EFFECT OF ADENOSINEDIPHOSPHATE ON INTENSITY OF RESPIRATION OF MITOCHONDRIA IN THE RABBIT BRAIN DURING POSTNATAL ONTOGENESIS

A. S. Kamysheva

UDC 612.65'82.014.21:612.26.014.46

Investigation of the mitochondria of the developing brain showed that exogenous adenosinediphosphate (ADP) considerably increases the oxidation of glutamate (by 3-4 times), but is almost without effect on the oxidation of succinate. Stimulation of respiration is manifested later in the mitochondria of the cortex (15 days) than in the mitochondria of the brain stem (5 days). In the latter, respiration is increased by a greater degree than in the mitochondria of the cortex.

In the absence of ADP at all stages of postnatal development the intensity of respiration of the investigated mitochondria is higher on succinate than on glutamate. In the presence of ADP the level of oxidation of each substrate in the mitochondria of cortex and brain stem varies with the animal's age.

* * *

Adenosinediphosphate (ADP) has been shown to have a marked activating action on respiration of the brain mitochondria. The intensity of this effect depends on the oxidation substrate, the activity of the corresponding dehydrogenases, the method of isolation of the mitochondria, the purity of this fraction, and other factors [6, 9, 13, 16]. Until recently, however, little information was available on the regulatory effect of ADP on respiratory activity of the developing brain [3, 5]. Such information could be important for establishing principles governing the development of regulation of mitochondrial respiration during morphological and physiological maturation of the brain.

The object of the present investigation was to study the effect of ADP in the mitochondria of the cortex and brain stem on oxidation of succinate and glutamate as substrates with different pathways of conversion in the respiratory chain of these organelles.

EXPERIMENTAL METHOD

Experiments were carried out on 70 rabbits of different ages: 1, 5, 15, 30, 60, and 120 days and sexually mature animals. Mitochondria of the cortex and brain stem were isolated by differential centrifugation (the TsLR-1 centrifuge) by a slightly modified method of Fonyo and Somogyi [11]. The isolation medium was 0.32 M sucrose solution in 0.02 M tris buffer. The intensity of mitochondrial respiration was measured manometrically in a Warburg apparatus at 26°. The incubation medium included the following components in final concentrations (pH 7.4) of : 12 mM tris buffer; 16 mM KH_2PO_4 buffer, 8 mM $MgCl_2$, 10 mM NaF, and 1.5 mM ADP (if included in the medium). The acceptor system consisted of glucose (50 mM) and hexokinase (Lawson, 0.4 mg per sample). The oxidation substrate was succinate or glutamate (17 mM). For reasons discussed in [5, 16], neither cytochrome c nor NAD was included in the incubation medium. The results were expressed per milligram mitochondrial protein. Protein was determined by Lowry's method [16].

EXPERIMENTAL RESULTS AND DISCUSSION

As Table 1 shows, the mitochondria of the rabbit brain can oxidize glutamate in the absence of ADP in the incubation medium. At all stages of development of the brain which were studied, except the first day, the intensity of respiration on this substrate in the mitochondria of the cortex was rather higher than in the mitochondria of the brain stem. Addition of ADP to the medium considerably increased the intensity of glutamate oxidation, to a lesser degree in the mitochondria of the cortex than in those of the brain stem.

Laboratory of Biohistochemistry, Institute of the Brain, Academy of Medical Sciences of the USSR, Moscow (Presented by Academician of the AMN SSSR S. A. Sarkisov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 4, pp. 43-46, April, 1969. Original article submitted January, 1968.

