EFFECT OF OXYGEN SUPPLY ON RESPONSE OF IDENTIFIED *Helix pomatia* NEURONS TO METABOLIC REGULATORS

M. O. Samoilov, V. P. Nikitin,
D. G. Semenov, V. N. Maiorov,
and V. V. Sherstnev

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During the last decades the view that cyclic purine nucleotides and prostaglandins play the role of universal regulators of cell metabolism, which act as mediators of interaction between cells and the external environment, as is clearly demonstrated under extremal conditions, has become firmly established [1, 2, 7, 8]. In particular, when the blood supply to the brain is disturbed, sharp changes in the concentrations of cyclic nucleotides and prostaglandins in the blood take place rapidly [6, 7, 8] and have a substantial effect on the pattern of intracellular metabolism [7]. Meanwhile, it is evident that cyclic nucleotides and prostaglandins also influence specific activity of nerve cells [4, 5, 9]. However, the fundamentally important question of the effect of these substances on the dynamics and mutual relations of metabolic and bioelectrical processes in the neuron in different functional states has virtually not been investigated.

The object of this study was to examine the dynamics of the state of the oxidation–reduction systems of identified molluscan neurons and their bioelectrical activity under the influence of cyclic AMP and prostaglandin E_2 (PGE_2), during variations in the oxygen supply.

EXPERIMENTAL METHOD

Neurons V_6, V_7, and LP1 1 of the isolated circumesophageal ganglia of *Helix pomatia* in winter served as the test object. The LYUMAM KF-1 contact microscope, by means of which a ganglion at a considerable depth from the surface can be examined in incident polarized light, was used for identification and cytophotometry of the neurons. Double multibarreled micropipets were applied to the cells under visual control. One served for intracellular recording of unit activity, the other for extracellular microiontophoretic injection of cyclic AMP or PGE_2. In some experiments a polarographic microelectrode was applied to the cells under visual control so that pO_2 could be determined close to the test neurons.

The neurons, stained with methylene blue ($7.5 \cdot 10^{-5}$ M), used as an intravital indicator of their redox state [3], were subjected to spectrophotometry in regions of 605 nm (one of the maxima of light absorption by oxidized forms of the dye) and 430 nm (to estimate the optical density of the cell). On the basis of these readings the dynamics of reduced forms of the dye, corresponding to changes in the level of reducing equivalents (RE) in the cell and reflecting the state of the redox systems of the neuron, was determined quantitatively. Hypoxia of the neurons was created by substituting nitrogen for oxygen in the physiological saline which permanently surrounded the ganglion in a special incubation chamber.

EXPERIMENTAL RESULTS

Two series of experiments were carried out. In series I (10 cells) the dynamics of the redox systems of the neurons and their bioelectrical activity was studied during the develop-

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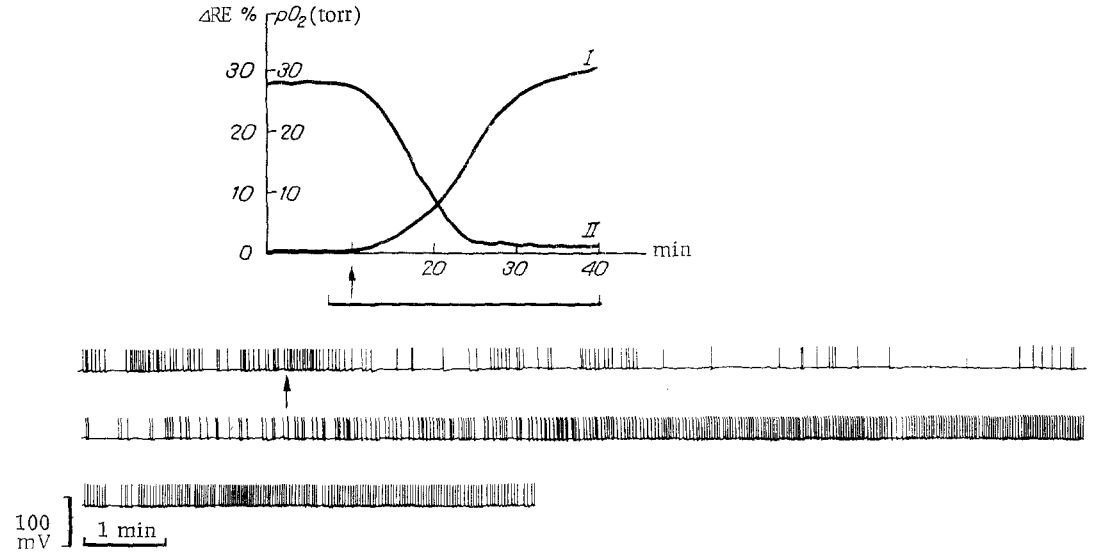

Fig. 1. Changes in intracellular RE (ΔRE) in spike activity of neuron V_6 during development of anoxia. Top graph shows changes in RE (1) and pO_2 close to neuron (2); horizontal line below abscissa indicates period of recording intracellular spike activity shown on trace below. Arrows indicate beginning of hypoxia.

