PHYSIOLOGY

Respiratory Response to Microinjections of GABA and Penicillin into Various Parts of the Ventral Respiratory Group

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Experiments on rats showed that local injection of GABA (10^{-4} M) into the rostral and caudal compartments of the ventral respiratory groups decreased the respiratory rhythm, but increased lung ventilation (especially injection into the rostral part). Penicillin (10^{-7} M) injected into the rostral division increased the tidal volume and practically did not change the respiratory rate, but its injection into the caudal part reduced the tidal volume and increased respiratory rate. These results indicate that GABAergic mechanisms including $GABA_A$ sites play an ambiguous role in the regulation of respiration at the level of the rostral and caudal parts of the ventral respiratory group.

Key Words: *ventral respiratory group; GABA; penicillin; breathing pattern*

GABA plays an important role in the mechanism of central respiratory neural network. This is confirmed by changes in respiration patterns induced by intracisternal injection of GABA and its derivatives [1] or their local application on CNS structures [5] including bulbar respiratory center (RC) [11]. The range of respiratory effects caused by GABA at the level of bulbar neurons includes the formation of reciprocal inhibition in neural ensembles of RC [3], suppression of respiratory rhythm [9] and changes in respiratory volume parameters [4]. Despite proven GABAergic modulation of respiration, the mechanisms of GABA receptors involvement in activity of functionally different RC parts are not fully understood and the role of inhibitory amino acids in RC function is still debated [1,6]. In particular, the role of GABAergic systems in the function of the ventral RC containing populations of neurons involved in respiratory rhythm and pattern formation remain unclear.

Here we studied breathing patterns under conditions of stimulation and blockade of GABA receptors in the rostral and caudal parts of the ventral respiratory group (rVRG and cVRG, respectively).

MATERIALS AND METHODS

Acute experiments were performed on 24 outbred rats weighing 200-300 g intraperitoneally narcotized with 1.6 mg/kg urethane. After tracheotomy animal's head was fixed in stereotaxis in ventral flexion. Trepanation of the occipital bone was performed to make an access to the dorsal surface of the medulla oblongata and to make microinjections into RC. GABA (10^{-4} M) and penicillin (10^{-7} M) were dissolved in artificial cerebrospinal fluid *ex tempore*. The substances in a volume of 0.2 μl were stereotactically injected into rVRG and cVRG [14]

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through a glass micropipette (tip diameter 15-20 μ) using an MSh-1 microsyringe.

The breathing pattern was evaluated by spirogram. To this end, a plastic cannula was introduced in the trachea and connected with the spirograph. The signal passed through an analog-to-digital converter and was displayed on a monitor. Recording was performed with the program PowerGraph 3.2 Professional (Interoptika-C) before and 1, 5, 10, 15, 20, 25, and 30 min after microinjection.

Significance of differences was evaluated by one-way ANOVA.

RESULTS

Decelerated respiratory rhythm was the most common respiratory effect of GABA-receptors activation in studied RC parts. However, the decrease in respiratory rate observed after GABA injections into rVRG was 2-fold more pronounced than injection into cVRG (21.3 and 9.5%, respectively, $p<0.01$). This difference was due to the fact that phases of the respiratory cycle changed in the reverse order. Namely, the duration of the inspiratory phase decreased regularly with a maximum shortening by 21.6% on the 20th minute after GABA injection into rVRG. This effect was associated with an increase in expiratory time by 24.6% from baseline (Fig. 1, *a*, left). In contrast, GABA injection into cVRG prolonged both respiratory phases, but to a different extent: the time of inspiration rapidly increased reaching the maximum by the 5th minute (by 32.4%), but the increase in the expiratory phase did not exceed 6% and this response developed not earlier than 15 min postin-

