EFFECT OF NIKETHAMIDE ON OXIDATIVE METABOLISM OF MITOCHONDRIA IN THE RABBIT CEREBRAL CORTEX

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The effect of nikethamide on oxidative metabolism of the total mitochondrial fraction from the gray metter of the rabbit cerebral cortex was investigated. Nikethamide inhibits oxidation of endogenous substrates and oxidation of exogenous glutamate, whether coupled with phosphorylation or uncoupled by 2,4-dinitrophenol. Nikethamide does not reduce the respiratory activity of the mitochondria during oxidation of exogenous succinate. The results demonstrate that in its action on cortical tissue nikethamide inhibits the activity of those mitochondrial dehydrogenases whose coenzyme is oxidized nicotinamide-adenine dinucleotide.

Nikethamide is widely used in clinical practice to stimulate the activity of the central nervous and cardiovascular systems. Since nikethamide is an analog of nicotinamide, the catalytically active group of the nicotinamide-adenine dinucleotide (NAD) molecule, it is important ot study the effect of this compound on the energy metabolism of animal tissues. Investigations showing the effect of nikethamide on the oxidative metabolism of heart tissue indicate that this compound inhibits hydrogen and electron transport by the respiratory chain of the mitochondria [1, 2].

In the investigation described below the effect of nikethamide was studied on oxidative metabolism of the mitochondria of the rabbit cerebral cortex.

EXPERIMENTAL METHOD

Male rabbits weighing 2.5 kg were used. The total mitochondrial fraction was isolated from the cortical gray matter. The isolation medium consisted of 0.21 M mannitol, 0.076 M sucrose, 0.01 M Tris, 0.002 M EDTA, pH 7.4 [3]. A 10% brain tissue homogenate was treated by differential fractionation: the nuclei were sedimented by centrifugation for 10 min at 1000 g and the mitochondria by centrifugation for 10 min at 15,000 g, washed twice with the isolation medium, and resuspended in the same medium. The incubation medium consisted of 0.21 M mannitol, 0.01 M Tris, 0.0002 M EDTA, 0.01 M KCl, and 0.025 M K_2HPO_4, pH 7.4. The intensity of oxygen consumption by the mitochondrial suspension was recorded by a polarographic method [1, 2]. The suspension of mitochondria (3-5 mg protein) was incubated for 40-80 sec at 26° C in 0.9 ml incubation medium. Glutamate or succinate (10 μ moles) were used as respiration substrates. The phosphate acceptor was adenosinediphosphate (ADP), 0.50-0.70 μ mole. Nikethamide (25% solution) was used in a dose of 40 μ moles and 2,4-dinitrophenol (DNP) in a dose of 0.03 μ mole.

The intensity of mitochondrial respiration was expressed as the number of microatoms of oxygen utilized by 1 g mitochondrial protein per minute. The protein content was determined by the biuret reaction. The results given are the mean values from 5 or 6 reproducible experiments.

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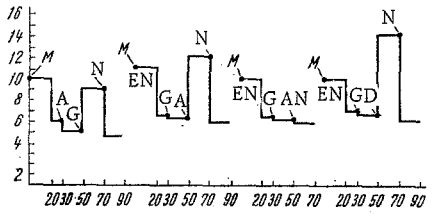

Fig. 1

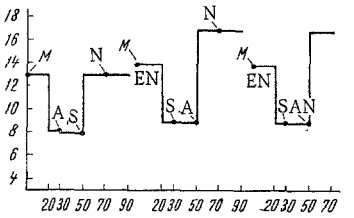

Fig. 2

Fig. 1. Effect of nikethamide on intensity of mitochondrial respiration of rabbit cerebral cortex during oxidation of glutamate. M – mitochondria; EN – endogenous respiration substrates; A – ADP; G – glutamate; N – nikethamide; AN – mixture of ADP and nikethamide; D – DNP. Abscissa, time (in min); ordinate, intensity of respiration (μ atoms O_2/g/min).

Fig. 2. Effect of nikethamide on intensity of mitochondrial respiration of the rat cerebral cortex during oxidation of succinate. S – succinate. Other symbols as in Fig. 1.

EXPERIMENTAL RESULTS

Washing the mitochondria twice in the course of their isolation did not completely remove all endogenous respiration substrates. Nikethamide inhibited the intensity of "endogenous" respiration of the mitochondria on the average by 30%.

Results showing the effect of nikethamide on activity of the mitochondrial respiratory chain during oxidation of glutamate are shown in Fig. 1. After incubation of the mitochondria for 30 sec, the course of exhaustion of the endogenous substrates, the intensity of oxygen consumption was reduced, and addition of gluamate to the experimental samples was the intensity of oxygen utilization increased. The intensity of mitochondrial respiration was unchanged until all the oxygen was used up unless nikethamide was added to the medium, when glutamate oxidation was completely suppressed. Glutamate was not oxidized in the brain mitochondria without ADP. If ADP was added together with nikethamide, respiration was not stimulated.

The results of investigation of the effect of nikethamide on mitochondrial respiration when uncoupled from phosphorylation by DNP are also given in Fig. 1. In these experiments glutamate was the respiration substrate. Nikethamide completely suppressed the activating effect of DNP on mitochondrial respiration. The fact that nikethamide inhibits mitochondrial respiration whether coupled with phosphorylation or uncoupled, is evidence that the transport of hydrogen and electrons is disturbed in these cases; the absence of control over respiration by ADP when nikethamide is also present in the medium is the result of this disturbance. Calculation of the phosphorylation coefficeint could give a more definite idea of the effect of nikethamide on respiratory phosphorylation in the brain mitochondria. However, probably because of the high adenosinetriphosphatase activity, conversion of the mitochondria from state 3 into state 4 could not be observed, and it was therefore impossible to calculate the phosphorylation coefficient from the ADP/O ratio and to estimate the phosphorylating activity of the brain tissue in the presence of nikethamide.

The results of experiments to study the effect of nikethamide on the activity of the mitochondrial respiratory chain during oxidation of succinate are shown in Fig. 2.

Nikethamide did not inhibit mitochondrial respiration during the oxidation of succinate. Like glutamate, succinate was not oxidized without ADP.

Addition of ADP, either alone or mixed with nikethamide stimulated mitochondrial respiration equally during the oxidation of succinate. In the experiments with succinate no conversion of mitochondria from state 3 into state 4 likewise was observed.

The results indicate that nikethamide inhibits mitochondrial respiration not only in the heart tissue [1, 2], but also in tissues of the cerebral cortex. The fact that nikethamide does not affect oxidation of succinate, whereas it inhibits oxidation of glutamate, suggests that nikethamide, in its action on the cerebral cortex just as on the heart, inhibits the activity of those dehydrogenases for which NAD is the coenzyme.

LITERATURE CITED

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