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Effect of exogenous gangliosides on the lipid composition of chick neurons in culture

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When exogenous gangliosides are added to the growth medium of neuronal cell cultures they are inserted into their plasma membranes and are afterwards metabolized in the cytoplasmic interior. The action of exogenous gangliosides brings important morphological and biochemical changes to neurons in culture. The present report shows that the treatment with exogenous gangliosides of a primary culture of chick neurons modified the distribution of fatty acids in phosphatidylinositol (PI), mainly that of arachidonic acid and the fatty acids of the $(n-3)$ series without affecting the other phospholipids. The composition of neutral lipids did not change but their content was increased up to 2–3-fold depending upon the concentration of gangliosides. The change of the growth medium from one containing fetal calf serum to a chemically defined one reduced dramatically the content of free fatty acids while the addition of gangliosides raised this content to normal levels. The increase in the amount of diacylglycerol (DG) confirmed the finding that gangliosides stimulate phosphoinositide degradation. Finally the fatty acid composition of DG suggests indirectly that this compound might be produced also by degradation of phosphatidylcholine and not only of PI.

Introduction

Gangliosides are integral components of the plasma membrane (for review, see Ref. 1) and are known to be involved in many biological functions of nerve cells such as neurotransmission [2,4], probably via their interaction with calcium ions [5], the reception of various neuromodulators [6,7], the recognition, the establishment and the maintenance of cell to cell contacts [8], and, finally, cell proliferation and maturation [9,10].

It has been shown that exogenous gangliosides can be inserted into the plasma membrane before their intake into the cell [11–13]. The addition of gangliosides to neurons in culture produces profound changes in the cell morphology the most important of which being sprouting of neurites [14,15]. This, however, is not accompanied by any remarkable variation in the overall content and metabolism of proteins and nucleic acids (Ferret, B. et al., unpublished data).

The effects produced by exogenous gangliosides must then be due to their action at a more specific level. In this respect it is noteworthy that exogenous gangliosides can regulate protein phosphorylation [16,17] and stimulate the metabolism of phosphoinositides [18], notably of inositol trisphosphate (IP_3). This implies an indirect role of gangliosides in the mobilization of intracellular calcium ions considering the involvement of IP_3 in such a mechanism [19].

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An increased metabolism of phosphoinositides led to the suspicion that gangliosides might also modify the content of DG, which is known to be an activator of protein kinase C [20]. Experiments were therefore undertaken in primary neuronal cell culture from chick embryos cerebral hemispheres to determine the possible effect of exogenous gangliosides upon the content and composition of neutral lipids as well as phospholipid.

Materials and Methods

Neuronal cultures from 8-day-old chick embryo's cerebral hemispheres were obtained following standard procedures [21] and grown, for three days, in 5 ml Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% fetal calf serum (Flow). At the 3rd day in vitro Dulbecco's medium was substituted with 5 ml of a chemically defined medium without fetal calf serum (CDM : DMEM containing per liter 5 mg insulin, 16 mg putrescin, 100 mg transferrin, 10^{-12} mol oestradiol, $2 \cdot 10^{-8}$ mol progesterone and $2 \cdot 10^{-8}$ mol sodium selenite), containing or not 10^{-5} M or 10^{-6} M of a mixture of gangliosides (G_{M1} 19.8%, G_{D3} 5.2%, G_{D1a} 39.6%, G_{D1b} 14.6%, G_{T1b} 17.6%, G_{Q1} 3.2%, Fidia). The CDM, with or without gangliosides was changed once more on the 5th day and the biochemical analyses were performed one day later. The concentration of the gangliosides were in this study respectively above and below the critical micellar concentration [11,22–25].

It has been shown that after 4 h of incubation of these cultures with 10^{-8} M of single species of gangliosides (G_{M1}, G_{T1b}; Ref. 26) labelled with tritium on the ceramide portion [27] or with a mixture of gangliosides similarly radiolabelled (unpublished results) about 96% of the radioactivity remains in the medium and decreases slowly there after to 85% after 72 h of incubation as in the present experimental conditions with a negligible decomposition of the sialoglycoconjugates.

