ENERGY METABOLISM UNDERLYING PHASIC CHANGES IN SPONTANEOUS NEURONAL SPIKE ACTIVITY IN HYPOXIA

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Nerve tissue in a surviving slice can regenerate spike activity with frequency and amplitude characteristics and can be unambiguously recorded in situ [2, 6, 9]. This enabled cerebellar slices to be used as a neuronal model with which to study the individual response of the nerve cell to hypoxia [1, 2]. Changes in spontaneous electrical activity (SEA) of neurons in slices of the cerebellum and cerebral cortex during hypoxia were found to be a phasic process, determined by the state of energy metabolism and reflecting activation and exhaustion of the routine adaptive mechanisms of the cell, maintaining its steady state in a medium deficient in oxygen [1, 3-5, 7].

The aim of this investigation was to study the role of the different components of energy metabolism in the formation of phasic changes in neuronal SEA in an experimental model of increasingly severe hypoxia, and to establish the limiting regions of this process.

EXPERIMENTAL METHOD

Freshly isolated surviving cerebellar slices 300-500 μm thick, obtained from mice and rats aged 15-21 days by the method described previously [2], were used in the experiments. After adaptation of the slices to the conditions of the medium (Earle's solution with glucose, saturated with carbogen, and kept at a constant temperature) for 40-60 min, Purkinje cells, which possess pacemaker properties, generated spike activity of continuous, interrupted-bursting, and bursting types with a frequency of 10-120 Hz and an amplitude of 0.2-1.0 mV for 1-2 h (Fig. 1a). A model of increasingly severe hypoxia was formed by lowering the pO_2 of the perfusion fluid to relative zero for 20 min. Immediately thereafter, reoxygenation of the perfusion fluid (also for 20 min) began. Continuous monitoring of pO_2 of the perfusion fluid was done polarographically with the aid of a Clark's electrode. SEA of the neurons (Purkinje cells) was recorded parallel with changes in pO_2. The effect of the various components of energy metabolism on SEA was assessed by inhibitor analysis. The action of inhibitors of glycolysis and of the respiratory chain, added to the perfusion fluid, on the dynamics of dependence of SEA on pO_2 was studied. Monoiodoacetate, potassium cyanide, sodium amytal, malonate, etc. were used in the work in concentrations with minimal effect on neuronal SEA.

EXPERIMENTAL RESULTS

During increasingly severe hypoxia even a small decrease of pO_2 (15-20%) led to primary inhibition of SEA by 20-25%. A further decrease in pO_2 in the perfusion fluid (by 30-65%) was accompanied by the appearance of a hyperactivation phase, expressed as an increase in SEA by 20-30% of its initial value. Only if pO_2 fell by 65% or more did depression of spike activity develop (Fig. 1b), and it was restored during reoxygenation very slowly: neuronal SEA increased sharply and reached its initial level or exceeded it only when pO_2 reached 70-80% of the initial value.

The principal energy-yielding substrate of the neurons in the system which we used, just as in vivo, is glucose, supplying pyruvate for the Krebs' cycle. This is confirmed by the fact that limitation or depression of glycolysis adversely affected not only neuronal SEA, but also the formation of phasic changes during increasingly severe hypoxia. Inhibition of glycolysis with

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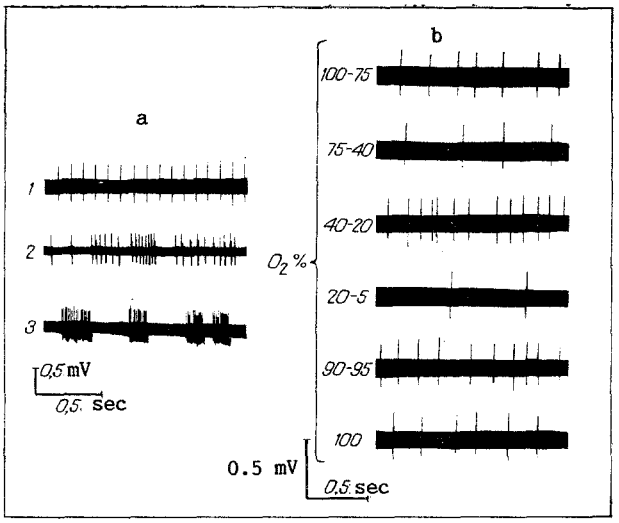

Fig. 1. Types of spike activity of cerebellar neurons in slice (a) and its changes during increasingly severe hypoxia (b). a: 1) Continuous, 2) continuous-bursting, 3) bursting type of activity; b: 1) initial activity, 2, 3, 4) spike activity during reoxygenation.

