GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Effect of Penicillin on Impulse Activity of Medullar Neurons

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Acute experiments on narcotized rats showed that intracerebral injection of penicillin increased the mean discharge rate in neurons of the bulbar respiratory center and neurons with rhythmic activity in the gigantocellular nucleus of the medulla oblongata. It also increased the number of neurons with high-frequency bursting activity. In most inspiratory neurons penicillin prolonged the discharges and increased the number of spikes in bursts. The population of inspiratory neurons became hyperactive and suppressed activity of expiratory neurons.

Key Words: *medulla oblongata; respiratory neurons; impulse activity; penicillin; apneusis; gasping*

Among the factors, which modern hypotheses recruit to explain the development of pathological types of respiration produced by central abnormalities, a particular role is given to attenuation of inhibitory influences in the network of bulbar neurons involved in respiratory control [6,9]. However, neurophysiological mechanisms underlying generation of respiratory rhythm remain unclear. It was found that disturbances in inhibitory synaptic processes in the medulla oblongata, which can be modeled with convulsants, are accompanied by enhanced background and evoked activity [1], increased number of excited neurons and their spatial redistribution [4]. In most cases, these changes in neural characteristics of medullar respiratory center (RC) induce apneustic pathological respiratory rhythm and in some cases provoke gasping [3].

Our aim was to study the contribution of integral impulse activity of the neurons from RC and gigantocellular nucleus (GN) belonging to the reticular formation in the medulla oblongata into the development of pathological rhythmicity provoked by cerebral microinjections into these structures.

MATERIALS AND METHODS

Experiments were carried out on 75 white male Wistar rats weighing 250-300 g. The rats were anesthetized with chloral hydrate (400 mg/kg intraperitoneally). The animals were kept under standard vivarium conditions with food and water *ad libitum* and 12-h light-darkness regimen. Electrical activity of the diaphragm was recorded with bipolar Nichrome electrodes connected to a VC-9 oscilloscope (Nihon Kohden), and stored on a magnetic type. To provide free access to the rhomboid fossa, the occipital bone was removed and the cerebellum was partially extirpated. The surface of medullar oblongata was covered with warm mineral oil protecting the brain from cooling and drying. Cerebral injections of benzylpenicillin sodium salt (50,000 U in 1 ml 0.9% NaCl) were performed into the regions of solitary tract nucleus, ambiguous nucleus, and GN using an oil microinjector delivering up to 1 μl solution via a 50-μ glass cannula. The ste-

