SOME ASPECTS OF AMMONIA FORMATION IN THE BRAIN OF DYING ANIMALS AND DURING RECOVERY AFTER RESUSCITATION

S. A. Khachatryan and A. A. Papyan

UDC 616-036.882-08-07:616.831-008-921.711-074

The dynamics of some indices of nitrogen metabolism in the brain was studied in dogs after lethal blood loss and in the postresuscitation period. The investigation showed that the main source of ammonia in the brain during the development of the organism and in the state of clinical death is glutamine. In the recovery period after resuscitation, however, glutamine becomes a remover of ammonia and the main source of ammonia becomes the labile amino groups of proteins.

KEY WORDS: clinical death; resuscitation; ammonia metabolism; brain.

Disturbances of carbohydrate and phosphorus metabolism in nerve tissue in the terminal state and after resuscitation are the aspects of this problem which have so far received the most study [1, 6, 10]. However, the correct management of the postresuscitation period calls for an investigation of other types of metabolism also. There are only isolated references in the literature to ammonia formation in brain tissue in terminal states and in the postresuscitation period [1, 2].

In the investigation described below the concentrations of ammonia and glutamine, glutaminase and glutamine systhetase activity, and the protein content in the brain tissue of dogs were studied during the terminal state and in the postresuscitation period.

EXPERIMENTAL METHOD

Experiments were carried out on 80 noninbred male dogs weighing 13-16 kg. Clinical death was produced by unrestrained arterial bleeding. Resuscitation was carried out by Negovskii's combined method. In all experiments followed by resuscitation, the duration of clinical death was 2 min 30 sec. The skull was trephined in the temporoparietal region under moderately deep morphine–ether anesthesia. Brain samples were removed during anesthesia, during agony, and at the end of 1, 5, and 10 min of clinical death. In the experiments with resuscitation brain samples were taken 1, 24, and 72 h, and 1 week after reanimation. Immediately after removal, the brain tissue was fixed in liquid nitrogen. Ammonia and glutamine were determined by microdiffusion distillation [9, 12]. Protein amido groups were determined in the residue. Readily hydrolyzed amino groups were determined after hydrolysis for 10 min in 1 N H_2SO_4 at 100°C, and protein amino groups more resistant to hydrolysis were determined after hydrolysis under the same conditions for 2 h. Glutamine synthetase [3, 11] and glutaminase activity of the brain also were determined. Reactive amino groups and SH groups of proteins were demonstrated histochemically [8]. The numerical results were subjected to statistical analysis [5, 7].

EXPERIMENTAL RESULTS

The ammonia concentration in the brain of the dogs was increased and the glutamine concentration reduced during the stage of agony (Table 1). With the development of clinical death these changes increased in severity. For instance, at the fifth minute of clinical death the ammonia concentration was 2.1 times higher than initially, whereas the glutamine concentration was reduced by 41%. The increase in the brain ammonia

Department of Pathological Physiology, Erevan Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. A. Negovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 6, pp. 686-688, June, 1977. Original article submitted August 24, 1976.

TABLE 1. Changes in Concentrations of Ammonia, Glutamine, and Protein Amido Groups and Also in Glutaminase and Glutamine Synthetase Activity in Brain Tissue after Lethal Blood Loss and in the Recovery Period (M ± m)

Index studied	Morphine–ether anesthesia	Agony	Clinical death		
			end of 1st min	end of 5th min	end of 10th min
Ammonia	0,59±0,025	0,92±0,041	1,12±0,053	1,24±0,061	1,43±0,077
n	13	13	13	13	13
P		<0,001	<0,001	<0,001	<0,001
Glutamine	6,28±0,23	3,92±0,20	3,56±0,13	3,5±0,11	2,9±0,16
n	13	13	13	13	13
P		<0,001	<0,001	<0,001	<0,001
Glutaminase	3,95±0,158	4,19±0,333	4,46±0,249	4,51±0,248	4,73±0,242
n	12	12	12	12	12
P		>0,5	<0,1	<0,1	<0,02
Glutamine synthetase	3,192±0,175	3,407±0,185	2,507±0,161	2,281±0,124	1,628±0,183
n	12	12	12	12	12
P		<0,5	<0,01	<0,001	<0,001
Readily hydrolyzed protein amino groups	19,75±1,104	18,55±1,637	15,27±1,276	13,37±1,060	9,63±1,042
n	12	12	12	12	12
P		>0,5	>0,01	>0,001	<0,001
Protein amino groups more resistant to hydrolysis	44,45±1,017	44,85±3,072	52,68±2,792	58,36±3,174	51,16±2,518
n	12	12	12	12	12
P		>0,5	>0,01	>0,001	>0,02

Index studied	After resuscitation			
	1 h	24 h	72 h	1 week
Ammonia	1,05±0,100	0,783±0,048	0,835±0,053	0,625±0,048
n	9	9	5	5
P	<0,001	<0,001	<0,001	>0,5
Glutamine	6,95±0,323	6,06±0,193	6,735±0,12	6,31±0,168
n	9	9	5	5
P	<0,2	<0,5	<0,1	>0,5
Glutaminase	1,43±0,147	1,802±0,157	1,88±0,123	3,1±0,134
n	9	9	5	5
P	<0,001	<0,001	<0,001	<0,001
Glutamine synthetase	2,169±0,254	1,852±0,183	2,104±0,077	2,985±0,144
n	9	8	5	5
P	<0,01	<0,001	<0,001	<0,5
Readily hydrolyzed protein amino groups	17,72±2,580	11,67±0,729	10,5±0,575	17,6±1,152
n	9	8	5	5
P	<0,5	<0,001	<0,001	<0,2
Protein amino groups more resistant to hydrolysis	44,16±1,683	49,69±2,419	49,0±1,919	45,7±1,823
n	9	8	5	5
P	>0,5	<0,1	<0,1	>0,5