The cells were washed three times with a 0.147 M NaCl solution at 4°C, collected in the same solution and then centrifuged (3000 × g, 15 min). The supernatant was discarded and total lipids were extracted from the pellet according to the procedure of Folch et al. [28]. Neutral lipids and phospholipids were separated by column chromatography with silica gel 60 (230–400 mesh, Merck) as adsorbent (4 × 30 mm). Neutral lipids were eluted with chloroform (10 ml); phospholipids were recovered by elution with methanol (10 ml) following a 15 ml wash with acetone/methanol (9 : 1, v/v). Neutral lipids were separated by thin-layer chromatography on silica gel 60 plates (Merck) developed in hexane/diethyl ether/acetic acid (70 : 30 : 1, by vol.). Phospholipids were separated by thin-layer chromatography on silica gel LK5 plates (Whatman) impregnated with boric acid according to Leray et al. [29]. Fatty acid methyl esters from each individual lipid were prepared [30] and analysed using a Perkin-Elmer Sigma 1 gas chromatograph (bonded fused silica open tubular column, 0.32 mm i.d. × 50 m, Superox. Alltech Assoc.) and a Sigma 10 chart integrator. The lipid amounts were estimated using heptadecanoate as an internal standard and the data are expressed as per cent molar concentration. Proteins were determined according to Lowry et al. [31].

TABLE I

DISTRIBUTION OF THE MAIN FATTY ACIDS IN PHOSPHOLIPIDS OF CHICK NEURONS IN CULTURE

Values expressed in mol% are means of two representative culture experiments (three Petri dishes each). Only the main fatty acids are listed. Σ, sum of the fatty acids. Satd., saturated fatty acids. n.d., not detectable. Only the fatty acids whose levels are higher than 0.4% are listed.

Fatty acid	PC	PE	PS
14:0	4.4	1.6	2.4
16:0	44.6	13.5	18.3
18:0	5.8	17.2	29.4
Σ satd.	55.0	32.3	50.2
16:1 (*n* − 9)	7.6	5.2	6.0
18:1 (*n* − 9)	25.1	18.7	20.1
20:3 (*n* − 9)	0.7	4.2	n.d.
Σ (*n* − 9)	33.4	28.2	27.2
Σ (*n* − 7)	6.4	5.9	1.1
18:2 (*n* − 6)	1.0	1.4	2.5
20:4 (*n* − 6)	1.6	14.3	2.6
22:4 (*n* − 6)	2.9	2.0	n.d.
22:5 (*n* − 6)	n.d.	0.7	n.d.
Σ (*n* − 6)	5.7	18.5	5.1
20:5 (*n* − 3)	n.d.	0.9	n.d.
22:5 (*n* − 3)	n.d.	3.1	1.9
22:6 (*n* − 3)	1.3	10.2	9.8
Σ (*n* − 3)	1.31	14.6	12.4

Results

The average protein concentrations were of 0.784 mg/Petri dish for control and 0.792 and 0.810 for 10^{-8} M and 10^{-5} M treated neurons, respectively (average of 12 determinations out of four independent experiments, S.D. < 8%) indicating that the treatment of the cultures with gangliosides had no effect on their protein content.

Since no statistically significant modification was seen in the fatty acyl chain composition of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS) as a function of ganglioside concentrations, the fatty acid composition of these phospholipids is given only for control cultures (Table I). The data hence show that in neurons in culture PC and PS were the most saturated phospholipid species, palmitic acid being the most abundant in the former and stearic acid in the later phospholipids. While the most abundant polyunsaturated fatty acids in PE were those of the $(n-6)$ series (mainly 20:4), the most abundant in PS were those of the $(n-3)$ series (mainly 22:6). Beside the saturated fatty acids, PC was also characterized by a high amount of non essential fatty acids (about 40% of monoenes of the $n-9$ and $n-7$ series). The quantitative distribution of these phospholipids was not modified by the ganglioside treatment and remained for PC, PE and PS 53%, 25% and 10% of total phospholipids, respectively.

