EFFECT OF HYPOXIA ON NICOTINAMIDE COENZYME CONTENT IN TISSUES OF NEWBORN RATS

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The concentration of nicotinamide coenzymes (NAD, NADH, NADP, and NADPH) was determined in tissues of the brain, heart, and liver of newborn rats exposed for 2.5 h to a hypoxic atmosphere containing 4% oxygen. A marked decrease in the NAD content, an increase in NADH, and a decrease in the NAD/NADH ratio by more than half were observed in the experimental animals and the changes were particularly marked in the brain and heart. Under the same conditions there was a decrease in the NADPH content, chiefly in the liver, and in the total reserves of NAD-phosphates in the tissues of the newborn rats studied. It is suggested that hypoxia has a substantial effect on the absolute and relative concentration of nicotinamide coenzymes in the tissues of newborn rats, resulting in changes in the level and direction of oxidoreductive processes under hypoxic conditions.

KEY WORDS: *Nicotinamide coenzymes: NAD, NADH, NADP, NADPH; hypoxia.*

Interest in the study of the content of nicotinamide coenzymes in the tissues in hypoxia is due to their important role in metabolism. With their function as hydrogen carriers, NAD and NADP and their reduced forms participate in cell respiration processes which are the first to be disturbed in oxygen deficiency. Hypoxia has been shown to cause changes in the ratio between oxidized and reduced forms of nicotinamide coenzymes in the tissues in favor of the latter [6, 8]. The investigations cited above were carried out on adult animals and were concerned chiefly with the NAD–NADH system.

This paper gives the results of a study of the contents of NAD, NADP, and their reduced forms in the tissues of newborn rats exposed to hypoxia.

EXPERIMENTAL METHOD

Hypoxia was produced in a special chamber through which a hypoxic gas mixture (at 32°C) containing 4% O_2, 2% CO_2, and 94% N_2 was passed at a constant rate. The respiration and heart rate (from the ECG) of the animals were recorded. The length of time that the animals spent in the chamber was 2.5 h. By that time the rats showed severe symptoms of hypoxia: cyanosis, a fall in respiration rate to 16 per minute, and, in individual animals, periodic breathing of the Cheyne–Stokes type. In some animals convulsions were observed. The heart rate reached 250 beats/min. Before the experiment began the respiration and heart rate of the experimental animals averaged 78 and 180 per minute, respectively.

The content of nicotinamide coenzymes was determined in brain, heart, and liver tissues frozen in liquid nitrogen immediately after removal. NAD was extracted with 2.5% TCA, NADP with 0.5 M $HClO_4$, and reduced nucleotides with 1 M KOH solution in ethanol. NAD and NADH were determined spectrophotometrically [5, 7]. NADP and NADPH were determined by the method of Slater et al. [10]. Reduced forms of the coenzymes were subjected to preliminary photooxidation in the presence of methylene blue.

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TABLE 1. Effect of Hypoxic Atmosphere on Content of Nicotinamide Coenzymes in Tissues of Newborn Rats (in μg/g wet weight; M ± m)

Tissue	Group of animals	n	NAD	NADH	NAD + NADH	NAD/NADH	n	NADP	NADPH	NADP + NADPH	NADP/NADPH
Brain	Control	6	145,00±9,17	42,00±5,73	187,00±10,20	3,4	6	12,0±3,3	18,00±4,14	30,0±6,7	0,7
	Experiment	7	94,00±20,13	86,00±16,0	180,00±18,8	1,1	6	7,00±2.62	12,00±1,86	19,0±4,3	0,6
	P		<0,05	<0,02	<0,5			<0,5	<0,5	<0,2	
Liver	Control	6	364,00±13,11	111,00±14,24	475,00±20,82	3,3	6	41,00±4.83	62,00±5,57	103,0±8,0	0,6
	Experiment	7	253,00±27,89	146,00±14,21	400,00±26,19	1,7	5	42,0±8,0	38,00±7,84	80,00±10,11	1,1
	P		<0,01	<0,2	<0.05			>0,5	<0,05	<0,1	
Heart	Control	6	459,00±52,64	160,00±12,65	619,0±64,7	2,9	6	52,00±9,21	56,00±10,06	108,00±21,99	0,9
	Experiment	7	339,00±14,30	281,00±18,09	620,0±34,7	1,2	5	38,00±5.14	44,00±7,02	84,0±9,4	
	P		<0,05	<0,001	>0,5			<0,5	<0,5	<0,5	

Legend. P relative to control group.

