ACTION OF SOME GANGLIOSIDES ON RESISTANCE OF MICE TO RABIES VIRUS

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Reports on the important role of glycolipids (gangliosides) in interaction between the host's cells and viruses, such as influenza virus, have recently been published [3, 7].

Considerable enhancement of the specific action of antirabic immunoglobulin, incorporated into phosphatidylcholine liposomes containing bovine brain gangliosides, was discovered by the writers previously [4]. It was suggested that one cause of the greater efficacy of antirabic immunoglobulin in these experiments is direct interaction between gangliosides and rabies virus. The communication is devoted to the testing of this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino mice of both sexes weighing 14-16 g. Gangliosides were isolated from brain, spleen, and erythrocytes [4-6]. Thin-layer chromatography was carried out in silica-gel from Merck (West Germany), in a system of chloroform–methanol–2.5 N aqueous ammonia (60:35:8) and the gel was developed with resorcinol reagent [2, 6]. Standard samples of gangliosides (Sigma, USA) were used for identification.

Rabies virus of the CYS strain, obtained from mouse brain, was used. A suspension of brain tissue containing the virus was treated in various dilutions with equal volumes of a solution of the gangliosides and, after incubation at 37°C for 30 min, it was injected into the brain of albino mice in a dose of 0.03 ml. The mortality of the animals was noted and activity of the virus calculated [1]. Control animals received an injection only of the virus-containing suspension in dilutions of 10^{-4} to 10^{-8} in a volume of 0.03 ml.

EXPERIMENTAL RESULTS

In the experiments of series I the mice were injected with a mixture of virus-containing brain suspension and the total fraction of brain gangliosides.

It will be clear from Table 1 that incubation of the virus with the total fraction of brain gangliosides significantly increased the survival rate of the animals. Injection of a brain suspension containing the virus in a dilution of 10^{-4} (incubation with the gangliosides was carried out in the same concentration), without treatment with gangliosides, led to a 100% mortality.

Correlation was found between the concentration of virus in the brain suspension and the quantity of added gangliosides. For instance, with low values of LD_{50} (from 50 to 100) the mice did not die when a concentration of 20-30 μg per mouse was used, whereas to inhibit virus with activity up to 7000 LD_{50}, the concentration of gangliosides had to be increased to 75 μg per mouse. The animals were not killed for 1-2 months after injection with a mixture of virus and gangliosides. Meanwhile mice receiving virus alone died on the 6th-8th day. The reason for this high affinity of rabies virus for brain tissue is not known. In the first stage we used gangliosides isolated from bovine spleen and erythrocytes. As Table 2 shows, splenic and erythrocytic gangliosides taken in the same proportions as brain gangliosides, gave no significant protective effect. The mortality varied from 80 to 100% depending on the value of LD_{50}.

High antiviral activity of bovine brain gangliosides was thus demonstrated. It was shown at the same time that ganglioside preparations isolated from other sources also

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TABLE 1. Survival Rate of Mice Infected with a Mixture of Brain Suspension Containing Rabies Virus and Total Gangliosides

Number of animals	LD_{50}	Quantity of gangliosides injected into one mouse, μg	Survival rate of mice	Control	
				survival rate of mice receiving injection of virus only	survival rate of mice receiving injection of gangliosides only
			%		
60	400	60	100	0	100
50	6900	75	90	0	100
50	1500	75	100	0	95
36	55	20	100	15	100
50	98	20	100	20	100
72	560	45	90	0	100

TABLE 2. Protective Effect of Splenic and Erythrocytic Gangliosides against Infection of Mice with Rabies Virus

Number of animals	LD_{50}	Source from which gangliosides obtained	Quantity of gangliosides injected into one mouse, μg	Survival rate of mice	Control	
					survival rate of mice receiving injection of virus only	survival rate of mice receiving injection of gangliosides only
				%		
50	200	Spleen	75	20	0	100
40	167	The same	75	0	0	90
45	59	» »	80	10	10	100
50	125	Erythrocytes	80	25	20	100
40	200	The same	75	16	0	95

TABLE 3. Survival Rate of Mice Infected with a Mixture of Brain Suspension Containing Rabies Virus and Various Ganglioside Fractions

Quantity of gangliosides injected into one mouse, μg	Survival rate of mice infected with mixture of virus and various ganglioside fractions, %					LD_{50}	Number of mice used when determining activity of each ganglioside fraction	Survival rate of mice (without treatment of virus with gangliosides), %
	GM_1	GD_{1a}	GT_1	GD_{1B}	GM_1			
75	20	50	95	25	10	160	40	5
100	15	55	90	25	5	147	45	0
50	20	47	85	15	5	210	25	5
150	15	53	95	15	10	177	50	10

possess considerable antiviral activity.