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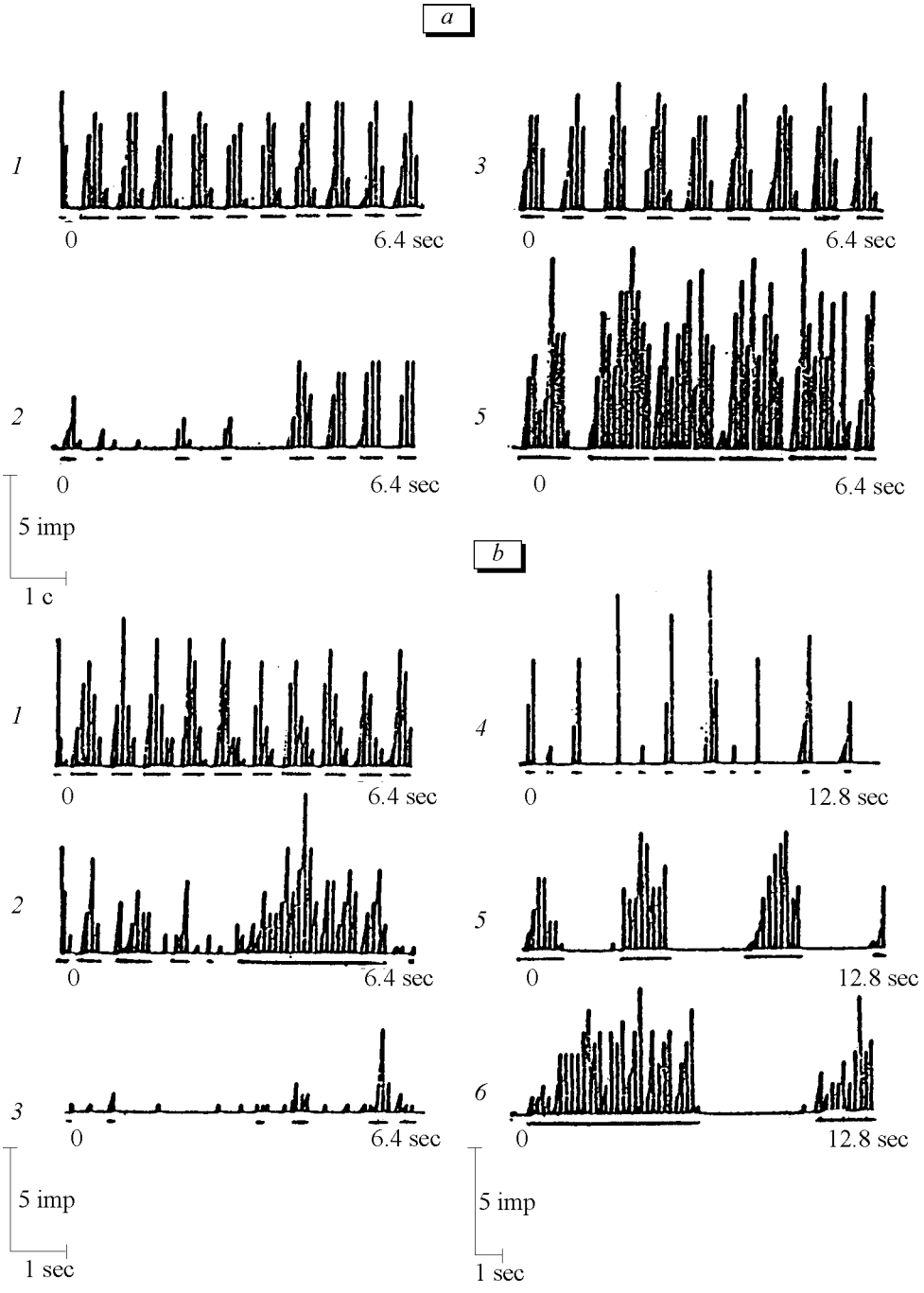

Fig. 1. Successive histograms of mean current discharge rate of two individual (*a* and *b*) inspiratory neurons before (*1*), immediately after (*2*) and after 1 (*3*), 3 (*4*), 5 (*5*), and 10 (*6*) min of penicillin injection. Here and in Fig. 3 the dash line corresponds to inspiratory phase of diaphragm activity.

reotaxic coordinates were calculated according to the rat brain atlas [11]. Activity of neurons in the examined structures before and after injection of the convulsant was recorded extracellularly with glass microelectrodes filled with 2.5 M NaCl solution according to the standard electrophysiological routine. The signals were amplified and stored on the magnetic type. Neural activity was processed on an ATAC-350 Averager (Nihon Kohden). The discharge patterns and the

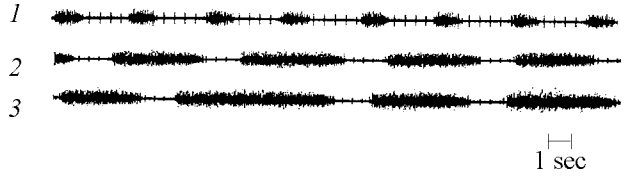

Fig. 2. Changes in electrical activity of diaphragm manifested by prolongation of inspiration and shortening of expiration characteristic of apneustic breathing. *1*) baseline activity; *2*) and *3*) diaphragm activity 5 and 10 min after cerebral injection of penicillin, correspondingly.

mean discharge frequency were determined, and the histograms of current mean frequency and interspike intervals were analyzed. In the control, the impulse activity of neurons was examined after cerebral microinjection of physiological saline.

The results were analyzed statistically using Student's *t* test.

RESULTS

Statistical analysis of interspike interval histograms of RC neurons before (n=53) and after (n=70) penicillin microinjection revealed 52% neurons with unimodal distribution of interspike intervals, 33% neurons with bi- or multimodal distribution, and 15% neurons with exponential distribution. Injection of penicillin into RC redistributed the shares of neurons with specific interspike intervals distribution: the fraction of neurons with unimodal and exponential distributions increased to 57% and 24%, correspondingly. Previously we showed a similar increase in the number of GN neurons with exponential distributions of interspike intervals [4]. This may attest to promoting the processes, which disorganize the impulse traffic in these structures. The total mean discharge rate of RC neurons increased from 13.02±0.85 to 15.95±0.77 Hz (p<0.05). In control experiments (n=30), impulse activity of neurons assessed by the total mean discharge rate and the ratio of neurons with individual type of activity did not change.

