ACTIVATION OF DEOXYCHOLATE SOLUBILIZED ADENOSINE TRIPHOSPHATASE BY GANGLIOSIDE AND ASIALOGANGLIOSIDE PREPARATIONS

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SUMMARY: ATPase was prepared from brain microsomes by solubilization with sodium deoxycholate and fractionated at different concentrations of ammonium sulfate. The Mg^{2+}-ATPase was activated by total brain gangliosides, disialoganglioside, monosialo - ganglioside, hematoside, total brain gangliosides obtained from a patient with Tay-Sachs disease and asialoganglioside. The effect was smaller on Ca^{2+} -ATPase and negligible on $(Na^{+} + K^{+})$-ATPase. Lactosyl-ceramide, glucosyl-ceramide, galactosyl-ceramide, ceramide and sialyl-lactose failed to produce activation.

INTRODUCTION

Gangliosides are present in gray matter of nervous tissue in concentrations one or two orders of magnitude higher than in other organs. Like phospholipids gangliosides are amphipatic components of membranes and because of this we decided to investigate whether gangliosides could activate delipidized ATPase preparations as phospholipids do. The results of this investigation are presented in this report.

MATERIALS AND METHODS

ATPase preparation. Brains from 20-30 day-old rats were used. The brain from one rat was homogenized in 10 ml of 0.32 M sucrose and centrifuged at 800 x g for 10 min. The resultant supernatant was centrifuged at 12000 x g for 30 min and the last supernatant centrifuged again at 100,000 x g for 1 h. The precipitate from this centrifugation (microsomal fraction) was homogenized in 4.8 ml of water and subjected to a fraction-

Abbreviations used: EGTA, ethylene glycol bis-(β amino-ethyl-ether)-N, N'-tetra-acetic acid; TCA, trichloro-acetic acid. Specific gangliosides are designated according to Svennerholm's nomenclature (2).

ation similar to that followed by Tanaka and Strickland (1). To the homogenized suspension was added 1.6 ml of 1.33 % sodium deoxycholate and after mixing by 2 or 3 strokes with the homogenizer piston, 0.20 g of solid ammonium sulfate per ml of fluid was added. The precipitated protein was centrifuged off at 12000 x g during 40 min and to the supernatant an equal volume of saturated solution of ammonium sulfate was added. The last protein precipitate was collected by centrifugation at 12000 x g for 40 min and dissolved in 2.5 ml of 20 mM Tris-HCl buffer, pH 7.4. The solution contained approximately 1 mg of protein per ml determined by Lowry's method (3) with bovine serum albumin as standard.

Preparation of gangliosides and ganglioside derivatives. Total brain gangliosides were prepared from a patient with Tay-Sachs disease and from rats by the method of Folch-Pi et al. (4). Hematoside (GM3) from dog red cells, monosialoganglioside (GM1), disialoganglioside (GD1a), asialoganglioside from rat brain and lactosyl-ceramide from calf brain were gifts from Dr. H.J. Maccioni and were prepared as previously described (5, 6). Human blood lactosyl-ceramide was a gift from Dr.F.A.Cumar and Mr.G.A.Roth who prepared it according to Yamakawa et al. (7). Gaucher spleen glucosyl-ceramide was a gift from Dr.J.A.Curtino who prepared it by the method of Radin and Brown (8). Sialyl-lactose was prepared according to Carubelli et al. (9). All other materials used were of commercial origin.

ATPase determination. Unless otherwise stated, ATP (Tris and Na^+ salt) pH 7.4, 1 µmol; $MgCl_2$, 0.8 µmol and enzyme solution at pH 7.4, 0.05 ml (approx. 50 µg of protein) were incubated in a total volume of 0.25 ml at 36°C for 20 min. The reaction was stopped by addition of 0.5 ml of 10% TCA and Pi determined by the method of Martin and Doty (10). For control purposes complete mixtures but for ATP or for enzyme solution were incubated and completed after the TCA addition.

RESULTS

Activation by gangliosides and asialoganglioside. Table I shows that gangliosides and asialoganglioside activated Mg^{2+}-ATPase whereas other compounds having just a part of the ganglioside structure, such as sialyl-lactose, ceramide, glucosyl-ceramide and lactosyl-ceramide, failed to activate. Hematoside is so far the smallest molecular weight glycosphingolipid that activated. The possibility that contaminating cations were responsible for the activation was discarded because mineralization of an active preparation turned it inactive. Possible contaminant phospholipids were also

Table I

ACTIVATION OF ATPase BY GANGLIOSIDES AND GANGLIOSIDE DERIVATIVES

The incubation mixture contained: ATP, 1 μmol; enzyme solution 0.05 ml (approximately 50 μg protein); $MgCl_2$, 0.8 μmol and Tris HCl buffer, pH 7.4, 20 mM in a total volume of 0.25 ml. Temperature 36°C, incubation time 20 min.