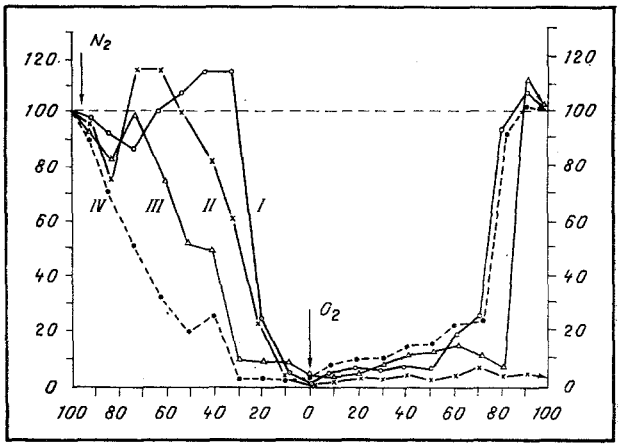

Fig. 2. Dynamics of changes in spike activity of cerebellar neuron of a mouse in slice under influence of hypoxia and of some inhibitors of energy metabolism. Abscissa, O_2 (in %); ordinate, spike activity (in %); broken line — initial activity. I) Hypoxia without substance, II) with malonate ($2 \cdot 10^{-3}$ M); III) with monoiodoacetate ($5 \cdot 10^{-5}$ M); IV) with KCN (10^{-4} M). Arrows indicate beginning of exposure to nitrogen (N_2) and carbogen (O_2).

the aid of monoiodoacetate led to a decrease in amplitude and frequency (by 20%) of neuronal SEA in the cerebellum under normoxic conditions and to the more rapid development of depression in experimental hypoxia. It can be seen in Fig. 2 that in the presence of even low concentrations of monoiodoacetate ($5 \cdot 10^{-5}$ M) the hyperactivation phase of neuronal SEA did not develop, and neuronal spike activity began to be depressed at much higher values of pO_2 than in the absence of the glycolysis inhibitor. Complete restoration of neuronal activity during reoxygenation also took place rather later than in the control, or not at all. With an increase in the monoiodoacetate concentration SEA was inhibited sooner and more strongly. Thus the contribution of glycolysis to maintenance of energy-dependent neuronal function was sufficiently large both during hypoxia and in the posthypoxic period. It evidently increased with an increase in the severity of hypoxia and was particularly marked in its terminal stage, when pO_2 had fallen to 70-80% of its initial value.

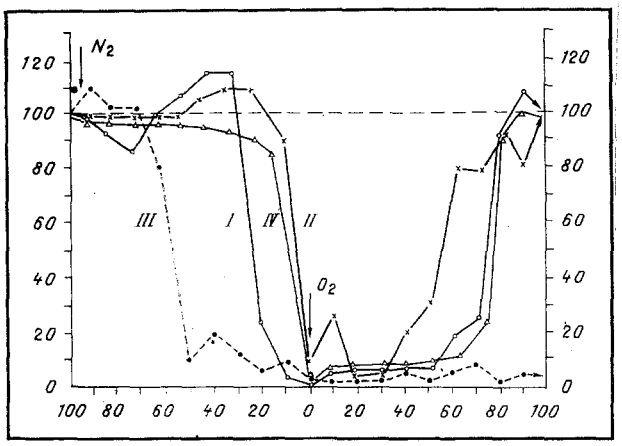

Fig. 3. Time course of changes in neuronal spike activity of mouse cerebellum in medium under influence of hypoxia and of certain drugs affecting oxidative metabolism. I) Hypoxia without substance; II) with vitamin K_3 (10^{-7} M); III) with semicarbazide ($5 \cdot 10^{-3}$ M); IV) with sodium amytal (10^{-4} M). Remainder of legend as to Fig. 2.

