GANGLIOSIDES OF GLIAL CELLS: A COMPARATIVE STUDY OF NORMAL ASTROBLASTS IN TISSUE CULTURE AND GLIAL CELLS ISOLATED ON SUCROSE-FICOLL GRADIENTS

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Received 14 October 1974

1. Introduction

Mammalian nerve tissue contains much higher levels of gangliosides than does non-neural tissue, and is, furthermore, characterized by appreciable amounts of tri- and tetrasiologangliosides, which are practically absent from non-neural cells [1].

At the present time, the only techniques available for separating neurons and glial cells from brain are microdissection and centrifugation on sucrose or sucrose-ficoll gradients. The first technique gives only minute amounts of material and therefore, is not convenient for most experiments. Using the centrifugation techniques, many authors [2–4] have shown that the fractions obtained are contaminated. In particular, the possibility of a contamination of the glial cell fraction with synaptosomes or membranes derived from synaptosomes, which are extremely rich in gangliosides [5], could seriously affect the distribution of gangliosides observed in isolated glial cells.

Glial cell cultures, which are free from contamination by other cells, seem to be a promising system for studying this problem. In order to throw some light upon the ganglioside composition of normal glial cells, we compared the gangliosides of astroblasts in culture with the glial cell fraction obtained from brain by centrifugation on sucrose-ficoll gradients.

2. Materials and methods

2.1. *Cell culture*

Primary cultures of astroblasts were obtained from new born rat cerebral hemispheres [6]. In these cultures, neurons and oligodendroglial cells progressively disappeared. After two weeks only astroblasts could be observed in the cultures.

Cultures in continuous lines originated from clone NN (Normal baby hamster astroglia) isolated by Shein et al. [7].

The cells were cultivated in Falcon dishes with Eagle-Dulbecco medium, supplemented with 10% foetal calf serum. NN cells were harvested at the beginning of the stationary phase and rat astroblasts after 14 days of culture. They were recovered by scraping, washed in 0.9% NaCl and freeze-dried.

2.2. *Isolation of glial cell fractions*

Glial cells and neurons obtained from the cerebral cortex of adult hamsters were separated by centrifugation on sucrose-ficoll gradients according to Sellinger et al. (8). Both fractions were then freeze-dried.

2.3. *Isolation and analysis of gangliosides*

Soluble proteins were first extracted from the freeze-dried cells with phosphate buffer 5 mM pH 7.2. Gangliosides were extracted according to Suzuki [9] or Tettamanti et al. [10], then purified by mild alkaline hydrolysis [11]. Total lipid sialic acid was measured by the technique of Miettinen and Takki-Luukkainen [12]. Gangliosides were fractionated by silicagel G thin-layer chromatography on pre-coated plates (Van der Eijnden, [13]). The gangliosides were detected with the Bial's orcinol–HCl reagent and their distribution determined by densitometry using a Vernon densitometer.

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3. Results and discussion

The lipid sialic acid concentration of NN cells is about 0.60 μg/mg dry weight. These results are similar to the values reported by Shein et al. [7] for hamster astrocyte nodules, by Dawson et al. [14] for the tumoral glial clone C6, and by Derry and Wolfe [15] on glial cells obtained by microdissection. On the other hand, our value is somewhat lower than those reported for glial cells fractions isolated from rabbit or rat brain by centrifugation techniques (Hamberger and Svennerholm, [16]; Norton and Poduslo, [17]).

Thin-layer chromatography of total NN hamster glial cells gangliosides, obtained by Suzuki's or Tettamanti's method, showed the presence of two major spots running as G_{M3} and G_{D3} markers. In addition, traces of other gangliosides were seen. Never tri- or tetrasialogangliosides were detected. The most abundant was the monosialolactosyl ceramide (table 1). This pattern is very different from that found in the glial cell fraction of the rabbit brain studied by Hamberger and Svennerholm [10] where G_{M3} and G_{D3} account for only a minor part of the total gangliosides. This difference could be explained by the fact that the glial cells which were analysed were isolated from the brains of different species. However, we obtained similar results to those of Hamberger and Svennerholm when we prepared glial cells from hamster brain by a centrifugation technique. In the glial cell fraction, the gangliosides were almost the same as those detected in the neuronal cell fractions as well as in hamster whole cortex (Table 2).