Cations[e]	Additions Sphingolipids or derivatives[a,b]	Activity %
-	-	100[c]
Na^+ - K^+	- (5)	150
Ca^{2+}	- (5)	130
-	gangliosides[f] (5)	390
Na^+ - K^+	gangliosides[f] (5)	435
Ca^{2+}	gangliosides[f] (5)	420
-	alkali treated gangliosides[d, f] (2)	515
-	disialoganglioside (GDIa)[g] (1)	300
-	monosialoganglioside[g, f] (2)	400
-	Tay-Sachs gangliosides[f] (2)	450
-	hematoside[g] (3)	300
-	asialoganglioside[f] (2)	200
-	mineralized gangliosides[f] (2)	100
-	ceramide[g] (2)	110
-	galactosyl-ceramide[g] (1)	100
-	glucosyl-ceramide[g] (2)	90
-	lactosyl-ceramide (from human blood)[g] (2)	80
-	lactosyl-ceramide (from brain)[g] (2)	100
-	sialyl-lactose[f] (1)	100

a Results are from experiments with different enzyme preparations. Since the enzyme preparations were variably activated by the same ganglioside solution, the conclusions should be drawn on a positive or negative basis only. Listed inactive substances were tested simultaneously with an activator.

b The amount added in each case was 100 μg. In parenthesis, number of determinations.

c In the 100% values, the Pi liberated varied from 0.025 to 0.180 μmol.

d Alkaline treatment: 5 mg of gangliosides were incubated in 1 ml of 0.1 N KOH during 3 h at 37°C. To eliminate the excess of K^+ the mixture was passed through Dowex 50.

e Na^+ added as NaCl (25 μmoles); K^+ as KCl (2.5 μmoles); Ca^{2+} as $CaCl_2$ (0.2 μmoles).

f Added in water solution.

g Placed in empty tubes in chloroform:methanol (2:1), dried under a N_2 stream and resuspended in water.

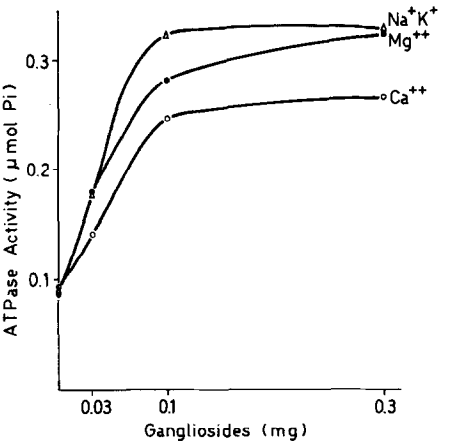

Fig. 1 : Activation of ATPase by gangliosides. The incubation system was as described in Table I with the following additions: For Mg^{2+} curve, none; for Ca^{2+} curve, 0.8 mM $CaCl_2$; for $Na^+ - K^+$ curve, 100 mM NaCl plus 10 mM KCl. In every case brain gangliosides from a Tay-Sachs patient were used as activators.

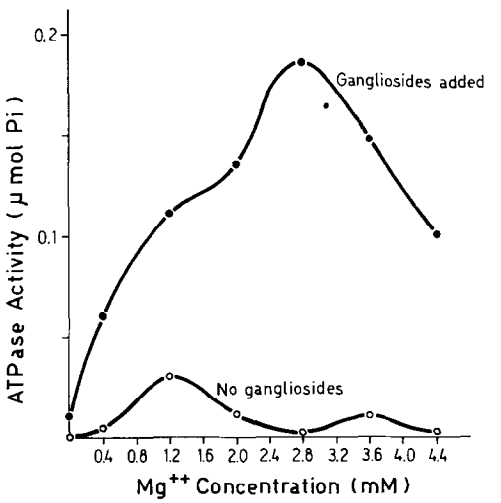

Fig. 2 : Activation of Mg^{2+}-ATPase by gangliosides. The incubation system contained ATP, 1 µmol; enzyme, 100 µg of protein; EGTA, 0.4 µmol; ouabaine, 0.125 µmol; gangliosides from a Tay-Sachs patient, 100 µg.

