EFFECT OF AN ORGANOPHOSPHORUS INHIBITOR ON THE FRACTIONAL COMPOSITION OF THE BRAIN GANGLIOSIDES

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The gangliosides of rats differ from those of rabbits in their lower content of the G_3 fraction, the principal disialoganglioside, and by their proportionately higher content of the G_2 and G_{2a} fractions, two other disialogangliosides. During poisoning by an organophosphorus cholinesterase inhibitor the content of gangliosides in the rat brain is unchanged, but in the rabbit brain it falls by 15%. No change takes place in the composition of the ganglioside fractions.

Poisoning by organophosphorus cholinesterase inhibitors (OCI), which disturb the mechanism of synaptic transmission of excitation [7], is accompanied by a marked decrease in the ganglioside content in the rabbit brain [5].

Since the gangliosides are a mixture of several components differing in the structure of the carbohydrate part of their molecule and by the number of N-acetylneuraminic acid (N-ANA) residues, the investigation described below was carried out to study the relationship between fractions of mono-, di, and trisialogangliosides in the brain of rabbits and rats by the method of thin-layer chromatography, and to study the effect of OCI on the composition of the ganglioside fractions.

EXPERIMENTAL METHOD

Experiments were carried out on male rabbits weighing 2.5 kg receiving an intramuscular injection of the OCI GA-3 (O-N-butyl-S-N-butylmethylthiophosphonate), synthesized by Godovikov and Abduvakhabov [1], in a dose of 10 mg/kg body weight. Analogous experiments were carried out on male albino rats weighing 250 g, receiving GA-3 in a dose of 5 mg/kg.

Lipids were extracted from a homogenate of the whole brain by the method of Folch et al. [8], followed by re-extraction by Suzuki's method [12]. Gangliosides were separated from the lipid extract by washing with 0.75% KCl solution. The upper aqueous phase containing gangliosides was purified by dialysis, and after its N-ANA content had been determined [14], it was lyophilized. The resulting residue was dissolved in 6-8 ml of a 1:1 mixture of chloroform and methanol, filtered to remove undissolved substances, and evaporated to dryness at 50°C in a current of air. The residue of gangliosides was dissolved in 0.3-0.5 ml of 1:1 chloroform and methanol mixture and kept at 2-4°C.

The chromatographic fractionation of the gangliosides in a thin layer of KSK silicagel, with particle size 10-20 μ, was carried out on plates measuring 6×18 cm^2 by Avrova's method [2] in a solvent system of $CHCl_3 : CH_3OH : H_2O : NH_3$ (60:35:8:1) for 50-60 min. The plates were developed with iodine vapor, identical fractions from three plates were combined, and the N-ANA content was determined in each fraction by the color reaction with resorcin without preliminary elution of the gangliosides [13].

The sphyngosine content in each fraction was determined by the method of Lauter and Trams [10] after elution of the silicagel with 30 ml $CHCl_3 : CH_3OH$ (1:1) and 10 ml CH_3OH.

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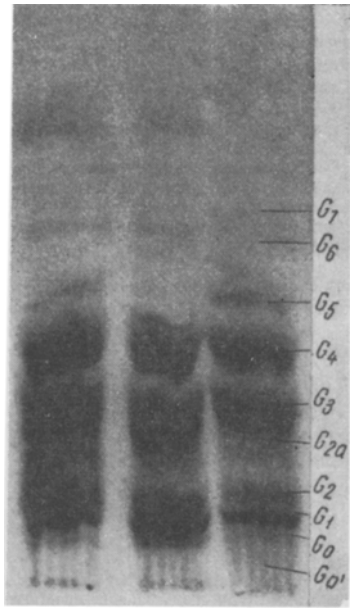

Fig. 1. Chromatogram of gangliosides from brain of normal rat, normal rabbit (Rab-1), and rabbit poisoned with OCI (Rab-2). Nomenclature of Korey and Gonatas [9].

EXPERIMENTAL RESULTS AND DISCUSSION

The rabbits developed definite toxic manifestations 7-10 min after receiving the lethal dose of OCI: salivation, tremor, dyspnea, fibrillation of skeletal muscles, and convulsions lasting 30-40 min. The ganglioside content in the whole brain of the rabbits poisoned with GA-3 was reduced from 7.5 ± 16 (M ± m) to 606±21 μg NaANA/g tissue ($P < 0.05$), i.e., by 15%.

In analogous experiments on rats, administration of OCI (the compound GA-3) did not induce convulsions and the total ganglioside content in the brain was unchanged (normal 712±31, after injection of GA-3 724±37 μg N-ANA/g). This confirms the previous conclusion that the ganglioside content in the brain is reduced during OCI poisoning only if the complete inhibition of cholinesterase is accompanied by severe convulsions [5]. Marked species differences in the response of animals to OCI poisoning have also been found by Dvorkin et al. [3]. OCI poisoning was found to cause marked hypothermia and to reduce the intensity of phospholipid metabolism in the brain in mice, but it had no such action on rats. Consequently, species differences in the reaction of the central nervous system to OCI poisoning consist not only of the development of symptoms of poisoning, but also of different effects on brain lipid metabolism.