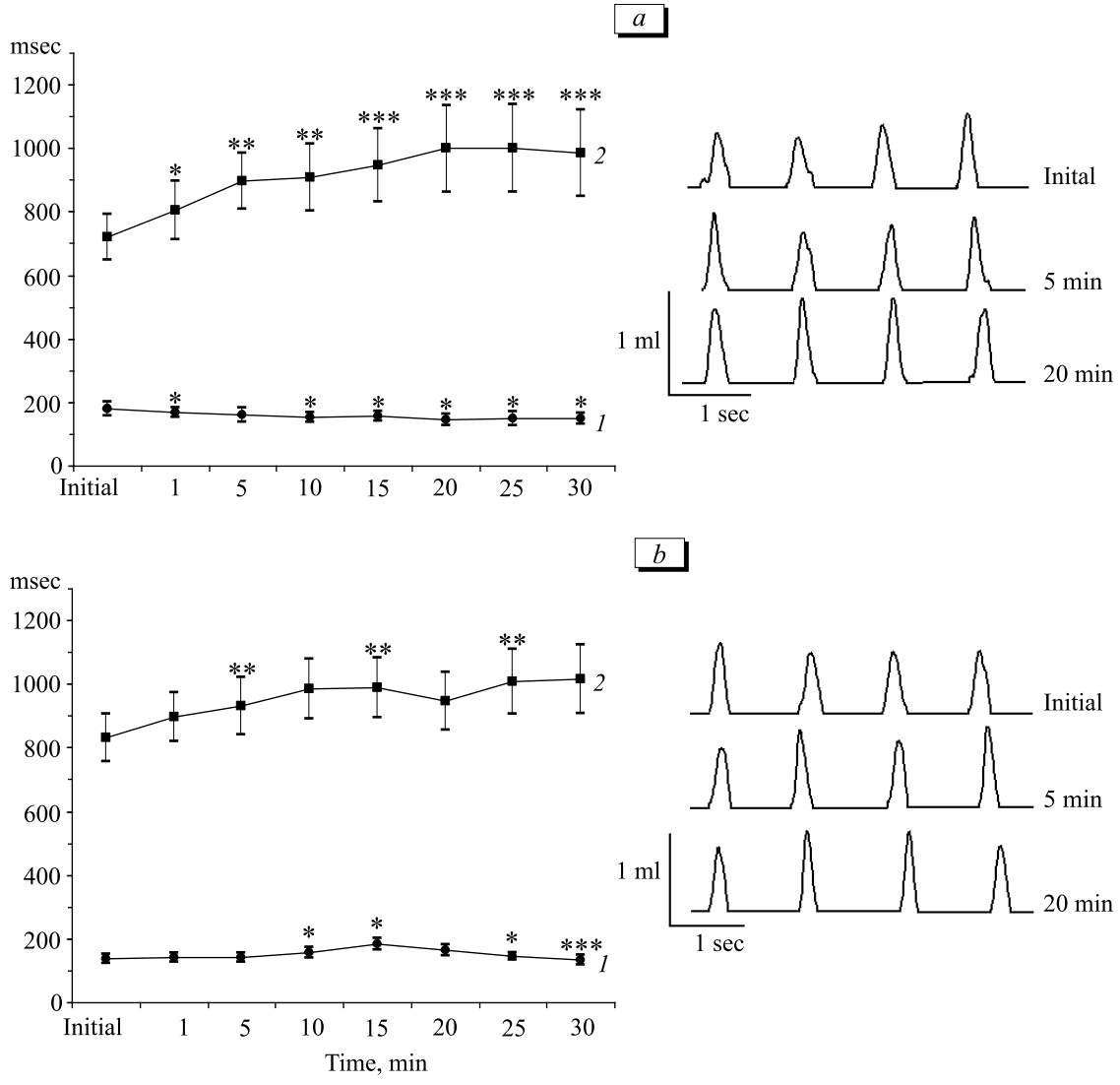

Fig. 1. Changes in breathing pattern at different times after microinjection of GABA (10^{-4} M) into rVRG (*a*) and cVRG (*b*). Here and in Fig. 2: left: dynamics of inspiration (*1*) and expiration time (*2*). Right: spirograms. *$p<0.05$, **$p<0.01$, ***$p<0.001$ in comparison with initial values.

jection (Fig. 1, *b*; left). Another feature of GABA action on rVRG and cVRG was that the changes in spirogram amplitudes were similarly directed, that is, tidal volume and pulmonary ventilation increased with increasing the time of GABA action. These responses were more pronounced after injections of GABA into rVRG. In this case, these parameters increased by 28.7 and 46.2%, respectively, by the end of observation, whereas after injections into cVRG, only by 18.8 and 35.8% (Fig. 1, *a*, *b*; right).

Injections of penicillin, a noncompetitive blocker of $GABA_A$-receptors, into RCA induced different changes in the breathing pattern. Injection into rVRG prolonged the expiratory time (by 28.4%) and shortened the inspiratory time (by 14.1%) on the 20th minute of exposure (Fig. 2, *a*; left). At the same time, the depth of inhalation increased by 23.5% as well as pulmonary ventilation, by 21.7% (Fig. 2, *a*; right). Blockade of GABA-receptors in rVRG induced a transient increase in respiratory rate followed by its decrease after the 10th minute, which clearly coincided with prolongation of expiration. Injection of penicillin into cVRG consistently produced more frequent breathing (by 19.1%), mainly at the expence of reduction of the expiratory time. Under these conditions, inspiratory time increased and tidal volume decreased by 13.8% (Fig. 2, *b*).