The main modification induced by gangliosides upon the fatty acid composition of phosphatidylinositol (PI) was a progressive decrease in the proportion of saturated fatty acids compensated by an increase in the proportion of several polyunsaturated fatty acids (Table II). Palmitic acid was the saturated fatty acid mostly affected by the ganglioside addition, 20:3 $(n-9)$ was more abundant (+46%) at 10^{-5} M ganglioside concentration, while more 20:4 $(n-6)$, 22:4 $(n-6)$ and 22:6 $(n-3)$ was found (+40%, +100% and +78%, respectively) at the lowest ganglioside concentration. The higher increase in the proportion of polyunsaturated fatty acids in the PI of neurons treated with 10^{-8} M gangliosides compared to

TABLE II

EFFECT OF EXOGENOUS GANGLIOSIDES ON THE DISTRIBUTION OF THE PHOSPHATIDYLINOSITOL ACYL CHAINS IN CULTURED CHICKEN NEURONS

Values represent means ± S.E. of three experiments (three Petri dishes each). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ for differences due to the ganglioside treatment (Student's t-test). Σ, sum of the fatty acids. Satd., saturated fatty acids. n.d., not detectable. Only the fatty acids whose levels are higher than 0.4% are listed.

Fatty acid	Ganglioside concentration (M)		
	0	10^{-8} M	10^{-5} M
14:0	2.4±0.1	2.6±0.1	0.7±0.0
16:0	18.7±0.7	14.2±0.6 **	11.9±0.5 **
18:0	24.1±1.2	25.9±1.0	26.9±1.1
Σ satd.	45.2±2.1	42.8±1.9	39.7±1.4
16:1 $(n-9)$	4.8±0.3	5.4±0.3	3.2±0.1
18:1 $(n-9)$	21.1±1.5	16.9±0.7	20.5±0.9
20:3 $(n-9)$	5.2±0.3	5.2±0.2	7.5±0.3 **
Σ $(n-9)$	32.0±1.3	28.9±0.9	32.7±1.2
Σ $(n-7)$	1.3±0.1	1.3±0.1	1.4±0.1
18:2 $(n-6)$	1.8±0.1	1.1±0.1	1.7±0.1
18:3 $(n-6)$	0.6±0.1	n.d.	n.d.
20:4 $(n-6)$	12.9±0.5	17.9±0.7 **	15.0±0.6
22:4 $(n-6)$	0.7±0.1	1.6±0.1 **	1.1±0.1 *
Σ $(n-6)$	17.6±0.7	20.7±0.8	19.9±0.7
22:5 $(n-3)$	0.4±0.1	1.4±0.1	1.3±0.1
22:6 $(n-3)$	2.6±0.1	4.8±0.2 ***	4.1±0.1 ***
Σ $(n-3)$	3.5±0.1	6.2±0.2 ***	6.1±0.2 **

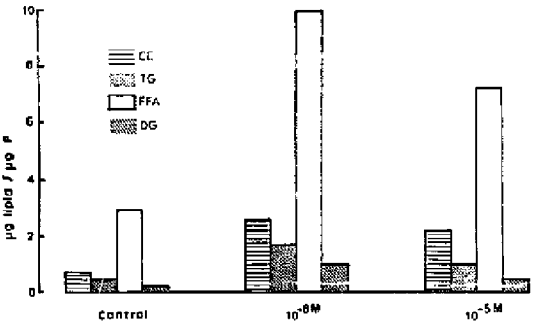

Fig. 1. Content of neutral lipids in neurons grown in CDM for three days and in the absence or presence of a mixture of gangliosides. CE, cholesterol esters; TG, triacylglycerols; FFA, free fatty acids; DG, diacylglycerols. In the abscisse the concentrations of of gangliosides. Values were obtained from two representative culture experiments (three Petri dishes each).

