MECHANISM OF THE PROTECTIVE EFFECT OF SUCCINIC SEMIALDEHYDE AND ITS DERIVATIVES IN HYPOXIA

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Preliminary injection of succinic semialdehyde into animals in experiments with hypoxia led to a lower concentration of free ammonia in their brain tissues than in control experiments in which the compound was not given. The enzyme system of the liver tissue catalyzes the oxidation of NAD·H_2 by succinic semialdehyde and its derivatives with a free aldehyde group. The conversion of succinic semialdehyde and its derivatives in the liver tissue leading to the formation of the oxidized form of NAD, which is deficient in hypoxia, and the prevention of accumulation of free ammonia in the brain tissue by preliminary administration of succinic semialdehyde are the possible mechanisms of the antihypoxic properties of succinic semialdehyde and its derivatives.

KEY WORDS: hypoxia; succinic semialdehyde -- antihypoxic properties; ammonia and its metabolism; brain and liver tissue; reduced and oxidized NAD.

Enzymic processes leading to the synthesis of the oxidized form of NAD become more important in hypoxia, in which this substance is deficient. Succinic semialdehyde and its derivatives increase the resistance of animals to hypoxia [6, 8] and help to restore the processes of oxidative metabolism, when disturbed in hypoxia, to normal [2, 7]. An enzyme system catalyzing the oxidation of NAD·H_2 by succinic semialdehyde and leading to the formation of NAD has been described in animal tissues [9].

The investigation described below was undertaken to make a further study of the mechanisms lying at the basis of the protective action of succinic semialdehyde and its derivatives in hypoxia.

EXPERIMENTAL METHOD

Albino mice weighing 20-24 g were used. Hypoxic conditions were created by placing the animals in closed chambers with a reduced oxygen concentration (to 8 vol.%) [1]. The preparations were injected intraperitoneally into the mice in a dose of 500 mg/kg 15 min before the animals were placed in the chamber. The mice were decapitated after a stay of 20 min in the chamber. Under these conditions mice not receiving the preparations died after 22-23 min, but those receiving the compounds with antihypoxic properties died after 50-60 min.

To determine the concentration of free ammonia in the brain tissue the animals were decapitated and the head frozen immediately with liquid nitrogen. The brain was extracted in the frozen state and homogenized in 5 volumes of 10% TCA solution. The free ammonia concentration in the supernatant was determined by isothermic distillation.

The soluble fraction of the mouse liver tissue was used as the enzyme preparation to catalyze the oxidation of NAD·H_2 by succinic semialdehyde or its derivatives. A 10% liver tissue homogenate was subjected to differential centrifugation and the soluble fraction was obtained by centrifuging for 60 min at

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TABLE 1. Oxidation of NAD · H_2 by Enzyme System of Liver Tissue in the Presence of Succinic Semialdehyde and Its Derivatives

No.	Compound	Specific activity
1	O=CH CH_2 CH_2 COOH	70
2	O=CH CH_2 CH_2 $COOC_2H_5$	80
3	O=CH CH_2 CH_2 CON⟨ ⟩	50
4	$(C_2H_5O)_2$ CH CH_2 CH_2 $COOC_2H_5$	0
5	$(CH_3O)_2$ CH CH_2 CH_2 CON⟨ ⟩	0
6	$(CH_3O)_2$ CH CH_2 CH_2 CON⟨ O⟩	0

110,000 g. The homogenization medium had the following composition: K_2HPO_4 0.1 M, mercaptoethanol 0.006 M; pH 7.2. The oxidation of NAD · H_2 was recorded in the cell of a type SF-4 spectrophotometer by the change in optical density at 340 nm. The reaction medium consisted of 3 ml of buffer solution, pH 6.2, of the following composition (in M): K_2HPO_4 0.1, NaCl 0.02, $MgCl_2$ 0.002, mercaptoethanol 0.006, NAD · H_2 0.0001, succinic aldehyde or its derivatives 0.01. The soluble fraction of the liver tissue was used in a volume of 10 μl (equivalent to 1-1.5 mg protein). The unit of activity was taken to be the amount of enzyme causing a change in optical density at 340 nm of 0.001 in 1 min. The specific activity was calculated as the number of units of enzyme per milligram protein of the soluble fraction. Protein was determined by the biuret reaction. The mean results of 6-8 equivalent experiments are given in the tables.

EXPERIMENTAL RESULTS AND DISCUSSION

To study the mechanism of the antihypoxic effect of succinic semialdehyde and certain of its derivatives [8], the ability of the enzyme system of the liver tissue to oxidize NAD · H_2 in the presence of these compounds was investigated. Besides succinic semialdehyde, its derivatives at the aldehyde and carboxyl groups were tested. The data in Table 1 show that the soluble fraction of the liver catalyzed the oxidation of NAD · H_2 both by succinic semialdehyde (No. 1) and by its derivatives preserving a free aldehyde group (Nos. 2, 3). In the presence of derivatives of succinic semialdehyde with a blocked aldehyde group, NAD · H_2 was not oxidized (Nos. 4-6).

