METABOLIC AMINO-ACID RESERVES AND AMINOTRANSFERASE ACTIVITY OF THE LIVER IN TERMINAL STATES AND IN THE EARLY PERIOD OF RECOVERY AFTER RESUSCITATION

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Experiments on 28 dogs showed that the content of free amino acids tested in liver homogenates is increased during clinical death and this is accompanied by a small increase in activity of aspartate- and alanine-aminotransferases. In the early recovery period a further accumulation of free amino acids accompanied by changes in their relative proportions is found in the liver. During the first hour after restoration of cardiac activity and respiration the transaminase activity in the liver tissue falls while that in the blood serum rises. After administration of insulin and glucose to animals in the recovery period the amino-acid level in their liver is lower than in untreated dogs and a tendency for it to return to normal is observed sooner.

An urgent problem in present-day reanimatology is the elucidation of the intimate mechanisms of the onset and course of "resuscitation sickness" [8]. There is experimental and clinical evidence that an important role in this syndrome is played by pathology of the liver [9]. After resuscitation marked disturbances of the antitoxic, protein- and prothrombin-synthesizing, and excretory functions of the liver develop in the early recovery period [15]. The RNA and DNA content in the liver tissue is reduced [5], activity of the uridyl-transferase system falls [14], and the portal blood flow is reduced [3]. Protection of the liver against hypoxia by perfusion with oxygenated blood considerably improves the prognosis of resuscitation [16].

The object of this investigation was to study the metabolic amino-acid reserves in the liver of dogs during clinical death and in the early period of recovery after resuscitation. Activity of aspartate- (2.6.1.1) and alanine-aminotransferases (2.6.1.2) was determined simultaneously in liver tissue homogenates and in the blood serum.

EXPERIMENTAL METHOD

Experiments were carried out on 28 male dogs weighing 12-14 kg. The femoral vessels were exposed on both sides under local anesthesia with procaine. A polyethylene catheter for drip infusion of 0.2% thiopental-sodium solution was introduced into one of the veins. Clinical death lasting 2-4 min was induced by free bleeding from the femoral artery after injection of heparin. Resuscitation was carried out by Negovskii's combined method. Considering the objects of the investigation, neither adrenalin nor glucose was added to the blood for infusion. In the course of the experiment the systemic arterial pressure, respiration, and ECG were recorded. Samples of liver tissue were taken before bleeding, during clinical death, and at various times of the recovery period (10, 20, 30, and 60 min). The tissue was fixed in liquid nitrogen. The serum transaminases in the arterial blood were investigated. The content of free amino acids was deter-

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TABLE 1. Dynamics of Changes in Content of Free Amino Acids in the Liver in Terminal States and in the Early Recovery Period after Resuscitation (M ± m)

Times of sampling	Amino acids (in μg/g)							
	lysine	histidine	aspartic acid + serine + glycine	glutamic acid + threonine	alanine	valine + methionine	phenylalanine	leucine + isoleucine
Initial background	51,8±4,0	68,0±3,0	142,3±4,7	77,7±3,2	102,0±6,0	54,7±3,2	59,3±3,0	57,1±1,4
Period of clinical death	87,9±2,1*	91,7±4,7*	190,6±4,7*	109,7±4,0*	156,4±5,3*	67,8±3,5**	82,5±2,7*	82,2±4,4*
Recovery period	140,3±6,0*	107,4±5,0*	205,6±4,6 †	136,8±4,5*	195,0±4,7*	86,1±3,1*	104,3±5,0*	93,3±5,0*

*P ≤ 0.001.
† P ≤ 0.01.

mined by descending strip paper chromatography [17, 18] with certain modifications [4, 10]. Activity of aspartate- and alanine-aminotransferases (GOT and GPT, respectively) was studied by the method of Reitman and Frankel [20] in Paskhina's modification and expressed in colorimetric units.

EXPERIMENTAL RESULTS AND DISCUSSION

The level of all the amino acids studied in the liver homogenates increased during the period of clinical death caused by arterial blood loss (Table 1). The increase was greatest for alanine and lysine and least for phenylalanine as well as for valine and methionine determined together.

In the early recovery period the content of amino acids in the liver homogenates continued to rise. At a time of stabilization of the arterial pressure 20 min after resuscitation, with the appearance of regular respiration, restoration of the normal ECG picture, and recovery of the corneal reflexes, the lysine content was 2.7 times higher than initially, the alanine concentration 94% higher, glutamic acid + threonine 75.6%, and phenylalanine 73.3% higher. The concentration of the amino acids studied still remained high in the liver 30 and 60 min after resuscitation. In the period of clinical death and, still more, soon after resuscitation, accumulation of free amino acids accompanied by changes in their relative proportions, possibly indicating protein renewal, were thus observed in the liver.

During clinical death the GOT and GPT activity in the liver homogenates was slightly increased. In the early recovery period, however, the activity of the aminotransferases fell. At the 10th minute after restoration of cardiac activity and spontaneous respiration, for instance, the GOT activity in the liver homogenates was reduced by 17.1% ($P < 0.001$) and the GPT content by 19.8% ($P < 0.001$).

