ELECTROPHYSIOLOGICAL ESTIMATION OF THE STRENGTH OF PROTECTIVE RESPIRATORY REFLEXES

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Projective respiratory reflexes are an important link in the general regulatory chain of the respiratory system. They differ from other respiratory reflexes in their extraordinarily great strength as a result of prolonged stimulation of the corresponding receptor zones of the nasopharynx, bifurcation of the trachea, bronchi, and lungs [1-3, 6].

The time course of respiratory reflexes can be studied only by methods possessing highly dynamic characteristics (microelectrode and stereotaxic techniques, electromyography).

The aim of this investigation was to study the strength of protective respiratory reflexes in cats under normal conditions and in a modified atmosphere.

EXPERIMENTAL METHOD

Experiments were carried out on 21 male and female cats weighing 2.4-3.0 kg, anesthetized with pentobarbital (40 mg/kg intraperitoneally). Discharges of the respiratory neurons were recorded in the region of the dorsal and ventral respiratory nuclei, according to Szentagothai's (1957) atlas. The thickness of the microelectrode tip was 3-5 μ and its resistance 20 kΩ. Electromyograms (EMG) of the diaphragm, the external and internal intercostal muscles, and the obliquus abdominis muscle, and the intrapleural pressure were recorded synchronously. The potentials were amplified and recorded with a Disa 13 A 69 electromyograph. The expiratory reflex was evoked by touching the vocal ligaments from the side of the trachae with a loop of silon fiber. The cough reflex arose in response to stimulation of receptors in the laryngopharyngeal and tracheobronchial regions with a silon fiber. A hypoxic gas mixture (10% O_2 in N_2) and a hypercapnic mixture (10% CO_2 in air) were used for breathing. Background activity and activity during recovery were recorded during breathing of atmospheric air.

EXPERIMENTAL RESULTS

The expiratory reflex (ER) was reflected in the EMG by a short and powerful (in amplitude and frequency of spikes) volley of the expiratory muscles. Characteristically the volley appeared to consist of two discharges: the first arose at the moment the silon loop was introduced into the larnyx, the second at the moment it was removed. Weak electrical activity appeared in the short interval between discharges in the diaphragm. This was the beginning of the stretch reflex of the lungs in response to their sudden collapse. However, the reflex could not be manifested to the full: it was depressed at its beginning by the second discharge of the expiratory muscles. Thus ER reappeared at the moment the silon loop was withdrawn from the larynx. Under these circumstances ER ended, but the characteristic after-response remained. This took the form of a prolonged volley with high-amplitude spikes in the diaphragm, appearing immediately after the end of ER. Strong inspiration after ER facilitated stronger expiration, which was followed by recovery of electrical activity (Fig. 1a). Judging by the time course of electrical activity of the respiratory muscles, a powerful focus of excitation appeared in the respiratory center (RC) during ER, and it was maintained during continuing stimulation of receptors located in the region of the larynx.

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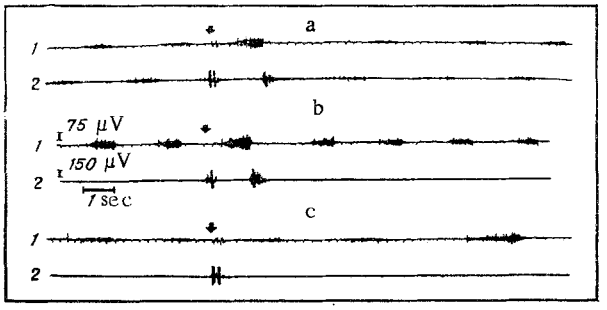

Fig. 1. ER during breathing of atmospheric air (a), a hypercapnic gas mixture (b), and a hypoxic gas mixture (c). Here and in Figs. 2 and 3: 1) EMG of diaphragm; 2) of obliquus abdominis muscle. Arrow indicates time of stimulation.

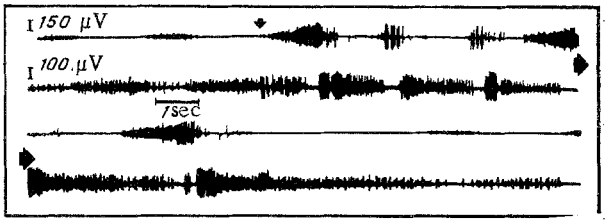

Fig. 2. Cough reflex evoked by stimulation of receptors of the laryngopharyngeal region.