These data raise the question of the quantitative ratio between aerobic and anaerobic glycolysis in different phases of the neuronal response to hypoxia. In the presence of KCN (10^{-4} M), a cytochrome oxidase inhibitor, changes in neuronal SEA were similar to those in the presence of monoiodoacetate: depression of spike activity began at pO_2 = 80-70% and increased with subsequent lowering of the oxygen concentration (Fig. 2). Consequently, despite the potentiation of glycolysis shown above in the presence of a low pO_2 the cytochrome oxidase pathway of oxidation also functioned under these conditions down to very low values of pO_2.

To discover which pathway of supply of reducing equivalents to the respiratory chain is utilized in neurons during hypoxia, the action of various inhibitors of energy metabolism was studied on the curve of neuronal SEA versus pO_2. In the presence of sodium amytal (10^{-4} M), an inhibitor of oxidation of NAD-dependent substrates (pyruvate) the phase of hyperactivation of neuronal SEA did not develop. However, terminal depression of SEA in this case occurred at lower values of pO_2 (Fig. 3). Malonate ($2 \cdot 10^{-3}$ M), a competitive succinate dehydrogenase inhibitor, on the other hand, had no significant effect on the hyperactivation phase, but accelerated the development of terminal depression (Fig. 2). These data show that the hyperactivation phase of neuronal SEA during increasingly severe hypoxia is connected mainly with oxidation of NAD-dependent substrates and the supplying of reducing equivalents to the respiratory chain through its NAD-dependent region, whereas in the region of terminal depression of SEA the role of this metabolic path is diminished and compensatory activation of the succinate oxidase path takes place. Thus functional activity of neurons directly depends on the energy metabolism of the cell. With an increase in the severity of hypoxia, mutual replacement of metabolic flows takes place, so that the respiratory chain is provided with energy substrates and reducing equivalents, which participate in compensatory maintenance of the energy status of the cell. As a result of this, a high intensity of SEA, characterizing the specific electrogenic function of neurons, is preserved within a wide region of pO_2 values.

The results are in full agreement with those of other studies [3-5, 7] which showed an increase in the contribution of amytal-sensitive respiration of brain tissue at pO_2 corresponding to the hyperactivation phase of SEA and to its depression, when pO_2 falls by 70% or more. This confirms also the view expressed by the writers earlier, that the primary disturbances in hypoxia are unconnected with depression of cytochrome oxidase activity, but are localized in the first complex of the respiratory chain [4, 5]. Alternative activation of the succinate oxidase path evidently compensates for the loss of this path. There is also other evidence that terminal depression of SEA at low pO_2 values is connected with a disturbance of the electron-transport function of the respiratory chain of neurons in the NADH-reductase — cytochrome B region, and accompanying limitation of the supply of oxidation substrates via the NAD-dependent path. Addition of the quinone derivative vitamin K_3 (10^{-7} M), which can shunt the respiratory chain in the NADH — COQ region and, as a result, can carry electrons from NADH-dehydrogenase to cytochrome *b*, to the perfusion fluid lengthens the hyperactivation phase and shifts the terminal depression phase into the region of lower values of pO_2, i.e., it lowers the sensitivity of neurons to oxygen deficiency and has an antihypoxic action.

However, whereas in hypoxia the succinate oxidase path can be activated, compensating for the energy deficit in the cell, potentiation of the formation of endogenous succinic acid – the substrate of this path – must also be postulated. In the brain, one possible path of its synthesis is the Roberts' cycle, in which successive conversion of glutamate into GABA, succinic semialdehyde, and succinate takes place. Proof of activation of these reactions under the conditions of experimental hypoxia which we used is given by the inhibitory action of semicarbazide ($5 \cdot 10^{-3}$ M), which specifically interrupts this path at the level of the glutamate-decarboxylase reaction [8, 10], on neuronal SEA. It will also be clear from Fig. 3 that semicarbazide affects the activation phase and prevents its formation. The lower the level of pO_2 the stronger the action of semicarbazide. Hence it can be concluded that activation of the succinate oxidase path in nerve tissue is strengthened gradually, with an increase in the severity of hypoxia. It is perfectly possible that in the initial period of lowering of pO_2 this process takes place parallel to intensive oxidation of substrates via the NADH-oxidase path and is not an alternative to it.

The use of different inhibitors of cell energy metabolism, acting under conditions of increasing oxygen deficiency, thus showed that the varied direction of the electrogenic response of the neuron (SEA) to hypoxia is due to compensatory activation of various metabolic flows connected with energy metabolism and aimed at maintaining the specific energy-dependent function of the cell over the widest possible range of pO_2 values.

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