THE AMMONIA AND GLUTAMINE CONTENT OF BRAIN TISSUE DURING ANEMIA RESULTING FROM LIGATION OF THE CAROTID ARTERIES*

M. N. Pertseva

From the Department of Pathological Physiology (Head – Prof. I. R. Petrov) and the Department of Biological Chemistry (Head – Prof. G. E. Vladimirov) of the S. M. Kirov Order of Lenin Military Medical Academy, Leningrad

(Received July 21, 1957. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

The present paper is devoted to the study of the effect of oxygen lack on the nitrogen metabolism of the brain. As an indicator of the state of the latter we took the content of preformed ammonia and the content of free glutamine in the tissue of the brain. According to a number of workers [10, 11, 12], anoxia is accompanied by stimulation of ammonia production in the brain. E. A. Vladimirova [2] found that with a profound degree of oxygen lack there is a fall in the ammonia production by the brain tissue.

EXPERIMENTAL METHOD

As experimental animals we took white rats weighing from 150 to 300 g. Incomplete anemia of the brain was obtained by application of ligatures to the common carotid arteries and subsequent division of these vessels. The operation was performed without general anesthesia. Cerebral anemia in rats is accompanied by two types of reaction [5, 7].

In one group of animals (convulsive group) periodic attacks of general motor excitation and convulsions developed, which were replaced by manifestations of severe inhibition. The other group of animals, after a brief period of gneral excitation, fell into a state of profound inhibition of the central nervous system (nonconvulsive group).

The brains of the convulsive group of rats were subjected to biochemical analysis 4-6 hours after operation, and those of the nonconvulsive group after 24 hours. These periods were selected in accordance with the observations made by K. G. Gromova et al. [5] in respect of changes in the metabolism of phosphorus compounds in the brain tissues of both groups of rats. The animals were frozen in toto in liquid oxygen during light ether anesthesia (5 minutes). Such brief anesthesia, as our control experiments showed, had no effect on the ammonia content of the brain. The tissue of the cerebral hemispheres, macerated in liquid oxygen, was treated with 5% trichloracetic acid to precipitate the proteins. The ammonia and glutamine content of the centrifugate were determined.

For determining the ammonia, we used the apparatus devised by S. D. Balakhovskii and Bruns [1] for estimation of the blood ammonia. This method was modified by us for application to brain tissue.

The ammonia was expelled from the centrifugate of brain after treatment with trichloroacetic acid by means of a borate buffer of pH 9.2-9.3 [2]. The ammonia content was estimated photometrically in the distillate with Nessler's reagent, using a graduated photometer or a photoelectric colorimeter M-1 with a blue filter. This method enables the ammonia content to be determined with an accuracy of a tenth part of microgram. The mean square error of the method is ± 4%.

* The substance of a dissertation submitted towards the degree of Candidate of Biological Sciences.

The brain centrifugate after trichloracetic acid treatment was hydrolyzed for 10 minutes with 2 N H_2SO_4 in a sealed tube on a water bath at 100°C [3] and the amide nitrogen of the glutamine was then estimated as ammonia.

EXPERIMENTAL RESULTS

Analysis of the brain tissue of normal healthy animals (10 white rats) showed that its preformed ammonia content averaged 0.43 mg% $N-NH_3$ with variation between 0.33-0.57 mg%, and the glutamine content averaged 7.08 mg% (6.21-9.04 mg%) $N-NH_2$.

Figures for the preformed ammonia content of the brain of white rats found in the literature vary considerably. Richter and Dawson [11] accept as normal 0.23 mg% $N-NH_3$ and 7.57 mg% $N-NH_2$ of glutamine, E. A. Vladimirova [2c] – 0.36 and 7.03 mg% and E. I. Gurina [1955] – 0.54 and 6.52 mg% respectively.

The control group of animals (7 white rats) was subjected to the same operation as the experimental animals up to the application of the ligatures to the vessels, but the ligatures were not tied nor the vessels divided. Until the time of removal of the brain for biochemical analysis (4-6 hours after operation for the convulsive group and 24 hours after for the nonconvulsive group), the behavior of the control rats was normal in every respect.