Increased aminotransferase activity was detectable in the blood serum during the period of preagony and agony. This effect was more clearly manifested in the early recovery period, in agreement with data in the literature [7]. Under these conditions a greater increase was found in the activity of GPT which, unlike GOT, is not firmly bound to the intracellular structures [19] and is easily liberated into the blood stream when the liver cells are damaged. As a result the ratio S_{GOT}/S_{GPT} fell from 1.6 in the control to 1.1 10 min after resuscitation.

Analysis of these results suggest that the increase in the intracellular reserves of free amino acids in terminal states is a manifestation of cell damage and of the inhibition of amino-acid utilization in metabolic reactions, including the synthesis of peptides and proteins. Another manifestation of cell damage is the decrease in GOT and GPT activity in the recovery period in the liver and the simultaneous increase in their activity in the blood serum. In this case the phenomenon of elimination of enzymes from the cell into the extracellular fluid and blood, familiar with respect to the myocardium and liver, is observed.

The observed accumulation of free amino acids during clinical death and resuscitation in other tissues also (the brain, for example [2, 13]) suggests that the increase in the intracellular amino-acid reserves in the liver is the result of the develop-

ment of a nonspecific disturbance of the "metabolic pool" of cells of various tissues in hypoxia. The increase in the amino-acid content in the tissues evidently determines the increase in their concentration in the blood serum, for unlike their transport into the cells, which takes place against the concentration gradient, their outward movement from the cell takes place by diffusion.

Under the conditions of severe hypoxia autolysis presumably is intensified in the cells as the result of disturbance of the integrity for increased permeability of the lysosomal membranes, followed by the liberation of hydrolytic enzymes, including tissue proteinases, into the cytoplasm. The "injury acidosis" thus developing [1] may be responsible for the disturbance of free amino-acid metabolism.

Another possible cause of the increase in the intracellular amino-acid reserves may be the inhibition of protein resynthesis. In terminal states this takes place as a result of exhaustion of the energy reserves of the cell, a decrease in the functions of the insular apparatus, and an increase in the activity of the liver insulinase [12].

In view of public reports that insulin and glucose are highly effective in resuscitation [6, 11], this combination was used in one series of experiments. Injection of insulin (0.3 unit/kg) simultaneously with glucose (40% solution, 3-5 ml/kg) immediately after the resumption of cardiac activity led to a lower level of amino acids in the liver tissue homogenates taken at various times of the recovery period than in the liver of untreated dogs ($P < 0.05$). In addition, when insulin and glucose were injected in the recovery period a tendency toward restoration of the normal metabolic reserves of amino acids was observed sooner.

LITERATURE CITED

1. A. D. Ado, Pat. Fiziol., No. 6, 12 (1972).
2. M. S. Gaevskaya, Biochemistry of the Brain during Clinical Death and Resuscitation [in Russian], Moscow (1963).
3. A. Ya. Evtushenko, Byull. Eksperim. Biol. i Med., No. 11, 16 (1972).
4. G. I. Zaitseva and N. P. Tyuleneva, Lab. Delo, No. 3, 24 (1958).
5. V. L. Kozhura, Dynamics of the Nucleic Acid Concentration in the Liver during Clinical Death from Blood Loss and in the Recovery Period after Resuscitation. Author's Abstract of Candidate's Dissertation [in Russian], Moscow (1969).
6. A. Labori, The Regulation of Metabolic Processes [Russian translation], Moscow (1970).
7. G. L. Lyuban et al., in: Endocrine Factors and Reactivity of the Organism during Resuscitation [in Russian], Novosibirsk (1963), p. 60.
8. V. A. Negovskii, Current Problems in Reanimatology [in Russian], Moscow (1971).
9. V. A. Negovskii, in: The Recovery Period after Resuscitation [in Russian], Moscow (1970), p. 11.
10. T. S. Paskhina, Modern Methods in Biochemistry [in Russian], Moscow (1964), p. 162.
11. S. A. Khachatryan, Zh. Eksper. i Klin. Med., No. 2, 13 (1968).
12. S. A. Khachatryan, in: Problems in Pathological Physiology [in Russian], Erevan (1966), p. 107.
13. S. A. Khachatryan and N. G. Episkoposyan, Pat. Fiziol., No. 6, 31 (1972).
14. G. S. Khachatryan et al., Biol. Zh. Armenii, 24, No. 11, 16 (1971).
15. V. M. Shapiro, Pat. Fiziol., No. 3, 32 (1969).
16. V. M. Shapiro, in: The Recovery Period after Resuscitation [in Russian], Moscow (1970), p. 99.
17. F. Bode, Biochem. Z., 326, 433 (1955).
18. K. V. Giri et al., J. Indian Inst. Sci., 35, 145 (1953).
19. R. Hook and C. Vestling, Biochim. Biophys. Acta, 65, 358 (1962).
20. S. Reitman and S. Frankel, Am. J. Clin. Path., 28, 56 (1957).