The most instructive phenomenon was the change in impulse activity of respiratory neurons. The time course of the changes in impulse activity of inspiratory neurons (IN) during the development of pathologic apneustic breathing is illustrated by the histograms of the current mean discharge rate (the figures demonstrate only the most indicative cases). In one case (Fig. 1, *a*) penicillin injection produced a short-term inhibition of IN discharges. In the following, IN resumed the rhythmic discharges with a certain prolongation of the bursts, which induced the prolonged bursts in electromyogram during apneustic inspirations (Fig. 2). In another case (Fig. 1, *b*), penicillin induced a single long-term discharge of IN followed by its short-term inhibition, after which the powerful short-term busts were observed during gasping. In the following, duration of IN discharges increased almost 10-fold, which was accompanied by the long-term diaphragm contractions reflected in myogram and prolongation of the expiratory phase.

The changes in impulse activity of expiratory neurons (EN) during the development of pathological respiratory rhythmicity were inhibitory. In one case, penicillin decreased the number of spikes in the bursts of EN (Fig. 3, *a*), while in other cases such decrease was also accompanied by shortening of the bursts (Fig.

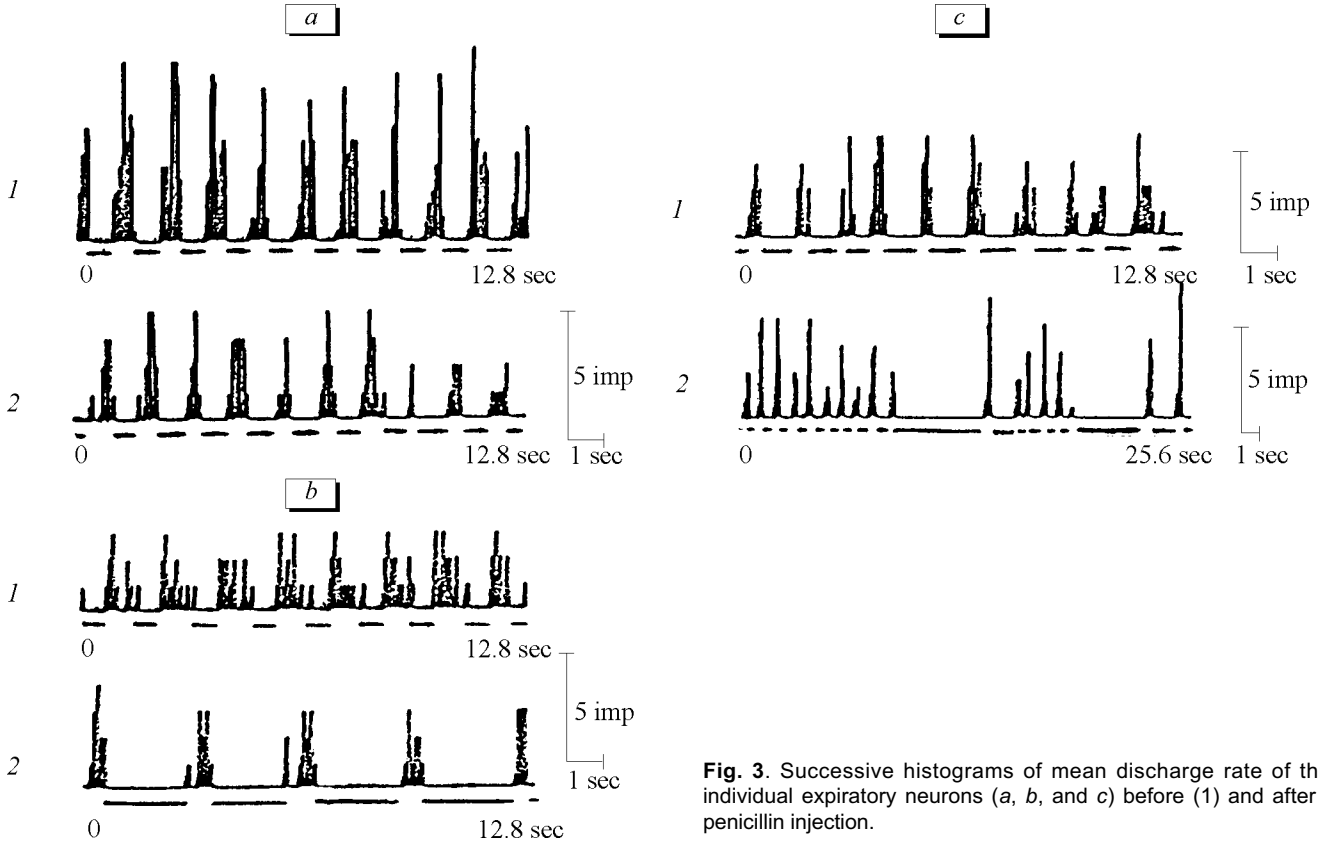

Fig. 3. Successive histograms of mean discharge rate of three individual expiratory neurons (*a*, *b*, and *c*) before (1) and after (2) penicillin injection.

TABLE 1. Ratios of the Numbers of GN Neurons with Various Activity Patterns in Discharge Rate Cluster Distribution Before/After Penicillin Injection

Mean discharge rate, Hz	Activity pattern					
	Rhythmic		Arrhythmic		Burst-like	
	abs.	%	abs.	%	abs.	%
0-6	16/19	23/11	3/24	14/40	9/19	28/22
7-12	24/55	34/33	10/16	46/27	13/21	41/24
13-20	17/42	25/25	7/17	31/28	4/21	12/24
21 and more	13/50	18/31	2/3	9/5	6/25	19/30

3, *b*) and inhibition of the activity during apneustic inspirations (Fig. 3, *b*, *c*).

The neurons of GN were characterized by rhythmic (64.0%), arrhythmic (13.5%), and burst (22.5%) activities. Penicillin modified the ratio of neurons with the above activity patterns: the fraction of neurons with rhythmic activity decreased to 53%, while the fraction of neurons with arrhythmic and burst activity increased to 20% and 27%, correspondingly.

