EFFECT OF SUCCINIC SEMIALDEHYDE ON SOME ASPECTS OF NITROGEN METABOLISM IN THE BRAIN TISSUE OF HYPOXIC ANIMALS

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Investigation of the concentrations of free ammonia, glutamine, and some free amino acids in the brain tissue of normal animals and of hypoxic animals receiving or not receiving succinic semialdehyde revealed a marked increased in the free ammonia and α-alanine concentrations in hypoxia compared with normal, a small increase in the γ-aminobutyric acid, no change in the glutamic and aspartic acid concentrations, and a decrease in glutamine. If succinic semialdehyde was given before exposure to hypoxia the concentrations of free ammonia, glutamine, and α-alanine were close to normal in this state. One possible mechanism of the antihypoxic effect of succinic semialdehyde is the conversion of this compound with the resulting synthesis of glutamic acid and glutamine, which leads to detoxication of the free ammonia that accumulates in the brain tissue during hypoxia.

KEY WORDS: brain hypoxia; nitrogen metabolism; succinic semialdehyde; antihypoxic effect.

Previous investigations have shown that succinic semialdehyde not only increases the resistance of animals to hypoxia but helps to restore normal oxidative metabolism and to abolish the accumulation of free ammonia in the brain tissue characteristic of the state of hypoxia [5, 6].

To continue the investigation of the mechanism lying at the basis of the antihypoxic effect of succinic semialdehyde the effect of this compound on certain aspects of nitrogen metabolism of the brain tissue was studied during hypoxia.

EXPERIMENTAL METHOD

Albino mice weighing 20-24 g were used. Hypoxic conditions were created by placing the animals in closed chambers initially containing 8 vol. % of oxygen; the duration of exposure to hypoxia was 20-25 min [4, 6]. Succinic semialdehyde was injected intraperitoneally into the mice in a dose of 500 mg/kg 15 min before the animals were placed in the chamber; physiological saline was injected into the control animals. The concentrations of free ammonia, glutamine, and of some free amino acids (alanine, aspartic, glutamic, and γ-aminobutyric – GABA – acids) in the brain tissue were determined. The animals were decapitated and the head quickly frozen in liquid nitrogen. To determine the concentrations of free ammonia and glutamine the frozen brain was homogenized in 5 vol. of 5% TCA. The free ammonia and amide nitrogen of glutamine (as the free ammonia after hydrolysis for 10 min in 2 N H_2SO_4 [2]) in the supernatant was determined by isothermic distillation by Conway's method. To determine the concentrations of the free amino acids the frozen brain was treated by Wood's method [10]. The amino acids were fractionated by high-voltage electrophoresis at 5000 V in the VÉF-5-0.36 apparatus. To determine the α-alanine concentration electrophoresis was carried out in a buffer solution, pH 1.9 (formic and acetic acids and water in the ratio of 1 : 4 : 45). To determine the concentrations of aspartic and glutamic acids and GABA electrophoresis was carried out in buffer solution, pH 4.5 (pyridine, acetic acid, and water in the ratio of 1 : 2 : 247). Whatman No. 3MM filter paper was used. Development

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TABLE 1. Effect of Succinic Semialdehyde during Hypoxia on Concentrations (μmoles/g wet weight of tissue) of Free Ammonia, Glutamine, and of Some Free Amino Acids in Brain Tissue (M ± m)

Experimental conditions	Free ammonia	Glutamine	Aspartic acid	Glutamic acid	GABA	α-alanine
Normal	0,6±0,06	5,3±0,35	3,3±0,18	10,8±0,52	3,1±0,1	1,1±0,12
Hypoxia	1,5±0,12	4,5±0,11	3,1±0,11	10,8±0,65	3,5±0,15	2,8±0,28
Hypoxia + succinic semialdehyde	0,8±0,09	5,0±0,31	3,4±0,14	10,8±0,59	3,0±0,16	1,4±0,15

after electrophoresis was with 0.5% ninhydrin solution in acetone. The stained spots of the amino acids were extracted with 0,005% solution of $CuSO_4$ in 75% ethanol and determined quantitatively on the spectrophotometer at 512 nm [1].

EXPERIMENTAL RESULTS AND DISCUSSION

As the results in Table 1 show, the concentrations of free ammonia and α-alanine were considerably higher in hypoxia than normally, the GABA concentration was a little higher, and the glutamine concentration was lower. No appreciable changes were found in the concentration of glutamic and aspartic acids.

