INVESTIGATION OF TRANSAMINATION REACTIONS IN THE BRAIN OF RATS INOCULATED WITH FIXED RABIES VIRUS

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(Received November 8, 1957. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

Enzymic transamination reactions, discovered and studied in A. E. Braunshtein's laboratory, and occupying a crucial position in nitrogen metabolism, have been estensively studied in various pathological conditions of man and animals. Nevertheless amino acid metabolism, and in the first place transamination reactions, in virus infections have so far received little attention. Such investigations would be of fundamental importance in view of the special features of viruses, the proliferation of which in living cells is regarded as a variant of synthesis of proteins and nucleic acids.

In the field of virology research has been done, to our knowledge, into the activity of transamination enzymes in the liver and in the serum of mice infected with virus hepatitis. Under these circumstances a fall was observed in the activity of the aminopherases in the liver tissue and a rise in their activity in the serum. This latter effect was also clearly shown by human serum, where it served as the basis of a diagnostic test for virus hepatitis (Botkin's disease) [4].

The aim of the present investigation was to ascertain the possibility of influencing the processes of transamination in the brain tissue of rats by means of fixed rabies virus.

EXPERIMENTAL METHOD

Experiments were carried out on white rats weighing 130-150 g. The vaccine strain used in the work was from the Moscow Pasteur station. The experimental animals were infected by intracerebral inoculation with 0.05 ml of a 10% suspension of infected brain, prepared in broth. Control animals were infected with the same suspension prepared from the brain of healthy animals. Usually at the end of the 5th day the experimental animal was in a state of agony, when it was killed and its brain removed for biochemical examination. At the same time the brain was taken from healthy animals to act as a control. For the investigation the cerebral hemispheres were used.

The brain was homogenized in the cold for 3 minutes. Each experimental sample contained 200 mg of brain homogenate and phosphate buffer at pH = 7.2. The first sample consisted purely of homogenate and 1 ml of phosphate buffer. To the second sample was added 10 μM of L-glutaminic acid or L-alanine, depending on the reaction to be tested. To the third sample was added a previously neutralized solution of a-ketoglutaric or oxalacetic acid in a concentration of 20 μM. The fourth sample contained solutions of amino acids and keto-acids in the above concentrations. The final volume of each sample was 1 ml. The oxalacetic acid was synthesized by the method of Krampitz and Werkman [5], and the α-ketoglutaric acid by the method of Friedmann and Kozauer [3].

TABLE 1

Activity of Glutamicoasparaginic Aminopherase in Brain Homogenates of Rats. Formation of Asparaginic Acid in μM/g Dry Weight of Tissue

Experiment No.	Control animals	Experimental animals	Suppression of synthesis, μM	Suppression of synthesis in %
1	79,04	64,61	14,43	18,3
2	70,15	59,64	10,51	15,0
3	66,69	55,66	11,03	16,5
4	69,15	61,28	7,87	11,3
5	75,09	63,12	11,97	16,0
6	72,12	65,21	6,91	10,0
7	71,14	58,65	12,49	17,7
Mean results	71,92	61,16	10,76	15,0

TABLE 2

Activity of Glutamicoalanine Aminopherase in Brain Homogenates of Rats. Formation of Glutaminic Acid in μM/g Dry Weight of Tissue

Experiment No.	Control animals	Experimental animals	Suppression of synthesis, μM	Suppression of synthesis in %
1	45,94	36,78	9,16	20,0
2	56,32	42,74	13,58	24,1
3	54,34	42,24	12,1	22,3
4	52,86	39,26	13,6	25,6
5	50,35	40,75	10,6	19,1
6	57,8	44,73	13,07	20,0
Mean results	53,6	41,68	12,0	21,8

A parallel series of control experiments was set up, using brain homogenate from healthy animals. Samples were incubated for 2 hours at 37°C. The reaction was stopped by the addition of 0.1 ml of 20% trichloroacetic acid and subsequent boiling of the samples for 3 minutes. Proteins were removed by centrifugation. The supernatant fluid was subjected to analysis. The transamination reactions were investigated by the method of paper chromatographic analysis of the amino acids. The biosynthesis of asparaginic acid from L-glutaminic and oxalacetic acids and of glutaminic acid from L-alanine and α-ketoglutaric acid were investigated. The amino-acids were estimated quantitatively by Bode's method, using a Spekker absorptiometer.