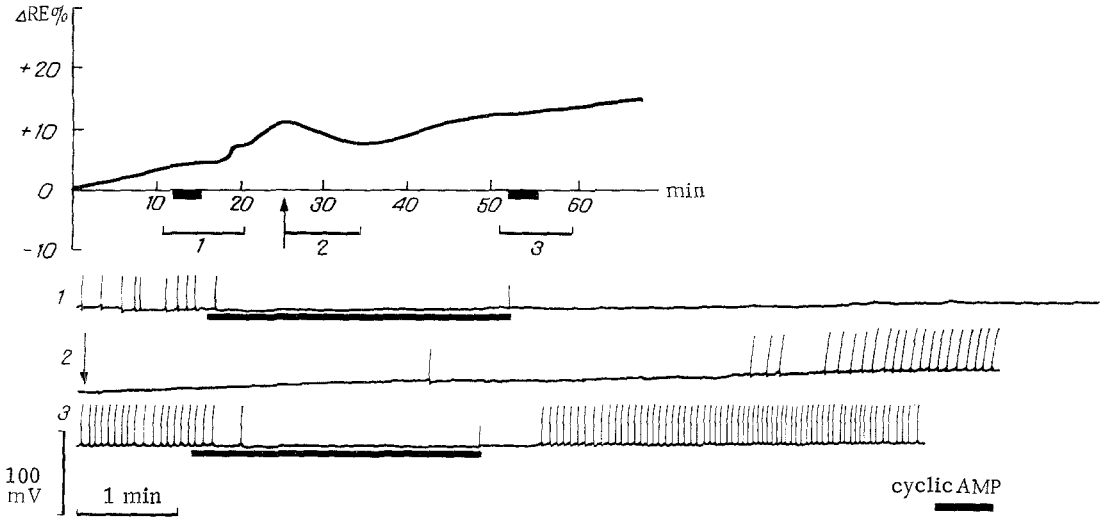

Fig. 2. Dynamics of intracellular RE (ΔRE) and spike activity of neuron V_6 under the influence of cyclic AMP and variations in oxygen supply. Graph above shows changes in RE; horizontal line along abscissa indicates periods of recording; spike activity shown on traces below (numbering of segments corresponds to numbering of traces). Bold lines on graph and traces denote periods of microiontophoretic application of cyclic AMP. Arrows indicate beginning of hypoxia.

ment of anoxia for 30-45 min. In series II (12 cells) these processes were investigated during the action of cyclic AMP or PGE_2, which were applied to the cell 10-15 min before the beginning of development of hypoxia, and also at the 30th minute of anoxia.

A graph showing changes in pO_2 near the neuron during saturation of the physiological saline with nitrogen is shown in Fig. 1 (curve 2). In neurons from series I, with a fall in pO_2 a progressive increase was observed in the RE level, to reach 25-30% of its initial value by the 30th minute (curve 1); a substantial rise in the RE level correlated with a marked increase in firing rate and with depolarization of the cells by 15-20%, which lasted until the end of the test period of anoxia.

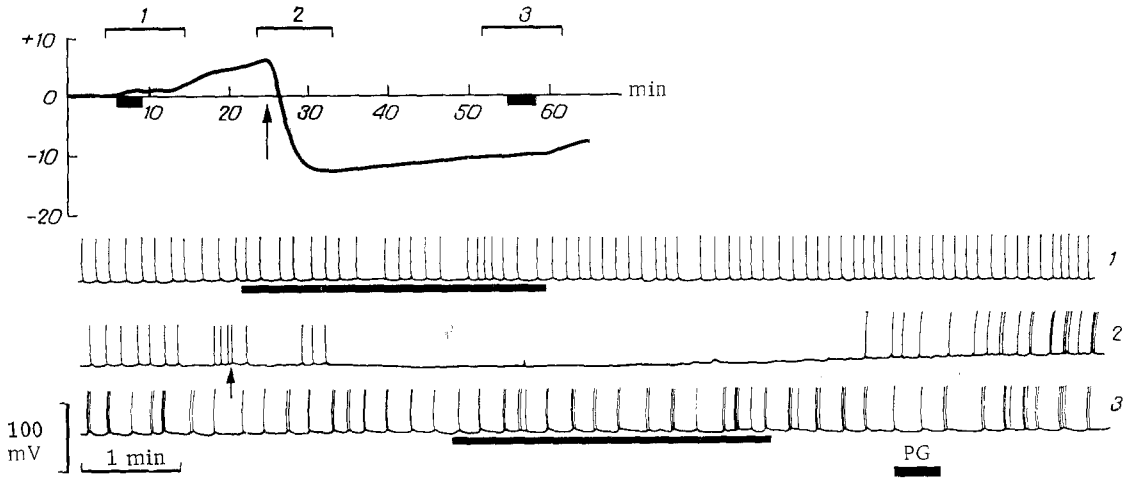

Fig. 3. Dynamics of intracellular RE (ΔRE) and spike activity of neuron V_7 under the influence of PGE_2 and various conditions of oxygen supply. Legend as in Fig. 2.

