EFFECT OF IMMUNIZATION AGAINST RABIES ON γ-AMINOBUTYRIC METABOLISM IN THE ANIMAL BRAIN

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Subcutaneous injection of fixed rabies virus into albino rats weighing 100-120 g is followed by a decrease in the γ-aminobutyric acid (GABA) concentration in the animals' brains. An increase in the activity of GABA-α-ketoglutarate transaminase also is observed in the brain tissue of animals vaccinated against rabies.

KEY WORDS: rabies; γ-aminobutyric acid (GABA); immunization.

Evidence has now been obtained that γ-aminobutyric acid (GABA) metabolism is connected exclusively with the activity of brain nerve cells and mainly concerned with inhibition [3, 7]. Pharmacological, hormonal, and other factors have been shown not to cause any appreciable deviation in the distribution of free amino acids (including GABA) in the animal brain [8, 9]. The absence of any sharp changes in the brain GABA level even in the seizure state indicates that constancy of the GABA level is very important for the activity of the nervous system.

Meanwhile the writers have shown that after intracerebral infection of animals with fixed rabies virus there is a statistically significant decrease in the brain GABA level and an increase in the activity of GABA-α-ketoglutarate transaminase [2]. It was accordingly decided to investigate the GABA metabolism of the animal brain after immunization with fixed rabies virus.

EXPERIMENTAL METHOD

The Moscow vaccinal strain of fixed rabies virus was used. Experiments were carried out on albino rats weighing 100-120 g immunized with decreasing dilutions of fixed rabies virus (from 10^{-6} to 10^{-3}) subcutaneously in the abdominal wall in doses of 1 ml daily for 14 days. None of the vaccinated animals developed the disease or died. Control animals were immunized with a placebo preparation made from uninfected rabbit brain under the same conditions. In the course of the experiment the immunized and control animals were decapitated on the 4th, 6th, 11th, and 15th days after the beginning of immunization and the brain was quickly removed and homogenized.

Glutamate decarboxylase (GDC; EC 4.1.1.15) activity was determined as described by Yakovlev [5]. The GDC activity was estimated from the increase in GABA in the experimental sample [1]. The incubation mixture (2.1 ml) consisted of 1 ml homogenate (200 mg fresh tissue) in 0.1 M Na, K-phosphate buffer, pH 7.0; 1 ml (1 μmole) of pyridoxal phosphate and 0.1 ml (50 μmole) of a solution of glutamic acid. The samples were incubated for 1 h at 37°C in an atmosphere of nitrogen. The reaction was stopped by addition of 8 ml 96% ethanol. The test solution was applied in volumes of 10 μl to Whatman No. 3 chromatography paper. Fractionation was carried out for 24 h.

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TABLE 1. GABA Concentration and GABA -α-ketoglutarate Transaminase Activity in Brain Tissue of Albino Rats on Second Day after Immunization with Fixed Rabies Virus

Statistical index	GABA concentration (in mg %)		Transaminase activity (in μmole α-glutamate/g tissue/h at 37°C)	
	expt.	control	expt.	control
M	19,5	24,4	28,7	25,7
±*m*	0,16	0,16	0,8	0,5
n	15	15	8	8
P	<0,05		<0,05	

Transaminase (4-aminobutyrate: 2-oxoglutarate aminotransferase: EC 2.6.1.19) activity was determined by a slightly modified method of Roberts [6]. The enzyme activity was estimated from the increase in glutamic acid [4]. The incubation mixture (1.2 ml) consisted of 1 ml homogenate (400 mg of fresh tissue) in tris-acetate buffer, pH 8.2; 0.1 ml (1 μmole) pyridoxal phosphate; 0.05 ml (40 μmole) GABA, and 0.05 ml (40 μmole) α ketoglutaric acid. After incubation (1 h, 37°C) the proteins were precipitated with TCA in a final concentration of 3%. The supernatant was washed with three portions of ether to remove TCA and applied in portions of 10 μl to the chromatogram.

EXPERIMENTAL RESULTS

In the experiments of series I the dynamics of the GABA concentration was investigated in the brain tissue of animals immunized with fixed rabies virus. The experimental results showed that on the fourth and sixth days after the beginning of vaccination no significant difference was present in the GABA concentrations in the brains of the vaccinated and control animals.

As Table 1 shows, on the 11th day (experiments of series II) after injection of the vaccine there was a significant fall in the GABA level in the brain of the immunized animals ($P < 0.05$), whereas the GABA concentration in the brains of the animals receiving the placebo was not appreciably different from normal. On the 15th day after the beginning of immunization and later no significant changes in the GABA concentration were observed in the brains. The decrease in the GABA level in the brains of the vaccinated animals was observed, it will be noted, before the appearance of virus-neutralizing antibodies in the blood. For instance, antibodies appeared on the 15th day after injection of the vaccine (neutralization index 1072). The blood antibody level remained high later during the experiments; On the 20th and 30th days the serum neutralization index was 1288, and on the 45th day it was 5012.

The fall in the GABA level on the 11th day after the beginning of vaccination shows that the brain responds to injection of fixed virus antigen much sooner than specific virus-neutralizing antibodies begin to appear in the blood.

In the experiments of series III the activity of the enzyme systems of synthesis and subsequent conversion of GABA was investigated in the brains of the vaccinated animals on the 11th day, i.e., when the GABA concentration in the brains had fallen. These experiments showed that the GDC activity in the brains of the vaccinated animals was indistinguishable from that in the brain tissue of the control animals. A study of the activity of GABA-α-ketoglutarate transaminase showed (Table 1) a statistically significant increase, consistent with the decrease in the GABA concentration in the brain under the same experimental conditions.

The results of these investigations thus indicate that the GABA system of the brain, which rarely undergoes changes even under pathological conditions, proved to be sensitive to rabies vaccination. This, in turn, is evidence that vaccination is an extremely powerful stimulus for the nervous system.

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