Spectral Parameters of Phrenic Nerve Activity in Mature Rats during Electrical Stimulation of Retrotrapezoid Nucleus

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In mature rats, electrical stimulation of the retrotrapezoid nucleus increased the amplitude and frequency of the high-frequency peak in the firing spectrum of the phrenic nerve, while the amplitude of medium-frequency peak and the amplitude ratio of medium- to high-frequency peaks decreased. These changes in spectral parameters were associated with accelerated increase in central inspiratory activity, decreased amplitude of phrenic nerve firing, and increased frequency of respiratory rhythm. It is hypothesized that being a relay structure of central chemosensitive mechanism, the retrotrapezoid nucleus regulates parameters of medium- and high-frequency spectral peaks of efferent electrical activity in the respiratory center together with the dorsal and ventral respiratory groups.

Key Words: *retrotrapezoid nucleus; phrenic nerve; spectrum analysis; rat*

Retrotrapezoid nucleus (RTN) located in the rostral ventrolateral aspects of the medulla oblongata is an important structure of the brainstem involved in the regulation of central inspiratory activity of the respiratory center [4,6]. Tonic and phasic neurons were found in this nucleus, whose activity is related to both phases of respiratory cycle, and which elevate their firing rate during hypercapnia [12]. The neurons of RTN perform the relay function in the central chemosensitive mechanism, which regulates activity of the respiratory center. The direct inputs from RTN neurons to neurons of the ventral and dorsal respiratory groups were identified [9]. Local acidosis in RTN region caused by acetazolamide or stimulation of glutamate receptors in RTN neurons by agonists of NMDA- or non-NMDA receptors elevated the frequency of the respiratory rhythm and the amplitude of phrenic nerve firing [11]. Under conditions of unilateral destruction of RTN in narcotized and decerebrated rats, the amplitude of phrenic nerve firing decreased and respiratory response to hypercapnic stimulation declined [13]. In spontaneously breathing narcotized rats, this destruction decreased the frequency of respiratory rhythm and eliminated increment in phrenic firing to hypercapnic stimulation [3,14]. Thus, while performing the relay function in the central chemosensitive mechanism, RTN neurons can affect the frequency and amplitude of efferent discharges of the respiratory center. These changes are related to changes in the spectral parameters of electrical activity of neurons in the respiratory center [1]. Since there are no data on the effect of RTN on spectral parameters of efferent firing of respiratory center, our aim was to fill this gap.

MATERIALS AND METHODS

Experiments were carried out under sodium etaminal narcosis (40 mg/kg intraperitoneally) on spontaneously breathing albino rats of both sexes (n=16) weighing 200-300 g. Body temperature was maintained at 37°C using a rectal thermometer and a heater. Ventral surface of the medulla oblongata was opened from the middle part of cranial nerve XII roots to the orifice of

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cranial nerve VII-VIII roots and 4.0-4.5 mm laterally to the median line.

Electrical stimulation of RTN neurons was performed via bipolar tungsten electrodes (interelectrode distance 50 μ) with series of rectangular pulses (repetition rate 100 Hz, amplitude 1-20 μA, duration 0.5 msec) generated by an ES-50-1 stimulator. A fragment of the right phrenic nerve was isolated to the length of 4-6 mm immediately after fusion of its roots C4-C5 and cut. The central end of the nerve was mounted on bipolar silver electrodes and bathed in warm mineral oil. The nerve signals were amplified, digitized, and fed into PC for monitoring and recording. The amplitude and temporal parameters of central inspiratory activity of respiratory center were analyzed [2].

Assessment of spectral density of respiratory discharges was performed after preliminary filtering of amplified electrical signal from the phrenic nerve (1.5-150.0 Hz) using fast Fourier transform at the sampling rate of 500 Hz. Spectrum analysis of phrenic nerve discharges was carried out with the epoch of 300 msec (1024 points, 3.9 Hz). In addition, two epochs of 150 msec (1024 points, 7.6 Hz) were used to analyze the first and second halves of the respiratory burst. The parameters of spectrogram were averaged from 10 successive respiratory bursts.

The data were processed statistically using Student's t test at $p<0.05$ and presented as $M\pm m$.