In about half of the neurons tested this process was preceded by a brief (5-10 min) and small reduction in spike frequency against the background of a virtually unchanged RE level in the cell.

The experiments of series II showed that under normoxic conditions the action of cyclic AMP and PGE_2 led to changes in the intracellular RE level, which as a rule initially rose (Figs. 2 and 3); the time course of this process was marked by specific distinguishing features. Under the influence of cyclic AMP this transition was frequently accompanied by spike-like oscillatory changes in the RE level. The predominant type of electrophysiological response during and after the action of cyclic AMP was inhibition of spike activity, whereas during the action of PGE_2 it was an increase in spike frequency (Figs. 2 and 3).

During the action of cyclic AMP the initial response of the neuron to anoxia took the form of lowering of RE and marked and prolonged inhibition of action potentials. The subsequent steady rise of the intracellular RE level was accompanied by the appearance or by an increase in the frequency of spike discharges. It will be noted that by the 30th minute of anoxia the RE level differed only very little from that found during normoxia. On repeated application of cyclic AMP during this period the response of RE was weak and a tendency was observed for them to stabilize at the level established by this time. The subsequent rise in the RE level was very small. In some cases a tendency was observed for the frequency of the spike discharges to increase (Fig. 2).

During the action of PGE_2 the response of the neurons to anoxia showed variability both of the metabolic disturbances (a sharp initial fall in the RE level followed by a rise or a gradual or progressive rise of the RE level) and functional reorganization of the firing pattern (complete inhibition or depression of spike activity followed by a change in the pattern); the sharp fall in the intracellular RE level as a rule correlated with deep inhibition of activity (Fig. 3). After 30 min of anoxia, during PGE_2 administration the RE level usually became stabilized, but this was followed by a small or substantial rise of its level. Under these circumstances the subsequent changes in spike activity either were slight or took the form of some decrease in its frequency. In particular it should be noted that after 30-45 min of anoxia hyperpolarization of the neurons by 15-35% compared with its initial level was found during administration of cyclic AMP or PGE_2.

This investigation thus revealed two principal facts. First, the response of the snail neuron to cyclic AMP and PGE_2 differed in different functional states associated with changes in the level of oxygen supply to the cell. Second, under the influence of these biologically active substances there was a substantial change in the character of the metabolic and bioelectrical shifts developing in the neurons during anoxia. This was reflected in changes in the dynamics of the state of the intracellular redox systems, the discharge frequency, and the membrane potential of the neurons. At the same time the response of neurons exposed to the action of cyclic AMP and PGE_2 showed definite specificity, and this was particularly marked as the oxygen deficiency developed. Preliminary administration of cyclic AMP to the

cell probably mainly initiates mechanisms preventing a marked rise in the RE level under these conditions. Meanwhile, under the influence of PGE_2 a broader spectrum of changes in the state of the redox systems of the neurons developed. In this case the pattern of unit activity was characteristically altered.

The variability of the cell responses is evidently associated both with differences in the type of the neurons identified and also with their original functional state. Further investigations will be devoted to the solution of this problem.

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FACTORS INFLUENCING THE ELASTIC RESISTANCE OF THE AORTIC COMPRESSION CHAMBER

V. L. Karpman and V. R. Orel

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Elastic resistance plays an important role in optimization of the cardiac contraction during ejection of blood from the left ventricle into the aorta. This value has a significant effect on the level of the input impedance of the arterial system [4] which, as we know [12], determines the afterload on the left ventricle.

According to Frank's definition [9] the elastic resistance (E) of the aortic compression chamber (ACC) is obtained by the equation

$$E = \frac{\Delta P}{\Delta V},$$

where ΔP is the pulse pressure (the difference between the systolic P_s and diastolic P_d arterial pressure); ΔV the increase in volume of the ACC during the period (s) of ejection of blood from the ventricle. However, no easy method has yet been devised for determining the value of ΔV and, consequently, the value of E. Some workers [8, 10, 14] have attempted to use for this purpose analysis of the wave phenomena arising in ACC during cardiac ejection. This approach to the problem proved ultimately to be not completely satisfactory: The values of E calculated by equations deduced by the authors cited above differed, other conditions being the same, by as much as 50-100%. Accordingly attempts to determine E by using only the principal hemodynamic indices appear to be more promising. This problem is solved in the present communication from this standpoint.

Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR. Department of Sport Medicine, Central Institute of Physical Culture, Moscow.

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