TABLE 1. Intensity of Respiration of Mitochondria of the Rabbit Cortex and Brain Stem in Postnatal Ontogenesis (μ atoms O_2/ mg mitochondrial protein/ h) Using Glutamate and Succinate as Oxidation Substrates (M ± m)

Age after birth (in days)	No. of expt.	Cortex			Brain stem		
		−ADP	+ADP	$\frac{\Delta O + ADP}{\Delta O - ADP}$	−ADP	+ADP	$\frac{\Delta O + ADP}{\Delta O - ADP}$
		Glutamate					
1	12	0.57±0.09	1.25±0.24	2.20	0.74±0.13	1.52±0.29	2.06
5	6	0.78±0.20	1.13±0.15	1.45	0.50±0.20	1.71±0.38	3.42
15	16	0.60±0.08	2.11±0.26	3.52	0.50±0.07	2.05±0.17	4.10
30	7	0.64±0.25	2.45±0.30	3.84	0.56±0.13	2.20±0.20	3.94
60	6	0.70±0.14	2.19±0.35	3.14	0.55±0.02	2.50±0.39	4.55
120	9	0.75±0.11	2.35±0.28	3.14	0.53±0.13	2.82±0.39	5.32
Sexually mature animals	14	0.72±0.08	2.34±0.24	3.25	0.61±0.14	1.84±0.28	3.02
		Succinate					
1	9	1.73±0.20	2.28±0.29	1.32	1.24±0.18	1.66±0.15	1.34
5	6	1.73±0.16	1.80±0.29	1.04	1.33±0.31	1.90±0.22	1.42
15	16	1.80±0.12	2.14±0.14	1.19	1.87±0.19	2.11±0.22	1.13
30	8	1.93±0.04	1.96±0.16	1.02	1.84±0.20	2.34±0.25	1.27
60	8	1.60±0.22	1.79±0.22	1.12	1.53±0.31	2.19±0.10	1.43
120	7	1.62±0.32	1.58±0.35	0.97	1.54±0.33	1.90±0.21	1.24
Sexually mature animals	10	2.26±0.20	2.18±0.33	0.97	1.29±0.31	2.01±0.12	1.56

Respiration in the cortical mitochondria was increased by 1.5-2 times on the 1st-5th day, and by 3-4 times in the period between the 15th and 120th days. These results show that the regulatory effect of ADP on glutamate oxidation is established during maturation of the brain. It is still relatively undeveloped in the cortical mitochondria at a time when mitochondria of the neuron bodies are predominant in tissue samples (1st-5th day) and reaches its maximum at the 15th-60th day, when mitochondria appear in processes of the nerve cells [1, 18] and their energy-producing function is at its most intensive. This function, like the membranes of the mitochondria, has not yet reached the adult type in a number of features [2]. Probably the properties of the mitochondrial membranes at this time are less concerned with the degree of the regulatory effect of ADP on respiratory activity of the mitochondria than with the degree of maturity and organization of the respiratory mechanisms themselves.

Evidence in favor of this view was given by the earlier appearance of a marked effect of ADP on mitochondrial respiration in the brain stem compared with the cortex, corresponding to the order of morphological, physiological, and biochemical maturation of these parts of the brain. On the 5th day after birth, for instance, the intensity of respiration in the brain stem mitochondria was increased by 3.5 times by the action of ADP, while the corresponding increase in the cortical mitochondria was only 1.52 times. The effect of ADP on respiration of the mitochondria in the brain stem was also increased in older rabbits (15, 30, and 120 days) – by 4-5 times.

In the mature mitochondria of the adult animal, ADP activated respiration of both types equally (3-3.25 times).

Different relationships were found in the case of oxidation of succinate (Table 1). Addition of ADP to the medium left the intensity of respiration of the cortical mitochondria practically unchanged at all periods of development investigated (except the first day, when ΔO was increased by 1.32 times). In the mitochondria of the brain stem ADP stimulated respiration by not more than 1.5 times.

The results showed that in adult rabbits ADP has practically no effect on oxidation of succinate in the cortical mitochondria and stimulates the oxidation of this substrate slightly in the mitochondria of the brain stem (by 1.5 times), in agreement with data in the literature for mitochondria of the whole rat brain [6, 16].

The absence of effect (cortical mitochondria) or the slight effect (mitochondria of the brain stem) of ADP on the oxidation of succinate is thus characteristic of these organelles at all stages of development of the brain and is exhibited from the first day after birth.