RESULTS

Initially, the firing rate of the phrenic nerve, the burst duration, and the mean period of respiratory cycle were 26.6±0.8 min^{-1}, 0.53±0.01 sec, and 2.25±0.9 sec, respectively. The power spectrum of the discharges had two peaks in the frequency bands of 29-45 and 70-81 Hz known as medium- (MFP) and high-frequency (HFP) peaks, respectively [7,10]. In 66% cases, both peaks were present in the power spectrum (Fig. 1, *c*). In other cases (34%), the power spectrum of phrenic firing had only MFP with a midband frequency of 37.7±1.9 Hz. Analysis of the fragments of respiratory bursts showed that the amplitude and midband frequency of MFP did not significantly differ in the first and second halves of the phrenic respiratory bursts. By contrast, in the second half of respiratory burst, the midband frequency of HFP (78.6±1.9 Hz) was significantly greater than that in the first half (72.0± 1.5 Hz). In addition, the amplitude of HFP was greater by 10.0±1.3% in the later half of the bursts (Fig. 2, *a*, *b*).

Electrical stimulation of the rostral ventrolateral divisions of the medulla oblongata induced maximum

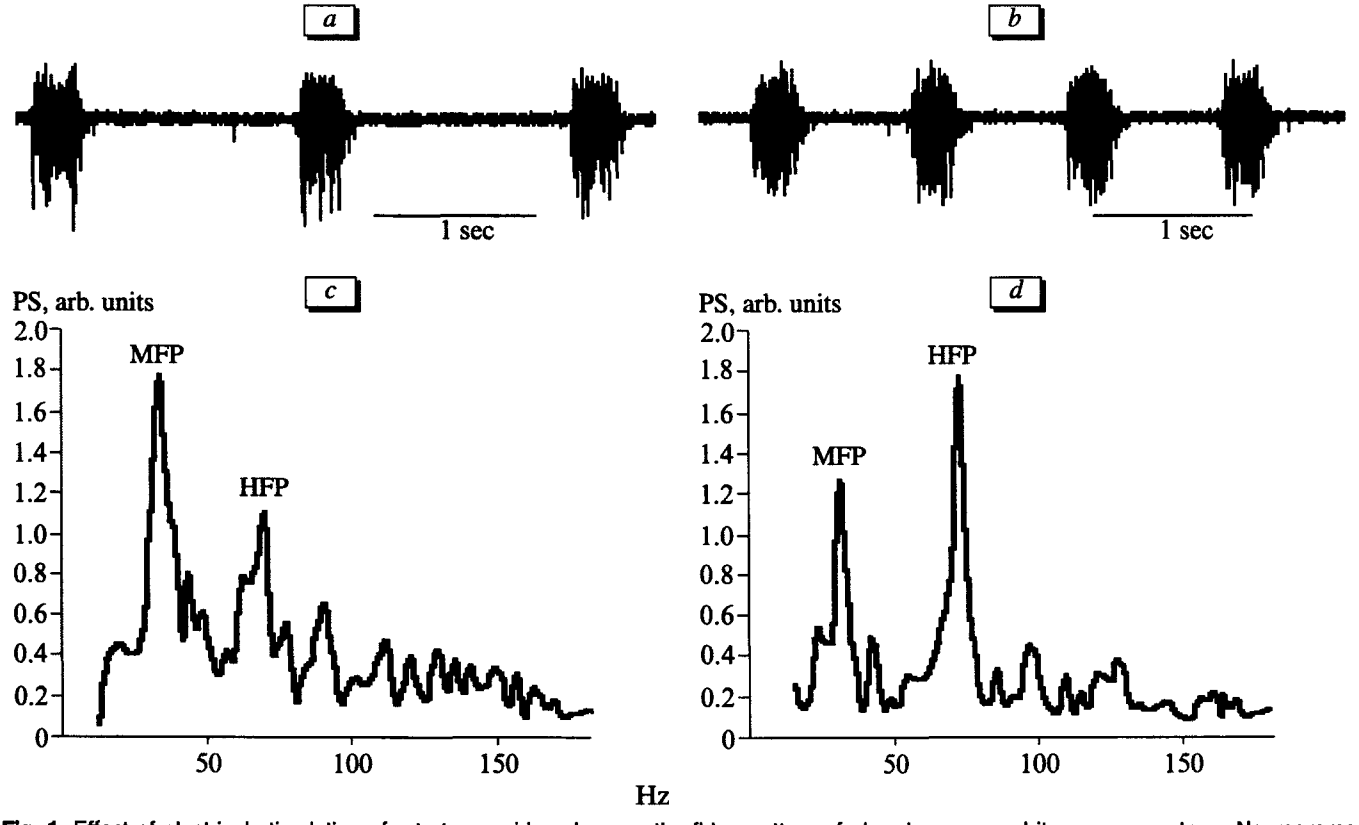

Fig. 1. Effect of electrical stimulation of retrotrapezoid nucleus on the firing pattern of phrenic nerve and its power spectrum. Neurograms and spectral plots of phrenic nerve discharges are shown before (*a*, *c*) and during (*b*, *d*) stimulation of retrotrapezoid nucleus. Here and in Fig. 2: PS, power spectrum; MFP, medium-frequency peak; HFP, high-frequency peak.

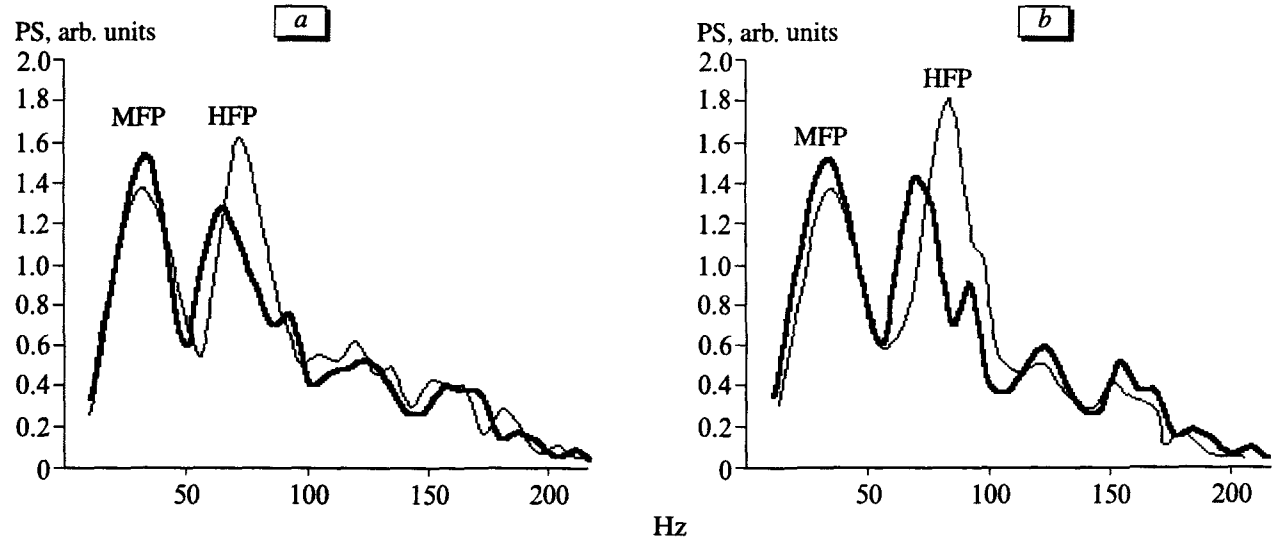

Fig. 2. Effect of electrical stimulation of retrotrapezoid nucleus on power spectrum of phrenic nerve discharges. *a*) power spectrum of the first half of respiratory burst before (solid line) and during (thin line) stimulation of the nucleus; *b*) corresponding plots for the second half of respiratory burst.

changes in phrenic nerve discharges, when the tip of the stimulating electrode was introduced at a depth of 200-500 μ below the ventral surface, 0-1 mm caudally to the trapezoid bodies, and 2-3 mm laterally to the median sulcus. Topographically, this zone was located immediately below the neurons of facial nerve at the supine position and corresponded to the location of RTN [9].