EXPERIMENTAL RESULTS

Results of the examinations are shown in Tables 1 and 2, and are expressed as μM/g of dry tissue.

As may be seen from Table 1, a fall in the biosynthesis of asparaginic acid was observed in brain homogenates of the rats infected with fixed rabies virus. This can also be seen in the chromatogram (Fig. 1), where the asparaginic acid stain in test No. 9 is more intense than in test No. 5.

As may be seen from Table 2, the biosynthesis of glutaminic acid took place 21.8% more slowly in the brain homogenates of the infected animals than in those of healthy animals. This difference is expressed here

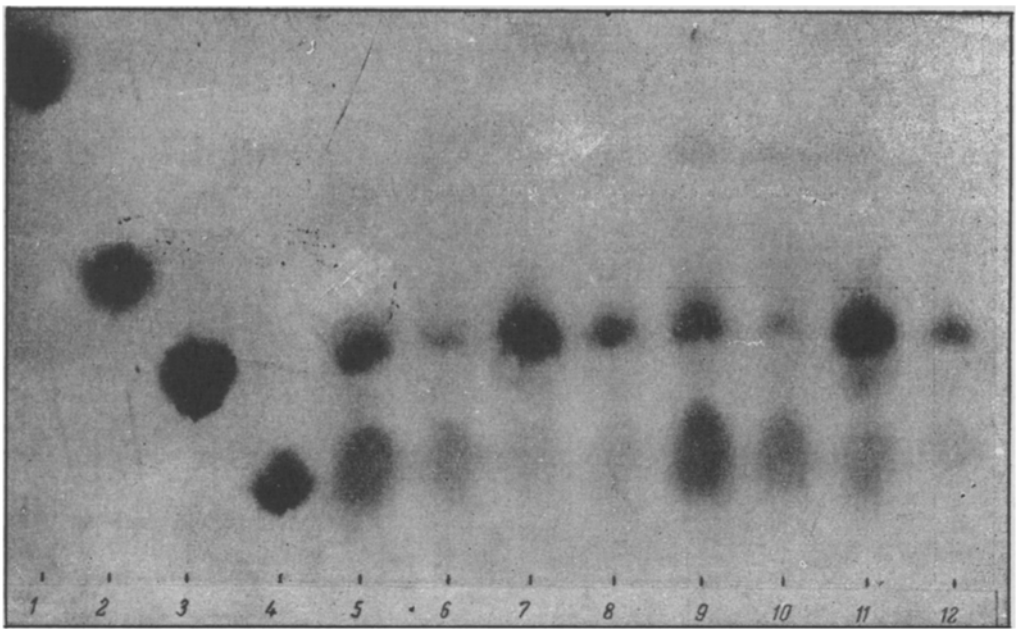

Fig. 1. Formation of asparaginic acid in brain homogenates of rats infected with fixed rabies virus (tests 5, 6, 7, 8) and in the brain of normal animals (tests 9, 10, 11, 12). Substrates for the tests: 5 and 9 — L-glutaminic and oxalacetic acids; 6 and 10 — oxalacetic acid; 7 and 11 — L-glutaminic acid; 8 and 12 — phosphate buffer. The numbers 1, 2, 3, 4 denote witnesses; γ-aminobutyric acid, alanine, glutaminic acid and asparaginic acid respectively.