Definite correlation was observed between the antihypoxic properties of succinic semialdehyde and its derivatives and the oxidation of NAD · H_2 in the liver tissue. Only succinic semialdehyde and its derivatives with a free aldehyde group increased the resistance of the animals to hypoxia [8], and it was in the presence of these compounds that the enzyme system of the liver catalyzed the oxidation of NAD · H_2 (Table 1). Probably the free aldehyde group enables these compounds to take part in processes increasing the animals' resistance to hypoxia.

The oxidation of NAD · H_2 by succinic semialdehyde is known to involve the participation of an NAD-dependent enzyme system: lactate dehydrogenase in the brain tissue [9] and alcohol dehydrogenase in the liver tissue [11]. The reaction products in these enzymic processes are oxidized NAD and γ-hydroxybutyric acid. If reduction of the aldehyde group into an alcoholic group is possible during the conversion of succinic semialdehyde into γ-hydroxybutyric acid with the participation of NAD · H_2, the analogous conversion of succinic semialdehyde derivatives with a free aldehyde group probably also may take place.

The oxidation of NAD · H_2 in liver tissue, leading to the formation of NAD, deficient in hypoxia, may thus be one possible mechanism responsible for the antihypoxic properties of succinic semialdehyde and of its derivatives with a free aldehyde group.

Considering the data obtained by Kretovich on the binding of free ammonia with succinic semialdehyde [5] and also the possibility of reductive amination of succinic semialdehyde into γ-aminobutyric acid in plant tissues [10], the protective effect of succinic semialdehyde in hypoxia may also be explained by the binding of ammonia, the increased concentration of which may be one of the causes of death from hypoxia. In fact a considerable increase in the content of free ammonia in the brain tissue was observed in hypoxia (Table 2). If the animal received a preliminary injection of succinic semialdehyde, the concentration of free ammonia in the brain tissue in hypoxia was reduced.

On account of the possible acceptance of free ammonia, the aldehyde group of succinic semialdehyde

TABLE 2. Concentration of Free Ammonia in Brain Tissue Normally, in Hypoxia, and after Preliminary Injection of Succinic Semialdehyde into Animals before the State of Hypoxia

State of Animals	NH_3 (in μg/g wet weight of tissue)
Normal	7
Hypoxia	30
Hypoxia, preliminary injection of succinic semialdehyde	12

can thus lead to increased resistance of animals to hypoxia. Meanwhile, the lowering of the free ammonia concentration in hypoxia may also be the result of the sedative and hypothermic effects caused by injection of succinic semialdehyde.

In animal tissues, besides the reduction of the aldehyde group into an alcohol group, as a result of which the succinic semialdehyde is converted into γ-hydroxybutyric acid, it may also be oxidized with the formation of succinic acid. In Kondrashova's opinion [3, 4], the function of succinic semialdehyde as a precursor of succinic acid is essential to its biological action. The accumulation of succinic acid produces resistance to hypoxia, for it is utilized preferentially on account of its easier entry into the respiratory chain compared with other substrates [3]. The possibility of oxidation of succinic acid under hypoxic conditions ensures a supply of high-energy compounds to the respiratory chain.

It can be concluded from the analysis of the results described above and of data in the literature that the antihypoxic action of succinic semialdehyde and its derivatives is based on the conversions of these compounds, as a result of which ammonia is bound, oxidized NAD deficient in hypoxia is formed and, possibly, succinic acid accumulates.

LITERATURE CITED

1. V. V. Zakusov and R. U. Ostrovskaya, Byull. Éksperim. Biol. i Med., No. 2, 45 (1971).
2. A. M. Zubovskaya and R. U. Ostrovskaya, in: Current Problems in the Biochemistry of Respiration and Its Clinical Aspects [in Russian], Vol. 2, Ivanovo (1972), p. 313.
3. M. N. Kondrasheva, in: Mitochondria [in Russian], Moscow (1972), p. 151.
4. M. N. Kondrasheva, in: Mitochondria [in Russian], Moscow (1973), p. 112.
5. V. L. Kretovich, Dokl. Akad. Nauk SSSR, 170, No. 5, 1212 (1966).
6. R. U. Ostrovskaya, N. M. Tsybina, T. V. Protopopova, et al., Khim.-Farmats. Zh., No. 12, 21 (1969).
7. R. Ostrovskaya, A. Zubovskaya, V. Parin, et al., Scand. J. Clin. Lab. Invest., Suppl. 126, 519 (1972).
8. R. U. Ostrovskaya, N. M. Tsybina, V. V. Parin, et al., Byull. Éksperim. Biol. i Med., No. 8, 68 (1973).
9. W. N. Fishbein and S. P. Ressman, J. Biol. Chem., 239, 357 (1964).
10. J. G. Streefer and J. F. Thompson, Plant Physiol., 49, 579 (1972).
11. M. Wolleman, Agressologie, 6, 613 (1964).