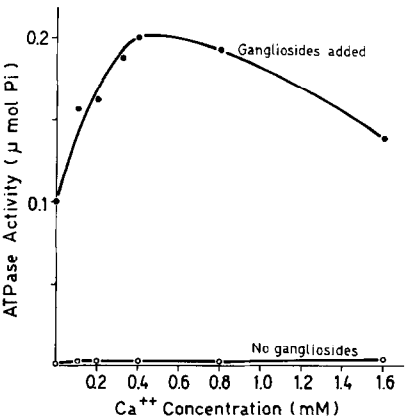

Fig. 3 : Activation of Ca^{2+}-ATPase. The incubation system was as in Fig. 2 but for the amounts of EGTA (0.025 μmol), ouabaine (0.025 μmol) and $MgCl_2$ which was the same for each tube (0.7 μmol).

eliminated as effectors because alkaline treatment in conditions that saponify phospholipids but not gangliosides had no effect on the activating capacity of the preparations (Table I).

The dependence of the activation on the concentration of gangliosides is shown in Fig. I. It also indicates that the major activation was on the Mg^{2+}-dependent enzyme. However, in the experiments shown in Fig. I no precautions were taken to eliminate Ca^{2+}- and $(Na^+ + K^+)$-ATPases in the series in which Mg^{2+}-ATPase was activated by gangliosides.

The effects on Mg^{2+}-, Ca^{2+}- and $(Na^+ + K^+)$-ATPases. The effect of gangliosides on Mg^{2+}-dependent ATPase was studied in the presence of ouabaine (0.5 mM) and EGTA (1.6 mM). It was assumed that any activity obtained in the presence of these substances was due to Mg^{2+}-ATPase. Fig. 2 shows that in these conditions the addition of gangliosides produced a great increase of ATPase activity.

The effect of gangliosides on Ca^{2+}-ATPase (which is known to require Ca^{2+} and Mg^{2+}) was studied at constant concentrations of ouabaine (0.1 mM), EGTA (0.1 mM) and $MgCl_2$ (2.8 mM) but at

concentrations of Ca^{2+} that varied from 0.1 to 1.6 mM. It was found that in the presence of gangliosides the ATPase activity was activated by addition of Ca^{2+} whereas in the absence of gangliosides that particular preparation showed no activity either with Mg^{2+} alone or with Mg^{2+} plus Ca^{2+} (Fig. 3). The increase produced by addition of Ca^{2+} to the system containing Mg^{2+} and gangliosides is assumed to be due to the effect of these last compounds on Ca^{2+}-ATPase.

The possible effect of gangliosides on $(Na^+ + K^+)$-ATPase was studied in the presence of constant concentrations of EGTA (0.1 mM) and $MgCl_2$ (2.8 mM) but with increasing concentrations (from 0.25 to 250 mM in Na^+) of a mixture containing 10 moles of Na^+ per mol of K^+. In these conditions we found only a very small activation that could be attributed to the $(Na^+ + K^+)$ - enzyme.

DISCUSSION

To decide whether the ATPase activation by ganglioside preparations was due to gangliosides themselves or impurities, we used preparations of diverse origins. Hematoside from dog red cells, rat brain gangliosides, human brain gangliosides from a patient of Tay-Sachs disease and an asialoganglioside preparation which was subjected to elaborate purification procedures (5), all had approximately similar activating effects per unit of dry weight. It is considered very unlikely that all these preparations contain a similar amount of a given impurity. Furthermore, cations and phospholipids that are the best known activators of ATPase were discarded as the effectors since mineralization completely inactivated the preparations and alkaline treatment in conditions which saponify phospholipids but do not affect gangliosides did not diminish the activating effect.

Failure to activate with ceramide, glucosyl-ceramide, galactosyl-ceramide, lactosyl-ceramide, or sialyl-lactose

indicates that in the conditions we have used the glycosphingolipid must be composed of a ceramide moiety plus a carbohydrate chain of at least three units to be able to activate. With most of the enzyme preparations used the main effect was elicited on the Mg^{2+}-ATPase; the effect was less on Ca^{2+}-ATPase and even smaller on $(Na^{+} + K^{+})$-ATPase.

We have no evidence of the relation between this effect of gangliosides and the functions of these compounds in the nervous tissue but since enzyme and activators are found in the same subcellular fractions this possibility should not be disregarded.

Acknowledgements

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