Legend. Ammonia, glutamine, and amido groups expressed in milligram percent ammonia nitrogen per gram wet weight of tissue; glutaminase activity in milligrams of ammonia nitrogen per gram wet weight of tissue; glutamine synthetase activity in micromoles glutamylhydroxamic acid per gram wet weight of tissue.

concentration evidently took place at the expense of a reduction in glutamine. Investigation of the activity of enzymes of glutamine metabolism showed a marked increase in glutaminase activity in the brain. However, the degree of this increase suggests that the decrease in the glutamine concentration in the brain tissue was due not only to its more rapid breakdown, but also evidently to a reduction in the rate of its synthesis. In fact, whereas during agony a small increase in glutamine synthetase activity was observed, by the end of the first minute of clinical death this had given way to inhibition, which deepened in successively later stages.

The combining site of this enzyme is known to contain two SH groups, and if these are blocked its activity falls sharply [14]. The experiments showed that at the end of the fifth minute of clinical death the intensity of the reaction for protein SH groups in residual neurons was much lower than in the anesthetized dogs. The possibility thus cannot be ruled out that a change in the content of free protein SH groups plays an important role in the fall of glutamine synthetase activity in the brain during terminal states.

Considering the important role of protein amido groups in ammonia metabolism [4, 13] and their high functional activity in the brain, their concentration was studied. During agony a tendency was observed for the quantity of labile amino groups to decrease and for the number of stable groups to increase, though this was not significant. These changes increased later. Labile amino groups can be considered to be one source of ammonia in brain tissue, whereas the groups more resistant to hydrolysis participate in the removal of ammonia.

With the development of clinical death the reaction for amino groups in proteins became somewhat weaker in sections through the cerebral cortex, and by the end of the fifth minute of clinical death this reaction was still present only in the nucleolus and the nuclear membrane. In some parts of the cytoplasm of the residual neurons a weak reaction also was still observed.

The ammonia concentration in the brain tissue 1 h after the appearance of the first spontaneous inspiration still remained high and the glutamine level was actually a little higher than that in the anesthetized dogs. The ammonia concentration fell 24 h after resuscitation, although not to its initial value. The brain ammonia concentration was restored after 1 week. Glutamine is evidently not the source of ammonia formation in the recovery period after resuscitation.

The investigations showed that the rapid recovery of the glutamine concentration in the postresuscitation period was due, not to restoration of its normal metabolism but, on the contrary, to the greater inhibition of breakdown than of synthesis. As a result, in the postresuscitation period glutamine can behave as a remover of ammonia.

The concentration of amido groups of brain proteins was unchanged 1 h after resuscitation, but after 24 h a decrease in the labile amino nitrogen and an increase in the concentration of stable amino groups were observed. The results of investigations of other functionally active groups of brain proteins 1 h after resuscitation showed a tendency for their concentration to recover.

The main source of ammonia in the recovery period after resuscitation is thus evidently the readily hydrolyzed amino groups of the brain proteins, whereas during agony its main source is glutamine.

LITERATURE CITED

1. M. S. Gaevskaya, Biochemistry of the Brain in the Terminal State and during Resuscitation [in Russian], Moscow (1963).
2. M. S. Gaevskaya and E. A. Nosova, Ukr. Biokhim. Zh., No. 3, 407 (1961).
3. Z. S. Gershenovich and A. A. Krichevskaya, Biokhimiya, No. 6, 715 (1956).
4. Z. S. Gershenovich, A. A. Krichevskaya, A. I. Lukash, et al., in: Proceedings of the Third All-Union Conference on Biochemistry of the Nervous System [in Russian], Erevan (1963), p. 91.
5. L. S. Kaminskii, The Analysis of Clinical and Laboratory Data [in Russian], Leningrad (1959).
6. V. A. Negovskii, Current Problems in Resuscitation [in Russian], Moscow (1971).
7. I. A. Oivin, "Statistical analysis of the results of experimental investigations," in: Trudy Stalingrad. Inst., 4, 4-8 (1957).
8. A. G. E. Pearse, Histochemistry: Theoretical and Applied, Little, Brown, Boston (1960).
9. A. I. Silakova, G. P. Trush, and A. Yakovleva, Vopr. Med. Khim., No. 5, 538 (1962).
10. S. A. Khachatryan, "Resuscitation during fever and certain changes in metabolism and in the endocrine system," Author's Abstract of Doctoral Dissertation, Erevan (1968).
11. W. H. Elliott, Biochem. J., 49, 106 (1951).
12. D. Seligson and H. Seligson, J. Lab. Clin. Med., 38, 324 (1951).
13. R. Vrba, Nature, 176, 117 (1955).
14. Wu Chung, Arch. Biochem., 106, 402 (1964).