EFFECT OF ADENOSINE DIPHOSPHATE ON PHOSPHORYLATION OF RABBIT BRAIN MITOCHONDRIA DURING POSTNATAL ONTOGENESIS

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The effect of exogenous adenosine diphosphate (ADP) on energy processes in the mitochondria of the developing brain depends on the substrate used (succinate, glutamate). In the case of succinate, exogenous ADP activates predominantly the phosphorylating mechanism of oxidation, whereas in the case of glutamate it activates respiration mainly. The degree of regulation of phosphorylation by ADP on these substrates is connected with the morphological features of the mitochondria and depends on the stages of development of the brain.

The ability of exogenous adenosine diphosphate (ADP) to activate respiration of the brain mitochondria of vertebrates has been described in the literature [9, 11]. Little information is available on the manner in which ADP regulates the intensity of respiration in the mitochondria of the developing brain [4, 6, 8].

The writer has shown [4] that exogenous ADP increases oxidation of glutamic acid in the mitochondria of the rabbit's brain by 3-4 times but has almost no effect on the oxidation of succinate. The intensity of respiration on each of these substrates in the organelles studied varies to a different degree with the animal's age.

Since during maturation of the brain mitochondria the intensity of oxidative phosphorylation coupled with respiration is known to increase [8, 16], it was decided to study the character of this effect of ADP on this process during the period of postnatal development.

EXPERIMENTAL METHOD

Respiration of a suspension of brain mitochondria was recorded manometrically for 20 min, after which it was suppressed by the addition of 50% TCA solution. The intensity of oxidative phosphorylation in the TCA extract was determined from the decrease in inorganic phosphate (the method of Ennor and Rosenberg in Kotel'nikova's modification [5]). The methods of isolation of mitochondria and of protein estimation and the composition of the incubation medium were the same as described previously [4].

EXPERIMENTAL RESULTS AND DISCUSSION

At all stages of postnatal ontogenesis investigated, phosphorylation coupled with glutamate oxidation was recorded in the mitochondria of the rabbits' brain in the absence of ADP in the incubation medium. Under these conditions the value of P/O was usually higher in the mitochondria of the brain stem (2.46-3.95) than in the mitochondria of the cortex (2.42-3.01).

Injection of ADP into the incubation medium activated the phosphorylation of this substrate (Fig. 1). In the early stages of postnatal ontogenesis (1st-5th days), stimulation of phosphorylation was less marked (about twice) compared with at the subsequent stages of development and in the state of sexual maturity

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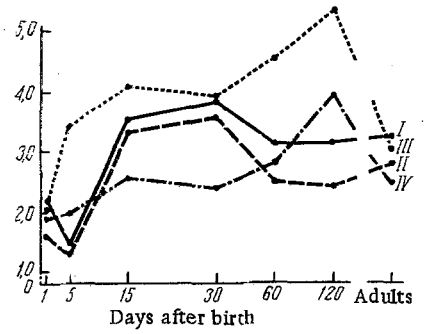

Fig. 1. Stimulation of phosphorylation and respiration of mitochondria of the cerebral cortex (I, II) and brain stem (III, IV). Substrate glutamate. I and III—respiration control; II and IV—stimulation of phosphorylation.

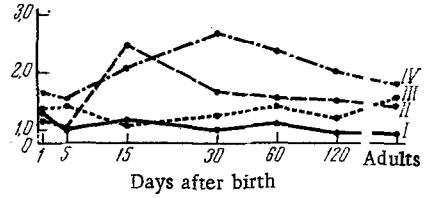

Fig. 2. Stimulation of phosphorylation and respiration of mitochondria of the cerebral cortex (I, II) and brain stem (III, IV). Substrate succinate. Legend as in Fig. 1.

(activation by 3-3.5 times). In rabbits aged 1-5 days, the increase in intensity of phosphorylation in the mitochondria of the brain stem under the influence of ADP was more marked than in the cortical mitochondria. However, in the animals aged 15 and 30 days and in sexually mature animals, on the other hand, the intensity of this process was increased more in the cortical organelles.