TABLE III

EFFECT OF EXOGENOUS GANGLIOSIDES ON THE DISTRIBUTION OF NEUTRAL LIPIDS WITH REFERENCE TO CONTROL NEURONS GROWN IN THE PRESENCE OF SERUM

Neurons were either grown for 6 days with DMEM supplemented with 10% fetal calf serum or 3 days in DMEM with serum and 3 more days in CDM. Values are expressed in % with reference to neurons grown with serum and were obtained from two representative culture experiments (three Petri dishes each). Abbreviations, see Fig. 1.

	Neurons grown in DMEM/serum	Neurons grown in CDM (ganglioside concentration)		
		0	10^{-8} M	10^{-5} M
CE	100	72	253	209
TG	100	30	109	65
FA	100	45	149	108
DG	100	34	148	75

10^{-5} M should be related to the physicochemical characteristics of the sialoglycolipids which below 10^{-7} M are more in a dispersed monomeric form than micellar [11]. This may facilitate their insertion into the membrane and consequently their metabolic action at the level of membrane elements.

The concentration of the four neutral lipids detected in cultured cells in the absence or in the presence of gangliosides is shown in Fig. 1. The treatment by gangliosides at a concentration of 10^{-5} M, increased 2-fold the amount of each lipid and, at 10^{-8} M, about 3-fold. Table III shows that the addition of gangliosides at both concentrations raises by a factor of three the amount of neutral lipids when compared to neurons grown solely in the presence of CDM. When expressed in molar percent (Table IV and V) and regardless of the ganglioside treatment, the free fatty acid composition was slightly affected. Cholesterol esters and triacylglycerols showed some changes, the proportion of stearic acid was increased in

TABLE IV

EFFECT OF EXOGENOUS GANGLIOSIDES ON THE DISTRIBUTION OF FATTY ACIDS IN THE FREE FATTY ACID GROUP (FFA) AND IN CHOLESTEROL ESTERS (CE) OF CULTURED CHICKEN NEURONS

Values are the means from two representative culture experiments (three Petri dishes each). Only the main fatty acids are listed. Σ, sum of the fatty acids. Satd., saturated fatty acids. n.d., not detectable. Only the fatty acids whose levels are higher than 0.4% are listed.

Fatty acids	FFA, ganglioside concentrations			CE, ganglioside concentrations		
	0	10^{-8} M	10^{-5} M	0	10^{-8} M	10^{-5} M
14:0	45.0	42.3	45.1	6.1	4.2	7.8
16:0	27.6	31.4	29.5	22.0	24.6	21.6
18:0	7.4	7.5	7.2	6.7	12.6	9.0
Σ satd.	80.8	82.2	82.7	35.5	44.6	40.4
16:1	2.7	2.7	2.5	25.1	11.7	18.1
18:1	13.7	13.1	12.5	23.7	22.5	27.7
Σ ($n-9$)	16.4	15.2	15.2	48.9	41.1	53.4
18:2	2.3	2.0	2.0	5.2	7.9	4.0
18:3	0.4	n.d.	n.d.	1.3	n.d.	n.d.
Σ ($n-6$)	2.6	2.5	2.0	7.2	8.3	4.0

TABLE V

EFFECT OF EXOGENOUS GANGLIOSIDES ON THE DISTRIBUTION OF ACYL CHAINS IN DIACYLGLYCEROLS (DG) AND TRIACYLGLYCEROLS (TG) OF CULTURED CHICKEN NEURONS

Values are means for two culture experiments (three Petri dishes each). Satd., saturated fatty acids. n.d., not detectable. Only the fatty acids whose levels are higher than 0.4% are listed.

Fatty acids	DG, ganglioside concentration			TG, ganglioside concentration		
	0	10^{-8} M	10^{-5} M	0	10^{-8} M	10^{-5} M
14:0	8.8	7.4	10.3	7.8	3.8	8.9
16:0	38.2	36.7	34.1	32.5	36.0	33.7
18:0	13.3	18.2	13.7	7.6	10.8	7.9
Σ satd.	60.5	66.3	60.4	48.5	51.9	51.6
16:1	10.8	11.4	12.0	15.8	13.9	14.4
18:1	21.9	20.3	19.1	25.4	28.0	26.5
Σ ($n-9$)	34.0	29.5	34.8	41.7	44.8	43.6
18:2	3.5	3.4	2.8	5.1	2.6	2.8
18:3	1.5	n.d.	n.d.	0.8	n.d.	n.d.
Σ ($n-6$)	5.0	3.4	3.1	6.6	2.8	2.8

cholesterol esters and DG only at the lowest ganglioside concentration, while that of linoleic acid increased in cholesterol esters and decreased in triacylglycerols. Also the amount of 16:1 decreased in cholesterol esters of cells treated with 10^{-8} M gangliosides.