TABLE 1

Ammonia and Glutamine Content of the Brain of Control and Nonconvulsive Groups of Rats 24 Hours After Operation (in mg% nitrogen per dry weight of tissue)

Control animals			Animals with induced anemia		
Experiment No.	$N—NH_3$	$N—NH_2$	Experiment No.	$N—NH_3$	$N—NH_2$
38	0.34	8.59	24	0.50	6.70
			30	0.46	8.30
			41	0.49	9.01
			42	0.57	7.27
40	0.40	8.38	44	0.57	7.31
			72	0.85	10.39
			75	0.80	10.02
61	0.40	9.87	96	0.47	8.36
			97	0.48	6.07
			99	0.45	8.19
Average	0.38	8.94	Average	0.57	8.16

The investigation (Table 1 and 2) showed that operative trauma causes slight changes in the content of the nitrogen-containing compounds under study in the brain tissue. In the 4-6 hour control the ammonia content was on the average 0.56 mg% of nitrogen (normal 0.43 mg%), but 24 hours after operation it had returned to normal (0.38 mg%). A slight rise in the glutamine content was observed in the 4-6 hour period (7.97 mg% $N-NH_2$) with a further rise at 24 hours to 8.94 mg%. In this connection we compared the results of the experiments on the rats with induced anemia with the values obtained in control experiments.

The nonconvulsive group of rats with induced anemia fell after operation into a state of severe inhibition and 24 hours later they began to show trophic disturbances in the form of blepharoconjunctivitis and falling out of the fur. In the brain of these animals 24 hours after operation an increase in the ammonia content by 50% over the controls was found. The changes in the glutamine content were inconstant in character and on the average they showed a slight tendency to fall (see Table 1).

The other group of animals reacted to the production of cerebral anemia by intense excitation and by the periodic occurrence of convulsions, in the intervals between which the rats were in a state of complete prostration. Their body temperature fell sharply.

In these experiments (Table 2) the mean content of preformed ammonia was increased more than 3 times over the control values. The ammonia content was particularly high (more than 4 times higher than the control) in animals with very severe convulsive attacks (experiments Nos. 102, 104, 105). In experiments Nos. 104 and 105 the rats were frozen in a convulsive state and in experiment No. 102 in a state of intense excitation, wich usually preceded a convulsion. The glutamine content of these animals was found to be less than the control value. A particularly sharp fall (by 30-35%) was observed in experiments Nos. 104 and 105.

TABLE 2

Ammonia and Glutamine Content of the Brain of Control and Convulsive Groups of Rats 4-6 Hours after Operation (in mg% of nitrogen per dry weight of tissue)

Control animals			Animals with induced anemia			State of the animal at the moment of removal of the brain for biochemical analysis
Experiment No.	N—NH_3	N—NH_2	Experiment No.	N—NH_3	N—NH_2	
80	0.64	7.42	31	0.77	8.35	Killed in the interval between convulsions in a state of inhibition
86	0.63	9.60	33	1.41	8.76	
			79	1.71	9.49	The same
91	0.57	7.97	102	2.08	7.09	Killed at a period of general excitation
103	0.43	6.90	104	2.08	5.69	Killed at a period of very strong convulsions
			105	2.55	5.19	
Average	0.56	7.97	Average	1.77[1]	—[1]	

*In view of the different trends in the changes in the glutamine content of the brain of the convulsive group of animals, mean values of this quantity are not deduced.

In the rats killed during a convulsion-free period, in a state of inhibition (experiments Nos. 31, 33, 79) the glutamine content was 5-20% higher than the control value.

Investigation of the brain tissue of the nonconvulsive group of rats from 4-6 hours after operation showed that changes in the ammonia and glutamine contents which could be attributed to anoxia could not be found at this period; they appeared only after 24 hours.

The results obtained show that during an inadequate supply of oxygen to the brain an increase takes place in its content of preformed ammonia. This rise was particularly noticeable in cases where the oxygen lack is aggravated by general excitation of the central nervous system and by convulsions. As V. I. Rozengart [9] showed, convulsions due to pharmacological agents lead to merely a very transient rise in the ammonia content of the brain, in spite of the continuing convuslive attacks, and do not cause changes in the glutamine content. Consequently, the marked and persistent increase in ammonia and the fall in the glutamine observed in the convulsive group are the results of anoxia.

The increase in the ammonia content of the brain under the experimental conditions studied may be attributed to stimulation of deamination processes. In anoxia there is a disturbance of oxidative phosphorylation, accompanied by breakdown of ATP with the formation of adenosinediphosphate and adenosinemonophosphate [4, 8]. The latter may act as a source of the ammonia in the brain during its oxygen lack.

Free glutamine, according to E. A. Vladimirova's findings [2b], also takes part in the formation of ammonia. It is possible that in our experiments also, part of the ammonia formed in the brain arises in consequence of glutamine breakdown, for the content of glutamine is in fact greatly reduced during aggravation of anoxia.

Alongside the other sources of ammonia and probably the most potent must be mentioned the amide groups of the proteins of nerve tissue [3]. It may be that in oxygen lack, expecially if accompanied by excitation of the central nervous system, the deamination of proteins in the brain is intensified, leading to the accumulation of considerable quantities of ammonia.