Comparison shows that under the influence of ADP the intensity of phosphorylation during oxidation of glutamate by the mitochondria of the brain stem increased by a lesser degree in the course of ontogenesis than the level of respiration (Fig. 1). Stimulation of these processes was by 2.5-4 and 2-5.4 times, respectively. Meanwhile, phosphorylation in the cortical mitochondria was activated by 1.3-3.6 times, and respiration by 1.5-3.8 times. As a result, at all stages of development the P/O ratio in the mitochondria of the brain stem was considerably reduced in the presence of exogenous ADP. In the cortical mitochondria, however, this was only a tendency.

Hence, depending on the type of brain mitochondria, exogenous ADP either affects respiration and oxidative phosphorylation proportionately during oxidation of glutamic acid (cortical fraction) or it activates respiration predominantly and lowers the level of coupling with phosphorylation (brain-stem fraction).

The cortical and brain-stem mitochondria in all age groups can carry out the oxidative phosphorylation of succinate in the absence of ADP. The intensity of this process in the mitochondria (in microatoms inorganic phosphorus per milligram mitochondrial protein per hour) was virtually identical in newborn (cortex 2.25, brain stem 2.14) and sexually mature rabbits (cortex 2.01, brain stem 2.9).

Exogenous ADP stimulates oxidative phosphorylation of succinate in both types of mitochondria (Fig. 2). In animals aged 15, 30, 60, and 120 days the effect of ADP was much more marked than in newborn animals and rabbits aged 5 days. Comparison shows that from the 15th day after birth the intensity of phosphorylation in the investigated fractions rises more under the influence of ADP than the intensity of respiration. This is reflected in the virtually equal increase in the values of the P/O ratio for the mitochondria of the cortex and brain stem. In the organelles investigated, exogenous ADP thus activates predominantly oxidation linked with phosphorylation in succinate metabolism at all stages of postnatal ontogenesis.

This investigation shows that the level and character of regulation of phosphorylation of individual substrates by ADP in the brain mitochondria are determined by the morphological and chemical properties of these organelles and depend on the stages of brain development.

In the early stages of ontogenesis (1st-5th days), when mitochondria of the bodies of the neurons predominated in the specimens, the degree of stimulation of respiration and of coupled phosphorylation by ADP was much lower than at subsequent stages of development (10th-15th day), coinciding with the appearance of mitochondria in the axon and dendrites [17]. At this time (the time of acquisition of vision), the velocity of oxidative phosphorylation of glutamate in the mitochondria of the brain begins to exceed that of succinate. The fact that ADP stimulates electron transport to a greater degree than energy formation in the case of glutamate utilization can evidently be explained by the removal of energy to meet endorganic requirements (the translocation of ions through the membrane, conformational changes in structures, the transdehydrogenase reaction, and so on). The ability of respiratory carriers to be oxidized without the formation of a high-energy component was demonstrated by the work of Green and Tzagaloff [13]. The facts described above evidently demonstrate the special role of glutamate in the metabolism of the recently matured neuron [2, 14].

At the same time, oxidative phosphorylation of succinate in the presence of ADP is accompanied by the transformation of energy predominantly in the oxidative chain of this substrate, a characteristic feature of the mitochondria of both fractions from the time of birth. This may be connected with the special role of succinate as a supplier of energy to maintain brain activity. Differences in the metabolism of individual substrates in the brain mitochondria have been confirmed polarographically.

These results are in agreement with data in the literature relating to differences in the biochemical properties of the brain mitochondria of growing and sexually mature animals [1, 10, 12], and they also indicate differences between the regulation of energy processes by ADP in the cortex and brain stem. The differences in the metabolic pathways of succinate and glutamate in the mitochondria of the developing brain are evidently associated with the character of compartmentalization of oxidase systems [15] and the accessibility of the corresponding substrates to them. The succinate system is known to be located both inside and outside the mitochondrial membranes, whereas the oxidation of glutamate takes place on the internal membranes.

Changes in the ultrastructural organization of the brain mitochondria in ontogenesis are also described in the literature [3, 7]. These facts, in conjunction with those described above, suggest that regular changes in the regulation of energy production in the mitochondria of the developing brain are interrelated with the morphological and chemical formation of these organelles to correspond to the energy needs of the organism at different stages of development.

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