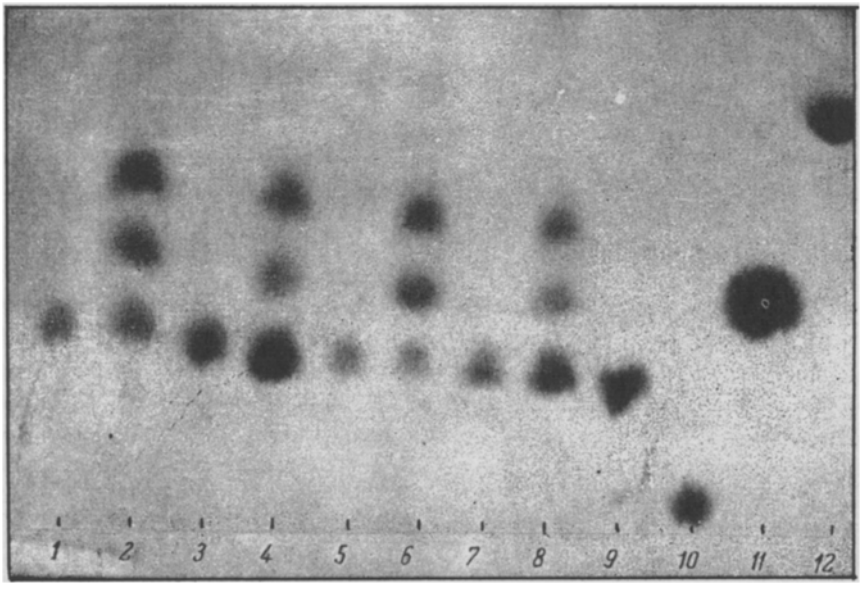

Fig. 2. Formation of glutaminic acid in brain homogenates of rats infected with fixed rabies virus (tests 5, 6, 7, 8) and in the brain of normal animals (tests 1, 2, 3, 4). Substrates for the tests: 1 and 5 — phosphate buffer; 2 and 6 — alanine; 3 and 7 — α-ketoglutaric acid; 4 and 8 — alanine and α-ketoglutaric acid. The numbers 9, 10, 11, 12 denote witnesses; glutaminic acid, asparaginic acid, alanine and γ-aminobutyric acid respectively.

A state of excitation was induced in the animals by the use of drugs: metamphetamine and caffeine sodium benzoate. Metamphetamine was injected subcutaneously in a dose of 1.5 mg/kg body weight of the animal, and caffeine in a dose of 50 mg/kg body weight.

A state of inhibition was produced by means of ether anesthesia, luminal, sodium amytal and pentothal. Luminal was given to the animals in a dose of 0.1 g/kg body weight. Sodium amytal was injected intravenously to a greater degree that during investigation of the activity of glutamicoasparaginic aminopherase. It should be pointed out that investigation of the activity of glutamicoalanine aminopherase with L-glutaminic acid and pyruvate also shows a fall in the formation of alanine from these substrates in brain homogenates of the experimental animals. The chromatogram shown (Fig. 2) illustrates the suppression of the synthesis of glutaminic acid in the infected brain (compare tests Nos. 4 and 8).

In control experiments the intracerebral injection of 0.05 ml of a 10% suspension of normal brain did not affect the aminopherase activity of the brain.

We were unable to detect any fall in the aminopherase activity at earlier stages of the disease. The experimental results obtained do not solve the problem of the cause of the fall in activity of the two most active aminopherases in the brain of rats infected with fixed rabies virus: does this take place at the expense of direct suppression of synthesis of apoenzyme by the proliferating virus, on account of changes in the level of protein synthesis in the brain infected with the virus, or as the result of pathological processes developing in the body during this infection? The influence of the virus on the aminopherase activity may be revealed only by its proliferation in a culture of isolated cells on synthetic media.

In this connection we may at present only state that in white rats infected with fixed rabies virus there is a fall in the activity of the transamination enzymes: in the case of glutamicoalanine aminopherase by 21.8%, and of glutaminicoasparaginic aminopherase by 15%.

SUMMARY

Homogenates of rat's brain affected with fixated rabies virus were examined. A decreased concentration of the 2 most active aminopherases was revealed: of glutamicoasparginic by 15% of glutamicoalaninic by 21.8% (in comparison with their quantities in the brain homogenates of healthy animals).

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