Later ER was studied against the background of a modified functional state of RC. For this purpose, instead of breathing atmospheric air, the animals breathed a hypercapnic or hypoxic gas mixture, whose mechanism of action on RC is well known.

During breathing of the hypercapnic gas mixture electrical activity of the diaphragm was sharply increased, whereas electrical activity of the expiratory muscles (obliquus abdominis and internal intercostal muscles) was depressed or ceased completely. Under these circumstances expiration was passive, due to the increased elastic pull of the lungs. Despite complete inhibition of electrical activity of the expiratory muscles, an ER appeared at the moment the silon loop touched the vocal ligaments. It was manifested, just as during normocapnia, as a double volley. Meanwhile the shape of the ER volley differed somewhat from normal. For instance, the amplitude of spikes of the first discharge increased gradually and it was appreciably less than the amplitude of spikes of the second discharge. The main distinguishing feature of ER was the fact that during the pause between discharges no action potentials appeared in the diaphragm (Fig. 1b). This can evidently be explained by the weakness of the first discharge and, consequently, the low expiratory volume, after which the lung volume was almost unchanged. This means that these stimuli were unable to induce the stretch reflex of the lungs, for it was suppressed by the second, stronger discharge of ER. The ending of ER was followed by a long volley of electrical activity in the diaphragm, expressed as deep inspiration, after which the breathing assumed its original character.

The process of origin and the course of ER in hypoxic hypoxia is particularly interesting. After breathing of the hypoxic gas mixture for about 3-5 min, electrical activity in the expiratory muscles was reduced and ceased completely. At this moment during stimulation of the receptors of the vocal ligaments, ER appeared very briskly and was indistinguishable in form from normal. Both discharges were expressive and powerful. Distinct electrical activity appeared in the diaphragm in the pause (between discharges), which was not observed in hypercapnia (Fig. 1c). This can evidently be explained on the grounds that during hypoxia ER is preceded by weak inspiration. Against this background ER leads to strong collapse of the lungs. In response to this collapse of the lungs a stretch reflex developed immediately. However, it was interrupted by the second discharge of ER. After the end of ER there followed a very weak, short volley of electrical activity of the diaphragm, with spikes of low amplitude. Hypoxia and the hypocapnia developing after ER probably inhibit not only the expiratory, but also the inspiratory activity of RC. Meanwhile hypoxia and hypocapnia had no inhibitory action on ER. This indicates its extraordinarily high stability and, consequently, its biological importance for man and animals.

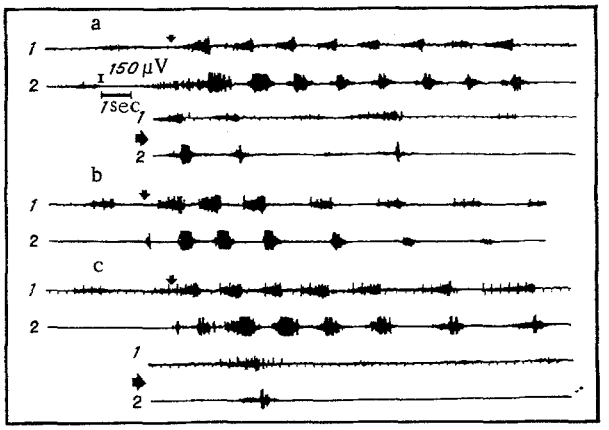

Fig. 3. Cough reflex evoked by stimulation of receptors of the tracheobronchial region during breathing of air (a), a hypercapnic gas mixture (b), and a hypoxic gas mixture (c).

The laryngopharyngeal cough reflex arose during stimulation of receptors of the laryngopharyngeal region. One of the remarkable features of the electrophysiological response of this reflex was that the phased alternation of discharges of the inspiratory and expiratory muscles was disturbed.