By means of thin-layer chromatography, the total gangliosides were separated into 9-10 fractions (Fig. 1) which were described by the nomenclature of Korey and Gonatas [9].

The results (Table 1) demonstrate that the 3 most mobile fractions ($G_{O'}$, G_0, and G_1) are trisialogangliosides. Next followed 3 fractions of disialogangliosides and 2 fractions of monosialgogangliosides. Because of their low content of N-ANA, the most mobile fractions G_6 and G_7 can be regarded as asialogangliosides with monosialogangliosides as impurities. The structure of all these and of some other gangliosides has been discussed in detail in a survey by Leeden [11] and illustrated by appropriate formulas.

The various fractions are unequally represented quantitatively in the brain (Table 2). About 90% of the total N-ANA was contained in 5 fractions (G_1, G_2, G_{2a}, G_3, and G_4), so that these can be regarded as the "chief" gangliosides of the rabbit brain. The remaining fractions ($G_{O'}$, G_0, G_5, G_6, and G_7) were present in such small quantities that the term "minor" gangliosides has been attached to them [11].

Comparison of the spectra of gangliosides in the control rabbits and rabbits poisoned with OCI showed no significant difference in the relative proportions of the fractions. This means that the OCI had no selective action on particular fractions of gangliosides but reduced their total content in the brain uniformly. Gangliosides from the rat brain were separated into 9-10 fractions identical in their mobility and N-ANA content with rabbit gangliosides. However, in rats the G_3 fraction (the "major" disialoganglioside) was present in much smaller amounts than in rabbits, and the amounts of the fractions G_2 and G_{2a} were correspondingly increased. This similarity between the qualitative and quantitative composition of the ganglioside spectra of the brain has also been found [15] in animals belonging to phylogenetically more distant species of warm-blooded animals. This fact supports the suggestion made by Kreps [4] that the chemical organization of nerve cell membranes in higher animals is based on a single common plan, produced at a particular stage of evolution and consolidated during the subsequent development of the animal world and the increasing complexity of its central nervous system.

In poisoning with a lethal dose of GA-3 the relative proportions of the ganglioside fractions were not significantly changed either in the rats or in the rabbits (Table 2). The same constancy of fractional composition of gangliosides during acute hypoxia was described previously by Romanova and Tumanova [6].

TABLE 1. Distribution of N-ANA and Sphyngosine among Fractions of Gangliosides from Rabbit Brain (in nmoles)

Test substance	Fraction of gangliosides							
	$G_0'+G_0$	G_1	G_2	G_{2a}	G_3	G_4	G_5	G_6+G_7
N-ANA	29,0	103,5	78,0	71,5	174,0	91,0	11,3	3,9
Sphyngosine	9,2	35,1	40,2	37,0	93,0	89,0	10,6	8,0
N-ANA / Sphyngosine	3,15:1	2,95:1	1,95:1	1,93:1	1,87:1	1,02:1	0,94:1	0,49:1
	Trisialoganglio-sides		Disialogangliosides			Monosialogangliosides		

TABLE 2. Distribution of N-ANA among Fractions of Gangliosides in Rabbit and Rat Brain under Normal Conditions and in GA-3 Poisoning (percent of total N-ANA)

Fraction	Gangliosides	Percent of N-ANA among fractions (M±m)			
		rabbits		rats	
		control (6)	GA-3 (5)	control (4)	GA-3 (4)
$G_0'+G_0$	Trisialogangliosides	5,5±0,6	5,2±0,3	4,2±0,47	6,7±0,95
G_1		21,3±1,4	19,3±1,4	21,6±0,65	18,7±1,30
G_2	Disialogangliosides	12,3±0,6	12,5±1,1	16,3±0,96	14,8±0,80
G_{2a}		9,8±1,1	10,8±0,7	12,8±1,14	12,8±0,50
G_3		31,1±1,5	32,6±0,9	25,4±1,20	28,8±1,50
G_4	Monosialogangliosides	14,3±0,7	14,6±1,5	14,1±1,40	14,4±1,60
G_5		2,9±0,4	3,3±0,6	2,6±0,80	2,8±0,70
G_6+G_7		2,7±0,8	1,6±0,7	2,8±1,05	1,6±0,26

Note. Numbers in parentheses show number of animals tested.

Maintenance of the constancy of the fractional spectrum of gangliosides located in nerve cell membranes is evidently a physiological necessity for maintenance of the functions of the central nervous system during exposure to powerful external influences.

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