Gangliosides in the Brain in Adult Down's Syndrome and Alzheimer's Disease

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ABSTRACT

Quantitative analysis of total gangliosides and of ganglioside composition by HPTLC has been carried out on the gray matter of frontal cerebral cortex of six brains from Down's syndrome (DS) adults, six age-matched controls, six Alzheimer's disease (AD) adults, and six controls matched for age with the AD brains, as well as on three DS and six control cerebellum specimens. In addition, the analyses were carried out on specimens of corpus callosum of five adult DS and five control brains. No abnormalities were found in the gangliosides of DS corpus callosum. In DS frontal cortex, the concentration of total gangliosides was reduced, and there was a decrease in the fraction of G_{T1b} and G_{D1b}, and an increase in those of G_{T1a}, G_{D3}, G_{M1} and G_{M2}; the ratio of total b-series to a-series gangliosides was decreased. Very similar abnormalities were found in the gangliosides of DS cerebellum. In AD frontal cortex, by contrast, the total gangliosides and their composition were normal by comparison with age-matched controls, with the minor exception of reductions in the fractions of G_{Q1b} and G_{T1L}. It is concluded that abnormalities in gangliosides exist in the brain in DS that are unrelated to AD-type pathology and may reflect developmental disturbances.

Index Entries: Down's syndrome, Alzheimer's disease, cerebral cortex, cerebellum, corpus callosum, ganglioside composition.

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ABBREVIATIONS

DS: Down's syndrome
NANA: *N*-Acetylneuraminic acid
ChAT: Choline acetyltransferase
AD: Alzheimer's disease
HPTLC: High-performance thin-layer chromatography

INTRODUCTION

Down's syndrome (DS), the most common known cause of mental retardation, is associated in the brain with a failure of normal development followed by a degenerative disorder, which in the brain eventually shows features virtually indistinguishable from Alzheimer's disease (AD) (Sinex and Merrill, 1982; Scott et al., 1983; Wisniewski et al., 1985; Coyle et al., 1987; Oliver and Holland, 1986; Brooksbank and Balázs, 1988; Mann, 1988). Not only is the adult DS brain small in overall size, with a simplified pattern of convolutions, but, in addition, certain parts, notably the cerebellum, are particularly underdeveloped. Although not pathognomic for DS, this underdevelopment of the brain is accompanied by a diminution of neuron numbers that especially affects granule cells, dendritic spine dysgenesis, and impaired myelination (for reviews, *see* Scott et al., 1983; Coyle et al., 1986).

During normal development of the brain, changes occur in both the concentration and the composition of gangliosides, (Suzuki, 1965; Vanier et al., 1971; Hakomori, 1981; Rösner, 1982; Ando, 1982; Yates, 1986), membrane glycolipids that are enriched in neurons (Ledeen, 1983). The composition of gangliosides differs considerably in different parts of the brain (Kraĉun et al., 1984).

Although no ganglioside abnormalities were detectable in the DS frontal cerebral cortex at midgestation (Brooksbank et al., 1989), this may have been too early in ontogenesis, especially for the later-developing granule neurons that seem notably deficient in DS. DS brains from infancy or childhood were not available to us, but it has been possible to examine frontal cerebral cortex, cerebellum, and corpus callosum in postmortem specimens from DS subjects who died in adulthood. As these brains were affected by Alzheimer neurodegeneration, it was necessary to examine the gangliosides in a series of non-DS AD, to determine whether any abnormalities found in the cerebral cortical gangliosides were also detectable in AD cerebral cortex. In addition, the ganglioside composition of adult DS cerebellum was analyzed and compared with non-AD controls, because the cerebellum is little affected by obvious neurodegeneration in AD (Tomlinson, 1980), but is maldeveloped in DS.

This work constitutes part of a more wide-ranging study of brain lipid abnormalities in DS and AD, which is being published separately (Brooksbank and Martinez, 1989).

MATERIALS AND METHODS

Human Specimens

Tissue was obtained from six postmortem brains from patients (aged 47–69 yr; mean 55.8 yr) with trisomy 21 that had been established by karyotyping. Frontal cortex (Brodmann areas 9 and 10) were dissected at −5 to −10°C from all six brains, corpus callosum from five of them, and cerebellar cortex from three.