Those workers who have studied this cough reflex have concluded that its character, as noted above, can be explained by simultaneous stimulation of receptors of the laryngopharyngeal and tracheobronchial regions [8]. The coughing attack in this case consists of 3-5 coughs. During the coughing attack paroxysmal contractions of the diaphragm took place (a very rare phenomenon).

It is a particularly interesting fact that a strong expiratory cough was often followed by prolonged expiratory apnea. This arose as a result of prolonged and strong stimulation of the receptors of the laryngopharyngeal region. At the periphery, apnea was manifested as continuous and intensified electrical activity in the expiratory muscles. At this moment the main groups of expiratory muscles were in a state of prolonged static contraction (Fig. 2).

During breathing of hypercapnic and hypoxic gas mixtures, despite complete inhibition of expiratory activity, a laryngopharyngeal cough reflex occurred and was similar in form to that observed during air breathing. The main difference was shortening of the coughing attack and a decrease in the force of the cough.

The tracheobronchial cough reflex appeared in response to stimulation of cough receptors of the vagus nerve (slowly adapting, fast-adapting, and intermediate) [8]. Under normal conditions, during breathing of atmospheric air, stimulation of these receptors evokes a prolonged cough reflex in cats. The tracheobronchial cough reflex began with a powerful volley of electrical activity in the diaphragm, followed by phased activity. This phased activity resembled in type the Hering-Breuer reflex: strong inspiration evokes equally strong expiration. The more powerful the first volley of the diaphragm (inspiration), the longer the coughing attack. In some cases it amounted to 7-17 individual coughs. After the end of the coughing attack electrical activity of the respiratory muscles fell sharply and recovered slowly. On the ending of the coughing session, humoral factors become involved in regulation [4-7]. Weakening of the humoral mechanisms was due to a disturbance of homeostasis during the prolonged coughing attack. The hypocapnia and respiratory alkalosis which arose under these circumstances inhibited the generation of spikes and their modulation into volleys of respiratory neurons. Respiratory arrest frequently occurred in these cases (Fig. 3a).

Under conditions of hypercapnia and hypoxia (Fig. 3, b and c), despite inhibition of expiratory activity of the respiratory muscles, a cough reflex appeared and followed the same course as in normoxia; only the force of the coughs and their duration were reduced.

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ROLE OF PROTEIN KINASE C IN THE REGULATION OF ELECTRICAL AND CONTRACTILE ACTIVITY OF SMOOTH MUSCLE: EFFECT OF PHORBOL ESTER

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Much progress has been made in recent years in the study of the role of phosphorylation of membrane proteins, induced by protein kinase C (PKC), in the regulation of cellular functions [10]. Nevertheless, many important aspects of this problem still remain unexplained. In particular, the role of PKC in the regulation of smooth muscle functions remains unexplained. It has been shown [6-9, 11] that direct activation of PKC by phorbol esters, simulating the action of the secondary messenger diacylglycerol, led to considerable changes in the mechanical resting tension and, in some cases, to marked weakening, and in others to strengthening of the contractile responses of a muscle to the action of mediators or of external Ca^{++}. However, the authors cited confined their investigations to the contractile activity of muscles, and did not attempt to differentiate between the effect of activation of PKC on systems of electrically and chemically excitable calcium (Ca) channels.

In the investigation described below, conducted on strips of guinea pig taenia coli, changes in electrical and contractile activity of the smooth muscles under the influence of depolarizing and hyperpolarizing electrical stimuli, induced by phorbol ester, were recorded simultaneously for the first time. The effect of phorbol ester on the effect of neurotransmitters also was investigated under conditions when spike activity was inactivated by potassium depolarization of the membrane. This last factor enabled the role of PKC in the regulation of the receptor-controlled system of Ca channels to be detected.

EXPERIMENTAL METHOD

Preparations of the smooth muscle of the guinea pig taenia coli were used as the test objects. The length of the muscle strip was 10-12 mm and its width 0.5-0.7 mm. The double sucrose gap technique [2] was used to record mechanical and electrical activity simultaneously at rest and during stimulation. Electrical signals were recorded by FOR-2 camera from the screen of an S1-18 oscilloscope and also on the KSP-4 automatic writing potentiometer. Contractile activity was recorded by means of the 6MKh2B mechanotron under near-isometric

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