Statistical analysis of histograms of interspike intervals of GN neurons before (n=124) and after (n=312) penicillin microinjection showed that convulsants increased the mean discharge rate of rhythmic neurons from 13.35±0.93 to 18.39±1.09 Hz (p<0.001). In control experiments, impulse activity of neurons (n=50) was assessed by the mean discharge rate of neurons with individual activity patterns. This activity and the ratios of the number of neurons with individual type of activity did not change.

The cluster discharge rate distribution of GN neurons revealed additional regularities in neuronal organization of the examined structure. For example, under normal conditions, most neurons with rhythmic activity fired at 7-20 Hz, neurons with arrhythmic activity fired at 7-12 Hz, and "burst" neurons discharged mainly at middle and low frequencies from 0 to 12 Hz (Table 1). Microinjection of penicillin changed these indices significantly: the share of neurons with rhythmic activity at very high discharge rate increased (Table 1); most arrhythmic neurons fired at low frequency of less than 6 Hz. The number of "burst" neurons increased, and most of them began to discharge at very high frequency of more than 20 Hz.

Thus, microinjection of penicillin increased the mean discharge rate of respiratory and reticular neurons in RC structures. In GN the mean discharge rate of rhythmic neurons and the number of arrhythmic and "burst" neurons increased. Similar changes of impulse activity were observed in neurons from the hyperactive structure in the experiments with pain syndrome models [2,7]. These observations are consistent with the data on possible participation of reticular neurons in the development of respiratory pathology: for example, tetanic stimulation of GN provoked inspiratory apneusis [10].

In most IN, duration of discharges and the number of spikes in each burst increased, so the population of IN became hyperactive. According to the hypothesis on pronounced contribution of reciprocal connections between the respiratory neurons [5,8] into generation of respiratory periodicity, this hyperactive IN inhibited activity of EN. The fact that a local disturbance in the mechanisms of inhibitory synaptic transmission within the limits of bulbar respiratory neural network leads to successive manifestations of apneustic respiration and gasping refutes the hypothesis on spatial separation of the centers responsible for generation of apnea, gasping, and apneusis.

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