SIALOGLYCOPROTEINS, GANGLIOSIDES AND RELATED ENZYMES IN DEVELOPING RAT BRAIN

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1. Introduction

It has been shown, that a similarity in regional distribution exists between sialoglycoproteins and gangliosides in bovine brain [1]. In rat brain as well as in bovine brain most of the gangliosides and the sialoglycoproteins are found in the mitochondrial and the microsomal fractions [2–4, 6]. Most evidence points to a localization in dendritic and axonal membranes as well as in their synaptic connections [2, 4–6, 7]. Dekirmenjian and Brunngraber [4] showed the distribution of gangliosides and sialoglycoproteins to be different after subfractionation of a fraction enriched in axones and nerve-ending particles.

We supposed that information about the distribution of these sialo-compounds could also be obtained from the developmental pattern. Therefore the levels of gangliosides and sialoglycoproteins during development were determined. For comparison the activities of sialidase (EC 3.2.1.18), CMP-NANA * synthetase, β-galactosidase (EC 3.2.1.13) and β-galactosaminidase were measured. Our data show that the rate of most rapid increase of both gangliosides and sialoglycoproteins is between the 4th and the 18th day. These changes more closely parallel the maximal rate of axonal than of dendritic increase [8].

2. Methods

TNO rats of ages between 0–150 days were used in this study. The number of brains pooled was 8 for 0–10 days old, 6 for 13–21 days old and 2 for 26–150 days old animals. Rats from different litters were pooled in order to average out any possible variations between different litters. Brains above the level of the pons were used. The pooled brains were homogenized in 9 vol. distilled water, freeze-dried and stored at −20° until use.

The dry weight was determined after drying over P_2O_5. DNA was extracted with a slightly modified Schneider procedure [9] and assayed according to Burton [10]. The gangliosides were separated from the sialoglycoproteins by sequential extraction of the tissue with 19 vol. chloroform-methanol (2:1, v/v) and 10 vol. chloroform-methanol (1:2, v/v) as described before [1]. NANA was determined according to Warren [11] and protein by the method of Lowry et al. [12]. β-Galactosidase and β-galactosaminidase activities were measured as described by van Hoof and Hers [13]. The conditions for CMP-NANA synthetase were the following: 0.04 ml of a 10% brain homogenate was incubated at 37° for 30 min with 0.60 μmole NANA, 0.80 μmole CTP, 1.60 μmoles GSH, 8 μmoles $MgCl_2$ and 32 μmoles tris-HCl buffer, pH 8.5, in a total volume of 0.2 ml distilled water. Excess NANA was reduced with $NaBH_4$ (4 mg in 40 μl distilled water, 10 min). $NaBH_4$ was removed with 40 μl acetone, to which 10% concentrated H_3PO_4 was added. CMP-NANA was determined as NANA [11]. Sialidase activity was measured at pH 4.0 with gangliosides or sialoglycopeptides as a substrate. The details of the method have recently been described [1].

* *Abbreviation:*
NANA: *N*-acetyl neuraminidase.

Table 1
Changes in wet and dry weight, DNA and protein in total rat brain during development.

Age (days)	Wet weight (g)	Dry weight (%)	Protein (mg)	DNA (mg)
0	0.164	12.6	9.59	0.547
1	0.200	11.9	10.7	0.568
2	0.286	11.6	15.6	0.595
4	0.289	11.2	15.2	0.564
6	0.453	12.4	25.4	0.622
8	0.618	13.1	37.4	0.779
10	0.735	14.9	48.5	0.884
13	0.858	16.8	66.9	0.997
15	0.863	17.7	69.0	0.861
17	0.955	18.7	84.0	1.110
19	0.997	19.8	90.2	1.066
21	1.025	20.5	97.4	1.112
26	0.972	21.0	95.3	–
36	1.155	23.0	112.0	1.028
76	1.260	23.2	127.3	1.116
150	1.335	22.9	127.0	1.044

3. Results and discussion

Table 1 illustrates the changes in wet and dry weight as well as protein and DNA content during post-natal brain development. For protein there is about a 13-fold increase from 0 to 150 days. Per g wet weight a 2-fold increase can be calculated, whereas per g dry weight the protein content is fairly constant during the whole period. Total post-natal DNA does not change until 4 days after birth. After that time DNA gradually rises till the 17th day. In the period from the 4th to the 17th day DNA increases about 2-fold. As 94–97% of the neurons in rat brain are present at birth [14], the rise in DNA mainly represents the formation of glial cells.

During postnatal development the gangliosides and the sialoglycoproteins show a similar course (fig. 1). In the period from birth up to the 4th day there is a small increase for both compounds: The ganglio-sidic NANA per brain changes from 0.178 μmole to 0.327 μmole and the glycoprotein NANA from 0.109 μmole to 0.237 μmole. The rate of most rapid increase of gangliosides and sialoglycoproteins is found between the 4th day and about the 18th day (for gangliosides in accordance with [17]). In this period also the largest proliferation of glial cells is

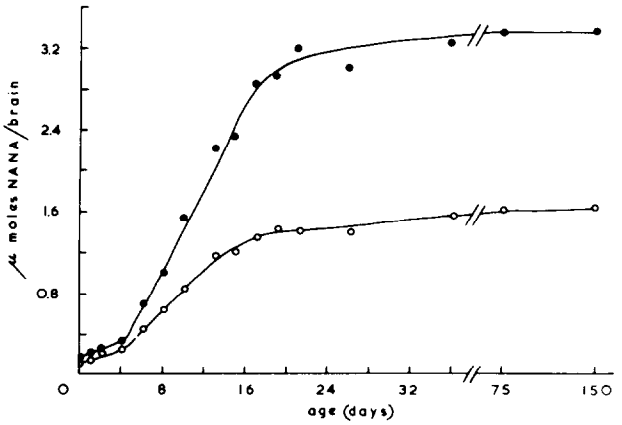

Fig. 1. Comparison of postnatal development of gangliosides (●——●) and sialoglycoproteins (○——○) in total brain. NANA was determined after hydrolysis at 80° with 0.1 N H_2SO_4 according to Warren [11].

