CONTENT OF N-ACETYL-1-ASPARTIC ACID IN BRAIN TISSUE AFTER HEAD INJURIES

M. Sh. Promyslov and Ya. M. Sokovnina

UDC 617.51-001-07:616.831-008.93-074

The content of N-acetyl-1-aspartic acid in the tissues of the cerebral cortex, cerebellum, and brain stem of rabbits was found to fall sharply for 1 h after head injury. Five days later the content of N-acetyl-1-aspartic acid in all parts of the brain was restored to normal.

N-acetyl-1-aspartic acid is found exclusively in the central nervous system. Investigations have shown that it is present in different parts of the brain of animals of different species [3,5,7-16]. From 80 to 110 mg% of N-acetyl-1-aspartic acid was found in the brain of mammals and birds. Its content reaches a maximum in the gray matter of the brain (124 mg%), but in other organs it does not exceed 1-3 mg%.

D'Adamo [4,17] showed that the N-acetyl-1-aspartic acid of the brain can act as donor of acetyl groups for biosynthesis of lipids during the period of myelinization (one-third of the carbon of fatty acids is synthesized at the expense of N-acetyl-1-aspartic acid). Bunyatyan [1] and collaborators showed that this acid can also act as acetyl donor in the brain for the synthesis of acetylcholine and of acetylated derivatives of glucosamine.

Tallan and McIntosh [11,14] consider that N-acetyl-1-aspartic acid can make good an anion deficiency in the brain.

Since N-acetyl-1-aspartic acid is a specific substrate of nerve tissue, changes in the concentration of N-acetyl-1-aspartic acid in tissues of the cortex, cerebellum, and brain stem were studied in the present investigation after head injuries.

EXPERIMENTAL METHOD

Experiments were carried out on male rabbits weighing 2300-2700 g; the animals were killed by injecting air into the marginal vein of the ear. The brain was quickly removed, weighed, and homogenized with 4 volumes cold 5% TCA solution. The residue was removed by centrifugation and washed with 2 volumes of 5% TCA 3 times, the washings being added to the first extract. The extract was then treated 3 or 4 times with ether to remove the TCA, and the traces of ether were removed by heating on a water bath at 80°. A standard solution of aspartic acid was treated in the same way. The pH of the supernatant and standard solution must not fall below 4. The content of N-acetyl-1-aspartic acid was determined as aspartic acid liberated during hydrolysis of the extract for 60 min with 2 N HCl on a boiling water bath. After the end of hydrolysis, the extract was neutralized with NaOH. Aspartic acid was determined by the method of electrophoresis. The supernatant before and after hydrolysis was applied to chromatographic paper. Electrophoresis was carried out in 0.1 M acetate buffer, pH 5.0, for 5 h. The quantitative estimation was carried out by Paskhina's method [2].

The investigations were carried out 1 and 24 h and 3 and 5 days after head injury. The injury was produced by a weight of 500 g falling freely from a height of 2.2 m on the head of the rabbit fixed in a certain position.

EXPERIMENTAL RESULTS

The content of N-acetyl-1-aspartic acid in the brain of the healthy rabbits was identical with values obtained by other investigators. However, 1 h after head injury, the content of N-acetyl-1-aspartic acid in

Laboratory of Biochemistry, Academician N. N. Burdenko Institute of Neurosurgery, Academy of Medical Sciences of the USSR, Moscow (Presented by Academician of the AMN SSSR S. E. Severin). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 2, pp. 46-48, February, 1969. Original article submitted December 6, 1967.

TABLE 1. Content of N-acetyl-1-aspartic Acid in Brain Tissue of Rabbits at Various Times after Head Injury (in mg% of fresh tissue)

Part of brain	Normal	Time after injury			
		1 h	24 h	3 days	5 days
Cortex	96,9±6,8	44,8±3,5	51,9±2,5	62,5±2,8	93,5±6,3
P		<0,001	<0,001	<0,01	>0,5
Cerebellum	69,8±4,3	31,1±2,9	38,1±3,9	46,4±2,1	63,6±3,8
P		<0,001	<0,01	<0,01	>0,2
Brain stem	61,6±3,2	43,0±2,2	39 ±3,7	46,2±4,7	61±2,5
P		<0,01	<0,01	<0,05	>0,5

Note. Significance of differences (P) calculated relative to normal.

the cerebral cortex was sharply reduced (by 54% compared with normal). A slight increase in its concentration in the cortex was observed 24 h after injury, with a further increase by the 3rd day and respiration almost to normal by the 5th day after head injury (Table 1).

Similar results were obtained during investigation of tissues of the cerebellum and brain stem. The results thus showed that the content of N-acetyl-1-aspartic acid in the tissues of all investigated parts of the rabbit's brain after head injury was sharply decreased. The changes were greatest in the cerebellum and cortex and least in the brain stem.

The content of N-acetyl-1-aspartic acid on the 5th day in the brain stem was completely restored; its restoration in the cortex amounted to 96% and in the cerebellum to 91%.

The changes observed in the content of N-acetyl-1-aspartic acid in the investigated parts of the brain after head injury is evidence that this compound is a metabolically active component of the brain, and it conflicts with the opinion of those workers who consider that this substance is metabolically inert [5,6,9,15, 18].

LITERATURE CITED

1. G. Kh. Bunyatyan, Ukr. Biokhim. Zh., No. 5, 679 (1965).
2. T. S. Paskhina, in: Modern Methods in Biochemistry [in Russian], Vol. 1, Moscow (1964), p. 162
3. A. Curatolo, P. D'Arcangelo, A. Lino, et al., J. Neurochem., 12, 339 (1965).
4. A. F. D'Adamo and F. M. Yatsi, J. Neurochem., 13, 961 (1966).
5. R. S. De Ropp and E. H. Snedeker, J. Neurochem., 7, 128 (1961).
6. J. P. Du Ruisseau, Canad. J. Biochem., 48, 763 (1960).
7. M. C. Fleming and O. H. Lowry, J. Neurochem., 13, 779 (1966).
8. K. B. Jacobson, J. Gen. Physiol., 43, 323 (1959).
9. F. Marcucci, E. Missini, L. Valrelli, et al., J. Neurochem., 13, 1069 (1966).
10. I. C. McIntosh and J. R. Cooper, J. Neurochem., 12, 825 (1965).
11. I. C. McIntosh and J. R. Cooper, Nature, 203, 658 (1964).
12. N. Okumura, S. Otsuki, and T. Aoyma, J. Biochem. (Tokyo), 46, 207 (1959).
13. N. Okumura, S. Otsuki, and S. Kameyama, J. Biochem. (Tokyo), 47, 315 (1960).
14. H. H. Tallan, S. Moore, and W. H. Stein, J. Biol. Chem., 224, 41 (1957).
15. J. K. Tews, S. H. Carter, P. D. Rox, et al., J. Neurochem., 10, 641 (1963).
16. Y. O. Tsukada, K. Vemura, S. Hirano, et al., in: Comparative Neurochemistry, Oxford (1964), p. 179.
17. F. Yatsi and A. D'Adamo, Fed. Proc., 24, 553 (1965).
18. F. Yatsi and A. D'Adamo, Neurology (Minneap.), 15, 285 (1965).