On the other hand, the increase in the ammonia content of the brain tissue during anoxia may also be explained by slowing of its removal through glutamine synthesis on account of the deficiency of ATP. In support of this hypothesis there is the reduction in the content of free glutamine which was found under these conditions, especially pronounced in the brains of the convulsive group of rats.

During oxygen lack, glutamine metabolism in the brain, as shown above, is disturbed. When anoxia develops slowly (in the nonconvulsive group of rats) the changes in glutamine metabolism are indistinctly expressed – there is a tendency for its content to fall. If this pathological process runs an acute course (in the convulsive group) this fall in the glutamine content is very marked. We consider that the fall in the glutamine content of the brain during anoxia may possibly be accounted for by interference with its synthesis from glutamic acid and ammonia in connection with the lack or even the total absence (in the convulsive group) of ATP, found in rats after ligation of the carotid arteries [4]. The small rise in the glutamine content found in isolated experiments may probably be explained in these animals by the particularly profound inhibition in the central nervous system, causing a certain predominance of processes of synthesis.

TABLE 3

Ammonia and Glutamine Content of the Brain of the Nonconvulsive Group of Rats (in mg% of nitrogen per dry weight of tissue) 4-6 Hours After Operation

Control animals (average of five experiments)		Experiment No.	Animals with induced anemia	
$N—NH_3$	$N—NH_2$		$N—NH_3$	$N—NH_2$
0.59	7.51	93	0.39	7.61
		94	0.44	7.75
		98	0.77	7.16
		Average	0.53	7.50

Changes which we discovered in the indices of nitrogen metabolism in the brain that we investigated during cerebral anemia may, we consider, be explained not only by oxygen lack but also by interference with the supply of nutrient substances and energy-producing materials to the brain and with the removal of metabolic products, which result from the disturbance of the cerebral circulation. Summing up, we may conclude that during deficiency of oxygen in the brain, caused by incomplete anemia, the quantity of preformed ammonia in the brain is increased in consequence of breakdown of ammonia-forming compounds during anoxia and of delay in the removal of the ammonia formed.

In glutamine metabolism under these conditions breakdown is slightly in excess of the processes of synthesis.

Convulsions in association with disturbance of the cerebral circulation aggravate these changes in the nitrogen metabolism of the brain: the ammonia content is increased particularly sharply and the glutamine content shows a marked fall.

SUMMARY

The author studied the effect of oxygen insufficiency occuring in the brain in "exclusion" of both common carotid arteries on the content of ammonia and glutamine in it. Ammonia was determined by the Balakhovskii-Bruns method modified by us for brain tissue, while amide nitrogen of glutamine was determined by ammonia content after 10-minute hydrolysis with 2 N H_2SO_4 at 100°C.

The brain tissue of white rats reacted to anemia by widespread stable inhibition. Increase in the quantity of ammonia by 50%, as compared to control, was revealed in the central nervous system. The glutamine content tends to decrease. These changes were more pronounced in the brain tissue of rats in which brain anemia is asso-

ciated with attacks of general excitation and cramps. The quantity of ammonia in this case increased 3 times, while the amount of glutamine is decreased by 30-35% in comparison with control values.

LITERATURE CITED

[1] S. D. Balakhovskii and I. S. Balakhovskii, Methods of Chemical Analysis of the Blood,* Moscow, 1953.

[2] E. A. Vladimirova, Biull. Eksptl. Biol. i Med. No. 2, 138-140 [1950].

[3] E. A. Vladimirova, Biochemistry of the Nervous System,* p. 47-62, Kiev, 1954.

[4] R. Vbra, Uspekhi Sovremennoi Biol., 41, 3, 321-352 [1956].

[5] K. G. Gromova, T. E. Kudritskaia, I. R. Petrov and V. S. Shapot, Biokhimiia 17, 1, 13-24 [1952].

[6] K. G. Gromova and V. S. Shapot, Doklady Akad. Nauk SSSR 78, No. 5, 941-944 [1951].

[7] I. R. Petrov, Fiziol. Zhur. SSSR No. 1, 9-18, [1955].

[8] I. R. Petrov, Z. A. Raikov and T. E. Kudritskaia, Fiziol. Zhur. SSSR No. 2, 107-116 [1957].

[9] V. I. Rozengart, M. N. Maslova and A. N. Paniukov, Doklady Akad. Nauk SSSR, 110, No. 1, 122-124 [1956].

[10] R. W. Gerard and O. Meyerhof, Biochem. Z. 1927, Bd. 191, S. 125-146.

[11] D. Richter and R. M. C. Dawson, J. Biol. Chem. 1948, v. 176, p. 1199-1210.

[12] H. Schwarz and H. Dibold, Biochem. Z. 1932, Bd. 251, S. 190.

* In Russian.