Electrical stimulation of RTN with 6-10 μA current decreased the period of respiratory cycle due to shortening of phrenic burst time and expiration pause between the bursts by 24.6±3.4% and 40.5±3.1%, respectively (Fig. 1, *a*, *b*). In addition, this stimulation modified the parameters of discharge pattern in the phrenic nerve, which reflected the changes in generation of inspiratory activity in the respiratory center. These parameters were the uprise rate of central inspiratory burst and duration of plateau of phrenic activity. They decreased by 17.7±2.7% and 33.7±3.4%, respectively ($p<0.05$). Electrical stimulation of RTN decreased the duration of postinspiratory phase in the phrenic nerve firing by 18.7±2.5%. These changes were observed against the background of decreased amplitude of the discharges by 18.3±3.1% relative to the baseline (Table 1).

Electrical stimulation of RTN decreased the amplitude of MFP by 12.2±1.4%, but increased the amplitude of HFP by 18.1±2.0% in comparison with the baseline. As a result, the amplitude ratio MFP/HFP decreased by 24.7±2.9% ($p<0.05$). In addition, this stimulation increased the midband of HFP (Fig. 1, *d*).

The spectrum analysis of the fragments of respiratory bursts during stimulation of RTN revealed a decrease in the amplitude of MFP by 14.3±1.9% in both halves of the bursts in comparison with the baseline (Fig. 2, *a*, *b*). In addition, the amplitude ratio MFP/HFP decreased by 25.8±3.2% ($p<0.05$). These changes were paralleled by an increase in the midband frequency of HFP in the first and second fragments of respiratory bursts. In fragment 1, stimulation of RTN increased the HFP midband from 72.0±1.5 Hz to 85.1±1.1 Hz ($p<0.05$), while in fragment 2 it shifted this parameter from 78.6±1.9 to 92.0±1.5 Hz ($p<0.05$, Fig. 2, *a*, *b*).

Most researchers explain the origin of HFP with activity of the dorsal respiratory group [5]. In our experiments, electrical stimulation of neuronal structures in RTN increased the frequency content and power of HFP in phrenic firing. These changes can reflect acti-

TABLE 1. Effect of Electrical Stimulation of Neuronal Structures in Retrotrapezoid Nucleus on Parameters of Phrenic Nerve Firing

Parameter	T_i, sec	+dt, sec	$+dt_{max}$, sec	ADH_{max}, rel. units	T_{pi}, sec
Prior to stimulation	0.360±0.013	0.210±0.005	0.150±0.008	33.1±1.3	0.170±0.004
During stimulation	0.270±0.004*	0.170±0.004*	0.100±0.004*	27.5±1.2*	0.130±0.003*

Note. T_i is duration of inspiratory phase; $+dt$, uprise time of central inspiratory activity to maximum; $+dt_{max}$, duration of plateau of phrenic nerve burst; ADH_{max}, plateau amplitude; T_{pi}, duration of postinspiratory phase. *$p<0.05$ compared to baseline values before stimulation.

vation of neurons in the dorsal respiratory group by RTN, which is corroborated by a decrease in duration of maximum amplitude plateau of phrenic bursts corresponding to the period of electrical activity of different types of inspiratory throughout neurons of the dorsal respiratory group.

The nature of MFP in efferent inspiratory activity of the respiratory center is not clear. It can be hypothesized that the decrease in the amplitude of MFP in the spectrum of phrenic firing during electrical stimulation of RTN is caused by modification of neuronal activity in the ventral respiratory group. This is indicated by direct connections of RTN neurons with cells in the ventral respiratory group [9]. This hypothesis is also corroborated by acceleration of the respiratory rhythm and shortening of postinspiratory activity in phrenic nerve discharges during electrical stimulation of RTN, since both processes relate to the work of the ventral respiratory group.

Electrical stimulation of RTN affected both components of the power spectrum of phrenic discharges (MFP and HFP) during the entire period of generation of inspiratory discharges by the respiratory center.

The revealed decrease in MFP/HFP ratio suggests that during electrical stimulation of RTN, its neuronal structures simultaneously modify electrical activity in neuronal populations of the ventral and dorsal respiratory groups. These changes can underlie accelerated increase of central inspiratory activity to its maximum, decrease in the amplitude of phrenic discharges, and increase in the frequency of respiratory rhythm. Probably, the role of RTN as the relay structure in the central chemosensitive mechanism consists in the regulation (together with the dorsal and ventral respiratory groups) of parameters of MFP and HFP in efferent electrical activity of the respiratory center.

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