The intensity of oxidation of different substrates by the mitochondria is a matter on which complete agreement has not been reached in the literature. According to some information [14], succinate is oxidized more rapidly than glutamate. The results of other investigations [7, 13] indicate that the mitochondria of the brain oxidize predominantly glutamate.

Our investigations show that the intensity of oxidation of different substrates depends on the morphological and chemical properties of the mitochondria. It differs in mitochondria of the cortex and brain stem and it depends on the stages of development. This is shown by the fact that succinate is oxidized more rapidly than glutamate in the presence of ADP in the cortical mitochondria of newborn rabbits and rabbits aged 5 days. In the period between the 30th and 120th days the intensity of respiration on glutamate becomes higher than on succinate. At the same time, the intensity of oxidation of both substrates in the mitochondria of the brain stem is practically identical (except from the 60th to the 120th days, when oxidation of glutamate is more intensive than that of succinate). In the absence of ADP the intensity of respiration of mitochondria of both cortex and brain stem on succinate is constantly higher than on glutamate.

We consider that the character of the effect of ADP on oxidation of these two substrates is determined, as well as by other possible factors, by the differences in distribution of adenine nucleotides in the mitochondria. The presence of endogenous ADP in these organelles has been demonstrated by Weinbach and Garbus [18]. Their reserves are evidently adequate in the sites of oxidation of succinate, especially in the cortical mitochondria, but they limit the oxidation of glutamate, which takes place in the deeper parts of these organelles [4, 11, 15]. The extent to which the observed differences in the degree of the effect of endogenous ADP on glutamate oxidation depend on differences in the energy metabolism of neuronal mitochondria and on the relationship between mitochondria of neurons and glial cells will be revealed by later investigations.

LITERATURE CITED

1. N. I. Dmitrieva and E. I. Kalinina, Zh. Evolyuts. Biokhim. i Fiziol., No. 2, 192 (1965).
2. Z. D. Pigareva, Abstracts of Proceedings of the 4th All-Union Conference on Biochemistry of the Nervous System [in Russian], Tartu (1966), p. 85.
3. A. A. Simonyan, Oxidative Phosphorylation in Mitochondria of the Brain and Liver of the Chick Embryo During Embryogenesis and the Postembryonic Period, Author's Abstract of Candidate Dissertation, Erevan (1966).
4. V. P. Skulachev, Relationship between Oxidation and Phosphorylation in the Respiratory Chain [in Russian], Moscow (1962), p. 37.
5. L. D. Abood, E. Brunngraber and M. Tylor, J. Biol. Chem., 234, 1307 (1959).
6. R. Balazs, D. Biesold, and K. Magyar, J. Neurochem., 10, 685 (1963).
7. R. Balazs, K. Magyar, and D. Riechter, in: D. Riechter (editor), Comparative Neurochemistry, London (1964), p. 225.
8. R. Cereijo-Santalo, Canad. J. Biochem., 43, 425 (1965).
9. A. P. Compello, D. O. Voss, S. A. Freire, et al., J. Biol. Chem., 239, 3942 (1964).
10. L. Ernster, O. Jalling, H. Löw, et al., Exp. Cell Res., Suppl. 3, 124 (1955).
11. A. Fonyo and J. Somogyi, Acta Physiol. Acad. Sci. Hung., 18, 191 (1960).
12. F. F. Jöbsis, Biochim. Biophys. Acta, 74, 60 (1963).
13. S. Løvtrup, in: Growth and Maturation of the Brain. I. Progress in Brain Research, Vol. 4, Amsterdam (1964), p. 237.
14. A. Lehninger, Mitochondria [Russian translation], Moscow (1966), p. 193.
15. S. Løvtrup and L. Svennercholm, Exp. Cell Res., 29, 298 (1963).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
17. J. Shade and C. Baxter, Exp. Neurol., 2, 158 (1960).
18. E. C. Weinbach and J. Garbus, J. Biol. Chem., 234, 412 (1959).