Accumulation of free ammonia and the decrease in the glutamine concentration in hypoxia could be the result both of a decrease in glutamine synthetase activity [3] and of an increase in glutamine breakdown [8]. The change in the glutamine concentration observed in hypoxia was not accompanied by any change in the concentration of glutamic acid, whereas the α-alanine concentration was considerably increased. The glutamic acid which accumulates during hypoxia is probably converted mainly into alanine. This is facilitated by the action of alanine aminotransferase in the direction of alanine formation [7] and the increased concentration of pyruvic acid [9], which causes the reversible reaction

$$\text{glutamic acid} + \text{pyruvic acid} \rightleftharpoons \alpha\text{-alanine} + \text{ketoglutaric acid} \quad (1)$$

to shift to the right.

If the animals received succinic semialdehyde before exposure to hypoxia the concentrations of free ammonia, glutamine, and alanine approached normal. Under conditions of normal respiration, the compound did not change these indices.

As was shown earlier [6] and confirmed by the present experiments, under the influence of succinic semialdehyde the binding of free ammonia during hypoxia was increased, and this was accompanied by a simultaneous increase in the glutamine concentration. Normalization of oxidative metabolic processes, modified in hypoxia [5], leading to preservation of high-energy phosphorus compounds, is the condition under which the increase in glutamine synthetase activity and the decrease in glutamine breakdown following injection of succinic semialdehyde are possible. As a result of these events in animals during hypoxia the glutamine concentration rises in the brain tissue and the concentration of free ammonia falls under the influence of succinic semialdehyde.

The detoxication of ammonia in the brain takes place chiefly through amination of glutamic acid (its conversion into glutamine) and by reductive amination of α-ketoglutaric acid by its conversion into glutamic acid [8]. Since amination of glutamic acid requires ATP, during hypoxia the reductive amination of α-ketoglutaric acid, a process not requiring the expenditure of energy, will predominate, more especially because free ammonia and NADH accumulate in the tissues [8]. What can be the role of succinic semialdehyde in the reductive amination of α-ketoglutaric acid, the reserves of which are exhausted in hypoxia [8]? After administration of succinic semialdehyde the concentration of α-ketoglutaric acid may be increased as a result of a transamination reaction:

$$\text{succinic semialdehyde} + \text{glutamic acid} \rightleftharpoons \alpha\text{-ketoglutaric acid} + \text{GABA} \quad (2)$$

The α-ketoglutaric acid formed is converted into glutamic acid by a reductive amination reaction, with the removal of free ammonia. Under these conditions succinic semialdehyde assumes the role in the detoxication of free ammonia accumulating in the brain tissues during hypoxia. The increase in the α-ketoglutaric acid concentration possibly also prevents excessive accumulation of alanine as a result of a shift of reaction (1) to the left.

Analysis of data obtained previously [6] and of those described in this paper leads to the conclusion that the antihypoxic action of succinic semialdehyde is based on conversions of this compound as the result of which

NAD, deficient during hypoxia, is formed and the excess of free ammonia is removed through the synthesis of glutamic acid and glutamine.

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EFFECT OF EXTREMAL STIMULATION ON BRAIN LEVEL OF CYCLIC AMP

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The content of cyclic adenosine-3',5'-monophosphate (cAMP) in the brain was investigated after electrical stimulation of immobilized rats, leading to the development of degenerative lesions in the internal organs. A marked decrease was found in the cAMP content in the rats' brain, which appeared 15 min after the beginning of extremal stimulation and remained at the same level during stimulation for 3 h. The lowering of the cAMP level was evidently connected with a deficiency of noradrenalin and, perhaps, of other biologically active amines also in the brain during electrical stimulation.

KEY WORDS: rat brain; cyclic AMP; electrical stimulation; degeneration of organs.

The appearance of neurogenic degenerative changes in the internal organs has been shown to be associated with disturbance of regulatory influences of the CNS and, in particular, of the hypothalamic region [1]. By means of biochemical analysis in addition to pharmacological, the processes taking place in the brain on the arrival of extremal impulses, leading to a disturbance of central trophic influences, were investigated more fully. In particular, during extremal stimulation a decrease in the level of biogenic amines and, in particular, of noradrenalin (NA) has been established [3]. Cyclic adenosine-3',5'-monophosphate (cAMP) is known to be an intermediary in the action of most biologically active substances and in the mechanism of their metabolic effects.

The object of this investigation was to study the concentration of cAMP in the brain during the development of neurogenic degenerative lesions arising as a result of extremal stimulation.

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