The last material that we have used in the study of glial cells gangliosides, was the primary cultures of rat astroblasts. In these cells total gangliosides amount was 0.5 μg/mg dry weight, similar to the NN level. Thin-layer chromatography showed the presence of only two spots moving as G_{M3} and G_{D3} which respectively account for 96% and 4% of the total gangliosides (table 1).

In view of the simple ganglioside pattern of glial cells in culture, it seems that the complex pattern obtained with glial fractions prepared by centrifugation methods could be the result of a contamination by a material rich in polysialogangliosides such as synaptic plasma membranes [5]. Nevertheless, we cannot exclude that mature glial cells have no other gangliosides than G_{M3} and G_{D3}. The cells we have studied are astroblasts, i.e. embryonic cells. A study of the differentiation of these cells into astrocytes is now necessary before we can affirm that glial cells are free of the complex gangliosides which are characteristic of nerve tissue.

Table 1
Distribution of gangliosides in cultured astroblasts

Gangliosides*	Clone NN**	Rat astroblast***
G_{M3}	62.7	96.0
G_{M1}	0.3	–
G_{D3}	36.5	4.0
G_{D1a}	0.5	–

Results are expressed as percentage of total sialic acid recovered. * Nomenclature of Svennerholm [18]. ** Mean of 5 experiments. *** Mean of 2 experiments.

Table 2
Distribution of gangliosides in various fractions of hamster brain

Gangliosides	Whole cerebral cortex*	Glial cell fraction*	Neuronal cell* fraction
G_{M3}	0	traces	traces
G_{M1}	19.8	15.4	12.2
G_{D3}	4.5	3.8	5.7
G_{D1a}	21.4	38.6	41.2
G_{D1b}	20.2	17.9	9.3
G_{T1}	26.2	17.9	26.3
G_{Q1}	7.9	6.4	5.3

Results are expressed as percentage of total sialic acid recovered. * Mean of two experiments.

Acknowledgments

The authors wish to thank Mrs P. Guerin and Mrs C. Ringeisen for their valuable technical assistance.

References

[1] Wiegandt, H. (1968) Angew. Chem. 80, 89.
[2] Cremer, J. E., Johnston, P. V., Roots, B. I. and Trevor, A. J. (1968) J. Neurochem. 15, 1361.
[3] Sinha, A. K. and Rose, S. P. R. (1971) Brain Res. 33, 205.

[4] Hemminki, K., Hemminki, E. and Giacobini, E. (1973) Inter. J. Neurasci. 5, 87.
[5] Breckenridge, W. C., Gombos, G. and Morgan, I. G. (1972) Biochem. Biophys. Acta, 266, 695.
[6] Booher, J. and Sensenbrenner, M. (1972) Neurobiol. 2, 97.
[7] Shein, H. M., Britva, A., Hess, H. H. and Selkoe, D. J. (1970) Brain Res. 19, 497.
[8] Sellinger, O. Z., Ascurra, J. M. Johnson, D. E., Ohlsson, W. G. and Lodin, Z. (1971) Nature New Biol. 230, 253.
[9] Suzuki, K. (1964) Life Sci. 3, 1227.
[10] Tettamanti, G., Bonali, F., Marchesini, S. and Zambotti, V. (1973) Biochem. Biophys. Acta 296, 160.
[11] Schneider, P. B. and Kennedy, E. P. (1968) J. Lipid. Res. 9, 58.
[12] Miettinen, T. and Takki-Luukkainen, I. T. (1959) Acta Chem. Scand. 13, 856.
[13] Van den Eijnden, D. N. (1971) Hoppe Seyler's Z. physiol. Chem. 352, 1601.
[14] Dawson, G., Kemp, S. F., Stoolmiller, A. C. and Dorfman, A. (1971) Biochem. Biophys. Res. Commun. 44, 687.
[15] Derry, D. M. and Wolfe, L. S. (1967) Science 158, 1450.
[16] Hamberger, A. and Svennerholm, L. (1971) J. Neurochem. 18, 1821.
[17] Norton, W. T. and Poduslo, S. E. (1971) J. Lipid Res. 12, 84.
[18] Svennerholm, L. (1963) J. Neurochem. 10, 613.