Discussion

It has been shown that the addition of a mixture of gangliosides to chick neurons in culture has a powerful neuritogenic effect [15,31,32] accompanied by a stimulation of the metabolism of phosphoinositides after incubation of the cells with radioactive inositol [18]. This observation led to the study of a possible effect of gangliosides upon the composition and distribution of the lipid moiety of membrane phospholipids. Moreover, the increased synthesis of IP_3 suggested that the DG part of PI might be affected as well by the treatment with gangliosides, hence resulting in a modification of protein kinase C activity, and subsequently, of protein phosphorylation. Therefore, the amount of neutral lipids has been analysed after the growth of neurons in the presence or in the absence of a mixture of gangliosides.

The results show that the addition of gangliosides had no effect on the amount and the distribution of phospholipids. The composition of fatty acids in the various phospholipids, instead, varies but only in PI where the proportion of saturated fatty acids decreased and polyunsaturated fatty acids increased, notably arachidonic acid and the fatty acid of the ($n-3$) series.

The content of neutral lipids was also markedly affected by the treatment with the sialoglycolipids. The change of the cell growth medium from the DMEM containing 10% fetal calf serum to the CDM reduced significantly the content of neutral lipids. The presence of gangliosides raised such a content to levels corresponding to those present in serum grown cells or even above (Table III). It should be pointed out that the growth of cells in CDM slows down their development when compared to DMEM supplemented with serum. Under these circumstances the finding further confirms the neurotrophic action of gangliosides.

The fetal calf serum used contains free fatty acids (with, notably, 7 to 9% of 14:0, unpublished observations) and the change to CDM growth medium, (which does not contain free fatty acids), must affect the neuronal content in neutral lipids. The large increase in cholesterol esters (Table III) might indicate an increased intraneuronal mobility of free fatty acids if one refers to the cholesterol esters as fatty acid transporters.

The composition of free fatty acids was not affected. The high amount of 14:0 corresponded to that found in the fetal calf serum and in neurons grown in the presence of this serum.

The composition of fatty acids in DG, when compared to that of PI and PC, suggests that this activator of protein kinase C derived mainly from the hydrolysis of PC rather than PI (compare Tables I and II with Table V). It remains however to be determined whether the DG produced from PC participates to the activation of protein kinase(s) C as the one produced from PI.

The present data confirm the hypothesis that the neuritogenic effect of exogenous gangliosides is mediated by a modification of the metabolism of PI. After treatment with gangliosides, the composition of fatty acids in neutral lipids and phospholipids was not affected, however, the overall content of both neutral lipids and phospholipids approached that of neurons grown in 10% fetal calf serum, further suggesting the protective role of these sialoglycolipids in the survival of neurons.