obtained. However, the rise of the gangliosides cannot be correlated with this proliferation as gangliosides are very low [5] or practically absent [15] from isolated glial cells. Sialoglycoproteins may be present in glial cells, although the bulk must be bound to neuronal components. This follows from the observation that in white matter, which is rich in glial cells compared to grey matter, the level of sialoglycoproteins is low [1]. A correlation with the outgrowth of axons and dendrites seems more likely, as after subfractionation gangliosides and sialoglycoproteins are found to be concentrated in the fractions containing nerve endings as well as axonal and dendritic fragments [2, 3]. This would be in accordance with the observations of Ears and Goodhead [8], who estimated the density of both axons and dendrites at several stages between birth and maturity. They found that the density of axons increases at a maximal rate between the ages of 6 and 18 days, whereas the rate of most rapid dendritic growth is between 18 and 24 days. Our results point to a closer correlation with the maximal rate of axonal than of dendritic increase.

CMP-NANA synthetase, which activates NANA for incorporation in glycoproteins and gangliosides, and sialidase, which splits off bound NANA from these compounds, show a different pattern during development (fig. 2). For comparison the increase of glycoprotein- and ganglioside NANA are given. All values are expressed per g wet weight. Sialidase

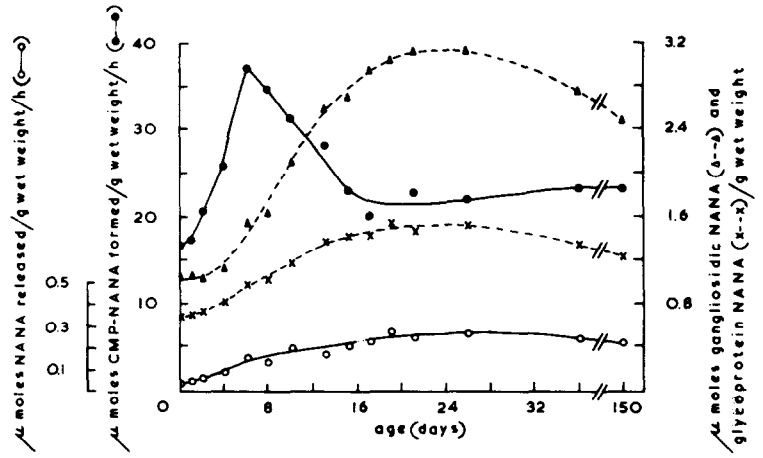

Fig. 2. CMP-NANA synthetase activity (●—●) was determined at pH 8.5; sialidase activity (○—○) at pH 4.0 with gangliosides as a substrate. Their changes in rat brain during development were compared with the change in gangliosidic NANA (▲---▲) and glycoprotein NANA (x---x). All values are expressed per g wet weight.

activity gradually rises during development and follows a similar course as the glycoproteins and the gangliosides. In fig. 2 only the activity with gangliosides as a substrate is shown. With sialoglycopeptides as a substrate comparable activities were obtained. The activity of CMP-NANA synthetase increases sharply from birth until the 6th day. Then the activity decreases. At about the 16th day the activity becomes fairly constant and hardly changes until maturity. The steep rise in CMP-NANA synthetase activity between birth and the 6th day (from 16.5 to 37.2 μmole CMP-NANA formed/g wet weight/hr) precedes the maximal production of gangliosides and sialoglycoproteins. The adult activity is reached when the gangliosides and the sialoglycoproteins obtain their maximal level.

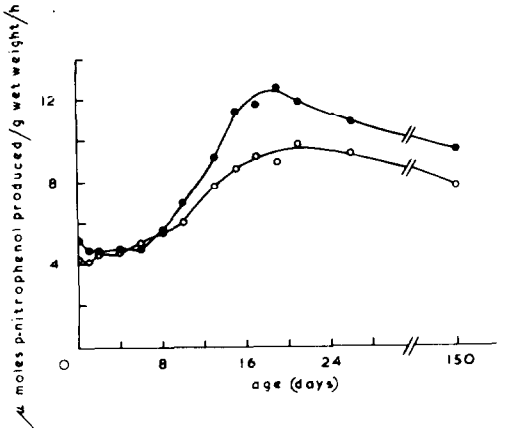

Fig. 3. Changes with age in β-galactosidase (○—○) and β-galactosaminidase (●—●) activities of rat brain. β-Galactosidase: substrate *p*-nitrophenyl-β-D-galactopyranoside, pH 3.5. β-Galactosaminidase: substrate *p*-nitrophenyl-2-acetoamido-2-deoxy-β-D'-galactopyranoside, pH 4.5. Incubation time 3 hr.

β-Galactosidase and β-galactosaminidase were investigated with the *p*-nitrophenyl derivatives of β-galactose and β-galactosamine as a substrate (fig. 3). Though the artificial substrates are less specific than the natural substrates [16] an indication is obtained about the enzymes engaged in the breakdown of gangliosides and glycoproteins. From the 4th to about the 20th day the activities per g wet weight per hr rise about 2-fold and 2.5-fold respectively. From the 20th day to maturity for both enzymes there is a small decrease in activity. Both enzymes show a development profile similar to sialidase, gangliosides and sialoglycoproteins (compare fig. 2). Our results are consistent with the view that both gangliosides and sialoglycoproteins are located in the synaptic region. The presence of sialoglycoproteins in glial cells has still to be investigated.

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