EFFECT OF POLIOMYELITIS VACCINE ON γ-AMINOBUTYRIC ACID METABOLISM IN THE ANIMAL BRAIN

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A single intramuscular injection or oral administration of poliomyelitis vaccine of immunological type II (Sabin strain) into albino mice (12-14 g) and cottontail rats (60-80 g) is followed by a transient but sharp increase in the γ-aminobutyric acid (GABA) concentration in the animals' brains. An increase in glutamate decarboxylase activity was also observed in the brain tissue of the cottontail rats after oral immunization. These changes in GABA precede the accumulation of specific antibodies in the serum.

It is now taken as proven that γ-aminobutyric acid (GABA) metabolism is entirely connected with the activity of the brain cells and, in particular, with their inhibition [4, 7]. Pharmacological, hormonal, and other agents have been shown not to cause any significant deviations in the distribution of free amino acids (inclusing GABA) in the brain of animals [8, 9]. The absence of any sharp change in the GABA level in the brain even in paroxysmal states is evidence that constancy of the GABA level is very important for the activity of the nervous system.

For these reasons it was decided to study GABA metabolism in the brain in response to intramuscular or oral administration of attenuated poliomyelitis virus. The starting point for the investigation was results showing that vaccination with living poliomyelitis vaccine is followed by changes in biochemical processes in the brain [2].

EXPERIMENTAL METHOD

The experimental animals were albino mice (12-14 g) and cottontail rats (60-80 g) which received a single massive dose (0.5 ml) of living poliomyelitis vaccine of immunological type II (Sabin strain), with a titer for monkey kidney cells of 10^{-7} TCD_{50}/ml intramuscularly or by mouth. The control animals received a placebo containing all the ingredients of the vaccine except the virus. In the course of the experiment the immunized and control animals were decapitated on the 3rd and 5th days from the beginning of vaccination, the brain was quickly removed, and the tissue homogenized.

Activity of L-glutamate-1-carboxylase (4.11.15) was determined as described by Yakovlev [5]. Activity of the enzyme was judged from the increase in GABA concentration in the experimental sample [1]. The incubation mixture (2.1 ml) consisted of 1 ml homogenate (200 mg fresh tissue) in 0.1 M sodium-potassium-phosphate buffer, pH 7.0, 1 ml of 0.02% pyridoxal phosphate solution, and 0.1 ml 0.5 M glutamic acid. The samples were incubated for 1 h at 37°C in an atmosphere of nitrogen. The reaction was stopped by the addition of 8 ml 96% ethanol. The test solution was applied in a volume of 10 μl to Whatman No. 3 chromatographic paper. Fractionation continued for 24 h.

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TABLE 1. GABA Concentration and Glutamate Decarboxylase Activity in Brain Tissue of Animals on 5th Day after Immunization with Poliomyelitis Vaccine of Immunological Type II (Sabin Strain)

Statistical index	Albino mice				Cottontail rats			
	intramuscular injection of vaccine		oral administration of vaccine		GABA concentration (in mg %)		Decarboxylase activity (in μmoles GABA/g tissue/h at 37°C)	
	GABA conc. (in mg %)							
	expt.	control	expt.	control	experiment	control	experiment	control
M	39,50	25,30	34,50	26,20	33,70	25,70	13,00	10,80
±*m*	2,60	0,95	2,03	0,93	1,56	0,97	0,36	0,50
n	15	10	10	10	12	11	8	8
P	<0,001		<0,01		<0,001		<0,01	

Activity of 4-aminobutyrate: 2-hydroxyglutarate-aminotransferase (2.6.1.19) was determined by the writers' modification of Roberts' method [6]. The activity of this enzyme was judged from the increase in the glutamic acid concentration [3]. The incubation mixture (1.2 ml) consisted of 1 ml homogenate (400 mg fresh tissue) in Tris-acetate buffer, pH 8.2, 0.1 ml 0.2% pyridoxal phosphate solution, 0.05 ml (40 μmoles) GABA, and 0.05 ml (40 μmoles) α-ketoglutaric acid. The samples were incubated for 1 h at 37°C. After incubation the proteins were precipitated with TCA in a final concentration of 3-4%. The supernatant was washed with three portions of ether to remove the TCA. The ether-washed supernatant was applied in volumes of 10 μl to the chromatogram.

EXPERIMENTAL RESULTS

In the experiments of series I, the concentration of GABA in the brain tissue of the vaccinated and control animals was investigated on the third day after vaccination and no difference was found between the experiment and the control. It follows from Table 1 that on the 5th day (experiments of series II) after injection of the vaccine there was a marked increase in the GABA concentration in the brain of the experimental animals. It is interesting to note that the increase in GABA concentration in the brain tissue of animals immunized with poliomyelitis vaccine always preceded the period of the highest blood level of specific antibodies. For instance, the serum antibody titer on the 15th day after vaccination was only 1:16, on the 20th day, 1:16, on the 30th day 1:16, and on the 45th and 60th days 1:64. The GABA concentration reached the control level on the 10th day. The GABA concentration in the control animals immunized with the placebo showed no significant deviations from normal. These results suggest the change in the GABA concentration on the 5th day is an early response of the brain to administration of the virus vaccine.

The GABA level in nerve tissue is determined by the relationship between two basic processes: decarboxylation of glutamic acid with the formation of GABA and transamination of GABA with α-ketoglutaric acid. The next task was therefore to determine the activity of the enzyme catalyzing the reaction of GABA formation in the brain tissue of the animals immunized with poliomyelitis vaccine of immunological type II. Tests were carried out on cottontail rats, for these animals are more sensitive to poliomyelitis virus.

It will be clear from Table 1 that a statistically significant increase in decarboxylase activity was found in the cottontail rats, and this corresponded to the increase in GABA concentration under the same experimental conditions.

Activity of the enzyme GABA-α-ketoglutarate transaminase in the brain tissue of the immunized cottontail rats, on the other hand, was indistinguishable from that in the brain tissue of the control animals.

These results indicate that the system of GABA metabolism in the brain and connected with its function undergoes definite changes during immunization with poliomyelitis vaccine, and these changes occur considerably earlier than the appearance of specific antibodies in the blood in response to administration of the vaccine.

LITERATURE CITED

1. E. L. Avenirova, M. N. Maslova, V. I. Rozengard, et al., Vopr. Med. Khim., 12, 633 (1966).
2. L. P. Gorshunova, M. I. Parasonis, M. Sh. Promyslov, et al., in: Proceedings of the 9th International Congress of Microbiology [in Russian], Moscow (1966), p. 646.
3. T. S. Paskhina, Biokhimiya, 19, 702 (1954).
4. M. Sh. Promyslov, T. V. Solov'eva, and R. I. Aniskina, Vopr. Med. Khim., 14, 619 (1968).
5. N. N. Yakolev, Ukr. Biokhim. Zh., 35, 175 (1963).
6. C. F. Baxter and E. Roberts, J. Biol. Chem., 236, 3287 (1961).
7. E. Flory, Naturwissenschaften, 40, 295 (1953).
8. E. Roberts, in: Inhibition in the Nervous System and γ-Aminobutyric Acid, London, (1960), p. 144.
9. E. Roberts and D. G. Simonsen, in: Amino Acid Pools, Amsterdam (1962), p. 284.