In the experiments of series II the effect of individual gangliosides isolated from the total fraction of the brain preparation, on resistance of mice to rabies virus was studied.

All gangliosides were taken in a dose of 75 μg per mouse. As will be clear from Table 3, GT_1 and GD_{1a} had maximal activity, and their administration delayed the development of the viral infection. It can be postulated that rabies virus has high affinity for nerve tissue gangliosides and, in particular, for gangliosides GT_1 and GD_{1a}.

It was interesting to study whether rabies virus infection in vivo can be delayed by means of gangliosides. For this purpose a series of experiments was carried out in which animals were infected with rabies virus in different concentrations, after which a solution of gangliosides was injected into the brain 2 and 6 h and 1, 2, and 3 days after injection of the virus. Injection of gangliosides was effective only during the first day, when activity of the virus was 50-150 LD_{50}. An increase in the dose of virus or its later injection did not lower the mortality. The survival rate was between 20 and 60% of the control (death of 100% of the animals). Thus injection of gangliosides into infected animals in the early stages may probably prevent the development of the infection.

There are two possible explanations of the results obtained in these experiments in vivo and in vitro. First, it can be postulated that binding of the virus with the ganglioside prevents its interaction with ganglioside-like receptor sites on the neuron membrane and facilitates subsequent destruction of the virus-ganglioside complex by the immune system. However, a second possibility must also be investigated, namely that the virus-ganglioside is highly immunogenic and induces rapid mobilization of antiviral immune mechanisms.

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SPECIFIC BINDING OF (+)-^{3}H-SKF 10047 BY MOUSE SPLENOCYTES

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The compound SKF 10047 (N-allylmetazocine), when administrated to laboratory animals, induces several characteristic responses, namely: a psychotic state, ataxia, inhibition of respiration, dilatation of the pupils, and tachycardia. On the basis of experiments on spinal dogs, Martin and co-workers [5] classed this compound as an agonist of one type (σ) of opioid receptors. In the CNS, stereoisomers of N-allylnormetazocine bind with different receptors: the (−)-isomer interacts mainly with μ-receptors and depresses the effects of morphine, whereas the (+)-isomer binds with type σ receptors and has a psychotomimetic action [3, 10-12]. Specific binding of SKF 10047 has been found in several peripheral organs [1]: in the liver, kidneys, spleen, heart, and gonads. A detailed study of binding sites of SKF 10047 in the rat liver has demonstrated their similarity with the σ-receptors of the brain.

In this investigation specific binding of the tritium-labeled (+)-isomer of SKF 10047 with mouse splenocytes was demonstrated. A study of the binding sites of (+)-SKF 10047 with splenocytes showed that they have a definite similarity to the σ-receptors of the mammalian brain and to binding sites on membranes isolated from rat liver.

EXPERIMENTAL METHOD

Male CBA, C57BL/6, BALB/c and, predominantly, DBA/2 mice were used in the experiments. Animals of the first two lines are known to be resistant to stress, whereas mice of the last two lines are highly susceptible to stress-induced lesions.

Mouse splenocytes were obtained by the method described previously [2] and were resuspended in buffer: Tris-HCl 20 mM, sucrose 0.25 M (pH 7.4), at 25°C (buffer 1).

Binding of (+)-SKF 10047 with splenocytes was studied as follows. Samples of 500 μl in volume contained 10^7 cells and also different concentrations of labeled ligand and of substances hypothetically competing with it for specific binding sites on the splenocytes. All components of the mixture were dissolved or suspended in buffer 1. Incubation was carried out in plastic test tubes at 37 or 0°C. After the end of incubation 1 ml of buffer 1, cooled to 4°C, was added to the sample, the mixture was filtered in vacuo through GF/C filters (Whatman, England), and the filters were washed with 10 ml of cold buffer, dried in air, transferred into scintillation mixture (toluene 2 liters, Triton X-100 1 liter, PPO 22.5 g, POPOP

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