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References

1 Ledeen, R.W. and Yu, R.K. (1976) in Glycolipid Methodology (Witting, A., ed.), pp. 187–214, American Oil Chemists Society, Champaign, IL.
2 Svennerholm, L. (1980) in Structure and Function of Gangliosides (Svennerholm, L., Mandel, P., Dreyfus, H. and Urban, P.F., eds.), pp. 533–544, Plenum Press, New York.
3 Massarelli, R., Wong, T.H., Harth, S., Louis, J.C., Freysz, L. and Dreyfus, H. (1982) Neurochem. Res. 7, 301–316.
4 Wierasko, A. and Seifert, W. (1986) Brain Res. 371, 305–313.
5 Rahmann, H. (1983) Neurochem. Int. 5, 539–547.
6 Ando, S. (1983) Neurochem. Int. 5, 507–513.
7 Berry-Kravis, E. and Dawson, G. (1985) J. Neurochem. 45, 1740–1747.
8 Yamakawa, T. and Nagai, Y. (1978) Trends Biochem. Sci. 3, 126–131.
9 Hakomori, S.I. (1981) Annu. Rev. Biochem. 50, 733–764.
10 Durand, M., Guerold, B., Lombard-Golly, D. and Dreyfus, H. (1986) in Gangliosides and Neuronal Plasticity (Tettamanti, G., Ledeen, R.W., Sandhoff, K. and Nagai, Y., eds.), pp. 295–307, Liviana Press, Padova.
11 Tettamanti, G. and Masserini, M. (1987) in Biomembranes and Receptor Mechanisms (Bertoli, E., Chapman, D., Cambria, A., Scapagnini, V., eds.), pp. 224–260, Liviana Press, Padova.
12 Facci, L., Leon, A., Toffano, G., Sonnino, S., Ghidoni, R. and Tettamanti, G. (1984) J. Neurochem. 42, 299–305.
13 Chigorno, V., Pitto, M., Cardace, G., Acquotti, D., Kirshner, G., Sormino, S., Ghidoni, R. and Tettamanti, G. (1985) Glycoconjugate J. 2, 279–291.
14 Ledeen, R.W. (1984) J. Neurosci. 12, 147–159.
15 Massarelli, R., Ferret, B., Gorio, A., Durand, M. and Dreyfus, H. (1985) Int. J. Dev. Neurosci. 3, 341–348.
16 Chan, K.F.J. (1987) J. Biochem. 262, 5248–5255.
17 Kreutter, D., Kim, J.Y.H., Goldenring, J.R., Rasmussen, H., Ukomadu, C., De Lorenzo, R.J. and Yu, R.K. (1987) J. Biol. Chem. 262, 1633–1637.
18 Ferret, B., Massarelli, R., Freysz, L. and Dreyfus, H. (1987) C.R. Acad. Sci. Paris 304, 97–99.
19 Michell, R.H. (1982) Cell Calcium 3, 285–289.
20 Nishizuka, Y. (1984) Science 225, 1365–1370.
21 Pettmann, B., Louis, J.C. and Sensenbrenner, M. (1979) Nature 281, 378–380.
22 Formisano, S., Johnson, M.L., Lee, G., Aloj, S.M. and Edelhoch, H. (1979) Biochemistry 18, 1119–1124.
23 Ulrich-Bott, B. and Wiegandt, H. (1984) J. Lipid Res. 25, 1233–1245.
24 Schwarzmann, G., Hoffmann-Bleihauer, P., Schubert, J., Sandhoff, K. and Marsh, D. (1983) Biochemistry 22, 5041–5048.
25 Corti, M., DeGiorgio, V., Ghidoni, R., Sonnino, S. and Tettamanti, G. (1980) Chem. Phys. Lipids 26, 225–238.
26 Dreyfus, H., Lombard-Golly, D., Aglea-Sbachnig, M., Guerold, B., Durand, M., Massarelli, R. and Ledeen, R. (1986) in Enzymes of Lipid Metabolism. II (Freysz, L., Dreyfus, H., Massarelli, R. and Gatt, S., eds.), pp. 577–590, Plenum Press, New York.
27 Ghidoni, R., Sonnino, S., Masserini, M., Orlando, P. and Tettamanti, G. (1981) J. Lipid Res. 22, 1286–1295.
28 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 487–509.
29 Leray, C., Pelletier, X., Hemmendinger, S. and Cazenave, J.P. (1987) J. Chromatogr. 420, 411–416.
30 Morrison, W.R. and Smith, L.M. (1964) J. Lipid Res. 5, 600–608.
31 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
32 Dreyfus, H., Ferret, B., Harth, S., Gorio, A., Freysz, L. and Massarelli, R. (1984) in Ganglioside Structure, Function and Biomedical Potential, pp. 513–524, Plenum Press, New York.