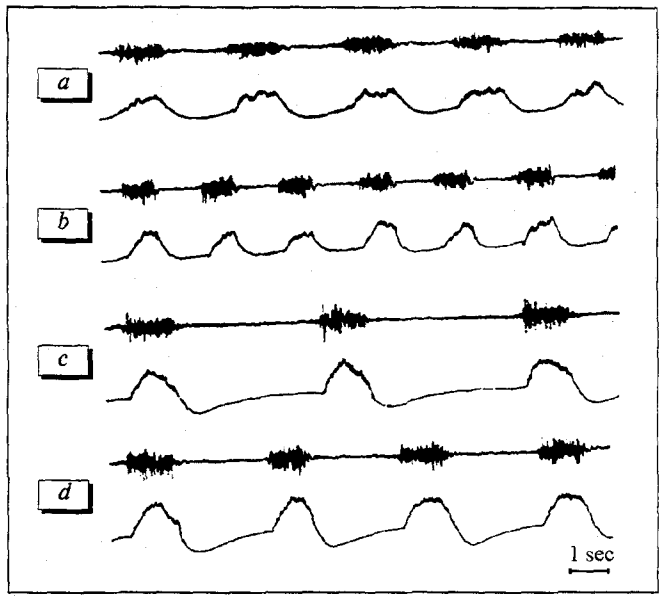

Fig. 1. Variations of phrenic nerve activity in response to hypercapnia before (*a* and *b*) and after (*c* and *d*) neuronal bilateral destruction (1 M glutamic acid) of the subretrofacial area. Upper curves: phrenic nerve activity; lower curves: envelopes of the curves.

TABLE 1. Responses of the Phrenic Nerve to the CO_2 Test Before and After Bilateral Application of 1 M Glutamic Acid to Subretrofacial Area ($M\pm m$)

Parameter	Baseline	CO_2 test	Acid applied for 10 min	CO_2 test
Respiratory cycle, sec	1.58±0.28	1.13±0.16*	3.27±0.38	2.06±0.63*
Inspiratory phase, sec	0.45±0.05	0.32±0.08*	0.66±0.04	0.43±0.14*
$+dt$, sec	0.27±0.03	0.15±0.05*	0.32±0.04	0.25±0.06
$+dt_{max}$, sec	0.18±0.02	0.17±0.03	0.31±0.03	0.18±0.07*
Postinspiratory phase, sec	0.20±0.03	0.20±0.03	0.25±0.04	0.23±0.05
Active expiration phase, sec	0.92±0.24	0.60±0.15*	2.33±0.37	1.38±0.51*
PNA_{ini}, rel. units	1.10±0.10	1.70±0.40*	1.40±0.10	1.40±0.10
PNA_{max}, rel. units	3.40±0.60	5.30±1.30*	4.6±0.7	4.70±0.10
PNA_{maxPI}, rel. units	1.80±0.40	2.9±0.30*	2.50±0.30	2.50±0.20
$+dt_{max}/+dt$, rel. units	0.70±0.01	1.17±0.24*	0.97±0.15	0.72±0.04*

Note. *$p<0.05$ relative to baseline. PNA = phrenic nerve activity. For other designations see text.

phase length by 117.8±44.8%, and $+dt_{max}/+dt$ by 39.3±22.7% ($p<0.05$); the amplitude of PNA discharges somewhat decreased but remained above baseline over that period (Table 1).

Histological examination of the sites to which glutamic acid had been applied in the SRF areas revealed irreversible destructive changes in their neurons near the VBS. These areas contained cells with severely deformed cytoplasm, a disintegrated nucleus with no nucleolus, and a homogeneous cytoplasmic basophilic substance that showed a tendency to hyperchromatosis. The contours of individual cells were indistinct. The mean depth of lesions was 581±163 μ.

Rats were exposed to hypercapnia both before and after glutamic acid application to the SRF areas. Phrenic nerve responses to hypercapnia after destruction of neuronal structures in these areas had the following characteristics (Fig. 1). The amplitudes of inspiratory and postinspiratory discharges and the time period during which these discharges reach the maximum did not change significantly. The PNA_{ini}, PNA_{max}, and PNA_{maxPI} were 30.7±11.9%, 30.2±9.1%, and 31.8±6.9% below their baseline values, respectively ($p<0.05$). The length of the postinspiratory phase showed no significant changes (both before and after glutamic acid application). The inspiratory phase and $+dt_{max}$ responded to the hypercapnic stimulus by decreasing their length, as did the expiratory phase ($p<0.05$).

Thus, neuronal structures of SRF area are involved in regulating the amplitude and velocity of the inspiratory mechanism. Bilateral local destruction of neuronal structures in this area by glutamic acid (1 M) applied to the rostral parts of the VBS increased the amplitude of PNA and markedly decreased the frequency of the respiratory rhythm generation. Under these conditions, hypercapnia had no effect on the PNA amplitude, but altered the plateau time of PNA and increased respiratory rate. The amplitude of central inspiratory activity is probably regulated by the central chemoreceptors with participation of structures present in the SRF area neurons.

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