Thus, the pattern of external respiration depends on activity of GABAergic mechanisms in rVRG and cVRG. Specific respiratory effects of GABA and penicillin on rVRG and cVRG (for example, opposite changes in inspiration and expiration) can be related, on the one hand, to some features of cell organization, since inspiratory neurons dominate in rVRG and exspiratory neurons in cVRG [3,10]. On the other hand, these effects can be due to different number of GABA-receptors or uneven density of GABA-sites of the certain class in these parts of RC. $GABA_A$-

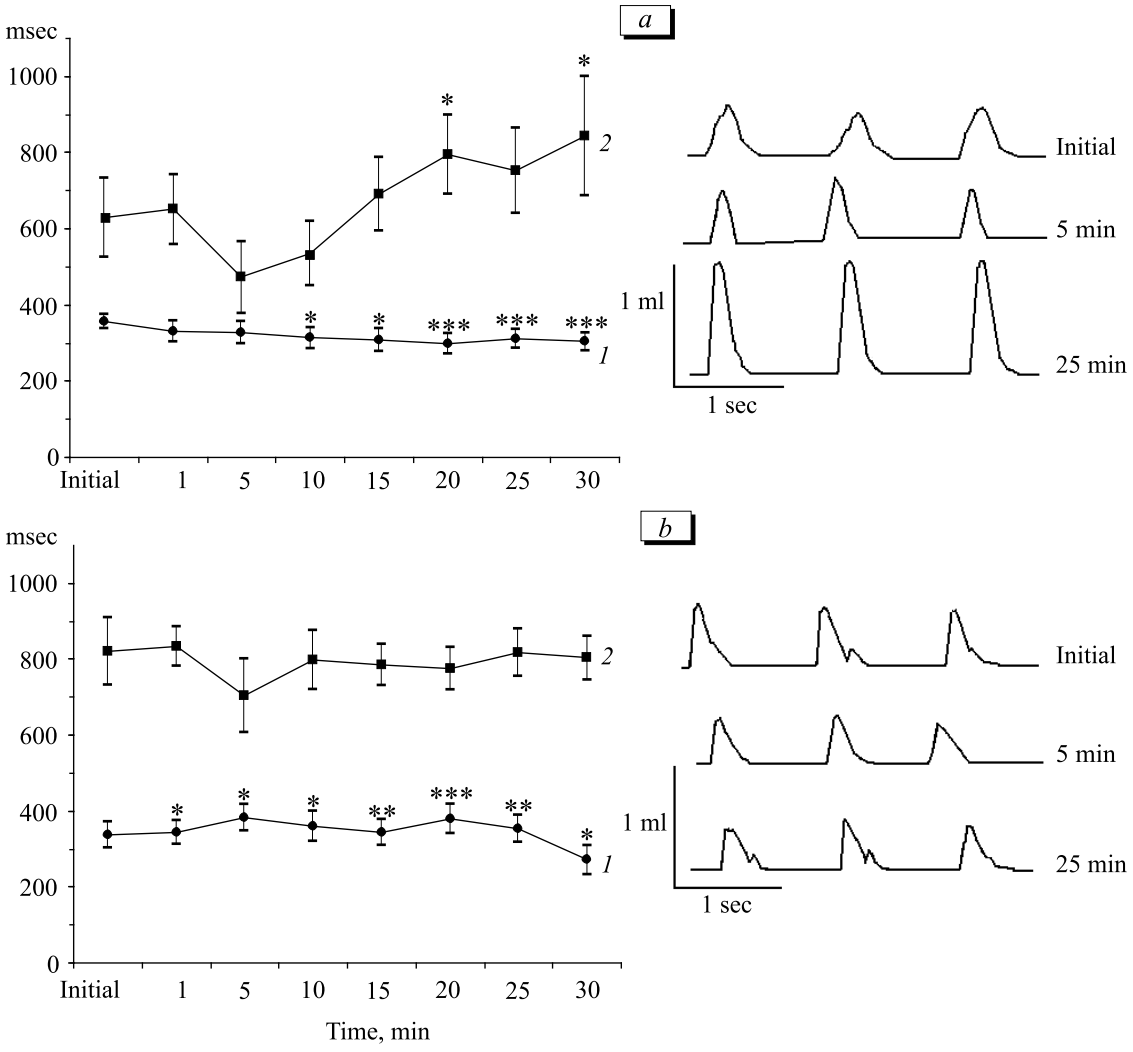

Fig. 2. Changes in breathing pattern at different times after microinjection of penicillin (10^{-7} M) into rVRG (*a*) and cVRG (*b*).

receptors play a more important role in the respiratory neural network, because they are involved in the processes of tonic inhibition required for the regulation of inspiratory rhythm and expiratory motor activity [11,13]. $GABA_A$-receptors enable phase waves of inhibitory potentials at rest and during discharge of respiratory neurons, change their activity pattern [12] and, thus, the breathing pattern in general [8]. Based on these data, the decrease in respiratory rate after GABA injection into rVRG can be explained by inhibition of inspiratory neurons mediated by $GABA_A$ receptors, while weakening of the respiratory rhythm after injection of the transmitter into cVRG was probably a consequence of $GABA_A$-ergic inhibition of expiratory neurons. This conclusion is consistent with the fact that penicillin that affects primarily $GABA_A$ receptors blocks GABAergic mechanisms in rVRG and cVRG. Based on the fact that the alterations in the breathing pattern after injection of GABA and its antagonist are more pronounced and considering the data from previous studies on injections of bicuculline into RC [2], we can assume that the density of $GABA_A$-receptors in rVRG is slightly higher than in cVRG.

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