EXPERIMENTAL RESULTS

As Table 1 shows, in all the tissues investigated from animals which had breathed the hypoxic gas mixture there was a statistically significant decrease in the content of the oxidized form of NAD compared with the control animals, and the difference was greatest in brain tissue (by 1.5 times; $P < 0.05$). The NADH content, on the other hand, was higher in the animals exposed to hypoxia, especially in the brain (twice as high as in the control; $P < 0.02$) and heart tissue (by 1.7 times; $P < 0.001$). An increase in the NADH concentration also was found in the liver, but it was not significant. Correspondingly the ratio between oxidized and reduced forms (NAD/NADH) was lower in the newborn animals exposed to hypoxia than in the control. The total content of NAD + NADH was reduced in the liver of the experimental animals ($P < 0.05$), but it was practically unchanged in the tissues of the brain and heart.

Changes in the content of NAD phosphates in the tissues of the newborn animals breathing the hypoxic gas mixture were less marked than changes in the NAD and NADH concentration. The NADP content in the liver tissue of the experimental animals was indistinguishable from the control level, but in the brain and heart, although some decrease was observed in the NADP content, the difference between the experimental and control groups was not significant ($P < 0.5$).

The NADPH content in the liver of newborn rats after inhalation of the gas mixture was 38% lower than in the control ($P < 0.05$). The decrease in the NADPH content in the brain and heart tissues was not so great ($P < 0.5$). The ratio between oxidized and reduced forms (NADP/NADPH) in the heart and brain tissues was virtually unchanged, but in the liver of the experimental rats it was almost doubled because of the greater change in the content of the reduced form. Differences between the total content of NAD phosphates in the experimental and control groups were not significant because of the great individual variations, but nevertheless there was a clear tendency for it to be lower in the tissues of the animals with hypoxia.

The increase found in the NADH content in the tissues of the young rats with hypoxia was due in all probability to activation of the various stages of glycolysis coupled with reduction of NAD and aimed at maintaining the required level of ATP. It has been shown [11] that when the oxygen supply to the heart muscle is deficient, the activity of glyceraldehyde-3-phosphate dehydrogenase, an enzyme catalyzing the reaction of glycolysis coupled with the formation of reduced NADH, in the heart muscle is increased. A decrease in the partial pressure of oxygen in heart tissue is known to lead to a sharp increase in the production of lactate and ATP, synthesized by the glycolytic pathway. NADH can be reoxidized to some degree in the final reaction of glycolysis, catalyzed by lactate dehydrogenase, which is accompanied by the utilization of the reduced form of NAD.

The decrease in NAD concentration taking place in the tissues of the experimental animals could be the result of its increased breakdown. The metabolic acidosis found under hypoxic conditions facilitates activation of NAD-glucohydrolase, which hydrolyzes NAD [3], for the pH optimum of this enzyme lies between 6.0 and 7.0. The rate of hydrolysis of NAD by glucohydrolase isolated from heart muscle has been shown [2] to be twice as fast as the rate of hydrolysis of NADP.

The decrease in the content of the reduced form of NADP, which is especially marked in the liver tissue, is perhaps connected with the fact that under the conditions of such a marked oxygen deficiency the dehydrogenase reactions of the pentose phosphate cycle, the main sources of supply of NADPH may be inhibited in the direction of pentose formation. Meanwhile there is another important fact, namely that under hypoxic conditions the activity of NADP-dependent malate dehydrogenase, an enzyme catalyzing the reaction of conversion of malic acid into pyruvic, coupled with accumulation of the reduced form of NADP in the cytoplasm [1], is reduced in the liver. In brain tissue, according to the authors cited, the change in activity of this enzyme was less marked. In the tissues of the brain and heart, metabolism of which is more strongly dependent on the oxygen supply, NADPH can be utilized in a transhydrogenase reaction which, in hypoxia [4], when respiration is inhibited, participates in energy-forming processes.

Hypoxia due to inhalation of an oxygen-deficient gas mixture for 2.5 h thus gives rise to significant changes in the tissue nicotinamide coenzyme system of newborn rats. The decrease in the content of the NAD form, increase in the reduced form (NADH), and the consequent decrease in the NAD/NADH ratio indicate that in newborn rats with hypoxia the necessary ATP level was maintained at the expense of glycolytic oxidoreduction. The decrease in the levels of NAD and NAD phosphates under the conditions of such severe hypoxia may in turn be one of the causes of the disturbance of oxidoreductive processes, e.g., of the Krebs' cycle and oxidative phosphorylation coupled with it, the oxidation of fatty acids, and other metabolic pathways concerned in the adaptation of the organism to hypoxia.

It can be concluded from these results that the absolute and relative concentrations of oxidized and reduced forms of nicotinamide coenzymes can characterize the degree and direction of the changes in oxidoreductive processes in newborn rats exposed to hypoxia.

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