Controls consisted of frontal cortex from the same areas from six normal (nontrisomic) patients who had died with no known neurological disorder (aged 48–69 yr; mean 56.8 yr), matched for age with the DS subjects. Secondly, a series of six frontal cortex specimens from postmortem brains of AD patients (aged 57–86 yr; mean 72.7 yr) was used as a control group to assess the possible association of typical AD neuropathology in DS with alterations in ganglioside composition. In turn, frontal cortex specimens from a series of six nonneurological controls (aged 62–86 yr; mean 75.0 yr) matched for age with the AD cases were analyzed. Control corpus callosum and cerebellar tissue was taken from normal nonneurological postmortem brains (aged 55–85 yr, mean 69.8 yr; and 48–70 yr; mean 56.8 yr., respectively) that were in the main different from those from which the cortex samples were obtained.

In all instances the time between death and autopsy was <72 h, cadavers had been refrigerated until autopsy, and the brains were frozen at −70°C thereafter. Before analysis, all tissue samples were rapidly homogenized with ice-cold phosphate-buffered saline (2.0 mL/g fresh tissue) in order to ensure that all parameters were derived from samples of uniform white matter content, and the homogenates were refrozen as pellets in liquid N_2 immediately after homogenization and stored at −70°C thereafter.

Extraction and Analysis of Gangliosides

All solvents used for extraction and chromatography were BDH Ltd. (Poole, Dorset, UK) "liquid chromatography" or "Aristar" grades, or their equivalent. All evaporations of extracts were carried out under a stream of N_2 at 55°C.

The method of extraction and analysis was that of Harth et al. (1978), with minor modifications. Samples of the tissue homogenate (about 200 mg, equivalent to about 67 mg of fresh tissue) were extracted with 20 vol

of chloroform–methanol (2:1, v/v), and the soluble material dried and redissolved in 0.5 mL of chloroform–methanol (1:1, v/v).

Total lipid-soluble *N*-acetylneuraminic acid (NANA, sialic acid) was determined by the resorcinol method of Svennerholm (1957), with extraction of color into *n*-butyl acetate-*n*-butanol (85:15, v/v) (Miettinen and Takki-Luukainen, 1959). The absorbance was read at 450 and 580 nm, and Svennerholm's (1957) correction was made with the value for the absorbance ratio of interfering substances taken as 7.5 (Ledeen and Yu, 1982).

Quantitative chromatographic analysis of individual gangliosides was carried out on Merck Kieselgel 60 10- × 10-cm HPTLC plates in the following sequence of systems, the plate being air-dried between each run:

1. Chloroform, to top of plate;
2. Chloroform–methanol–water (70:30:4, v/v) *twice*, each to 1.5 cm from top of plate;
3. Chloroform–methanol–0.25% (w/v) aqueous KCl (60:35:8, v/v) *twice*, each to 2.5 cm from top of plate.

Gangliosides were visualized with the resorcinol reagent of Svennerholm (1957) and applied as a fine mist with three passes of an atomizer. This was followed by heating in an oven at 110°C, and the spraying and heating were repeated.

On each plate, using a Camag Linomat IV automatic spotting apparatus (Camag, Muttenz, Switzerland), four samples of extract (two each containing known amounts of about 1.0 μg and one each containing 1.5 μg and 2.0 μg of total NANA, respectively) were spotted on duplicate 0.5-cm lanes, and single 0.5-cm lanes were spotted with 5.0, 10.0, 15.0 and 20.0 μL of a solution in chloroform–methanol (1:1) of a mixture of pure gangliosides G_{M1}, G_{D1a}, G_{D1b} and G_{T1b} (each 50 μg/mL) (Fidia Research Laboratories, Adano, Italy).

Scanning densitometry was carried out as described in the accompanying paper (Brooksbank and Martinez, 1989). Several scans of each lane were made, with great care being taken to adjust the computed background in order to optimize the integration of the area of the minor components of the extracts.

Identification of individual gangliosides other than those in the standard mixture was inferred from their relative mobilities, by reference to published chromatograms, as shown for frontal cortex in Fig. 1 (cf Ando et al., 1978; Ueno et al., 1978; Kundu, 1981; Ledeen and Yu, 1982). As alkali-labile gangliosides were included in the analyses, and these, at least in rodents, constitute a significant proportion of the total (e.g., Ghidoni et al., 1980; Zanetta et al., 1980) and may not have been separated from the designated components, some degree of uncertainty in identification is accepted, as it is in most other published surveys of ganglioside composition. The least polar gangliosides, G_{M3} and G_{M4},

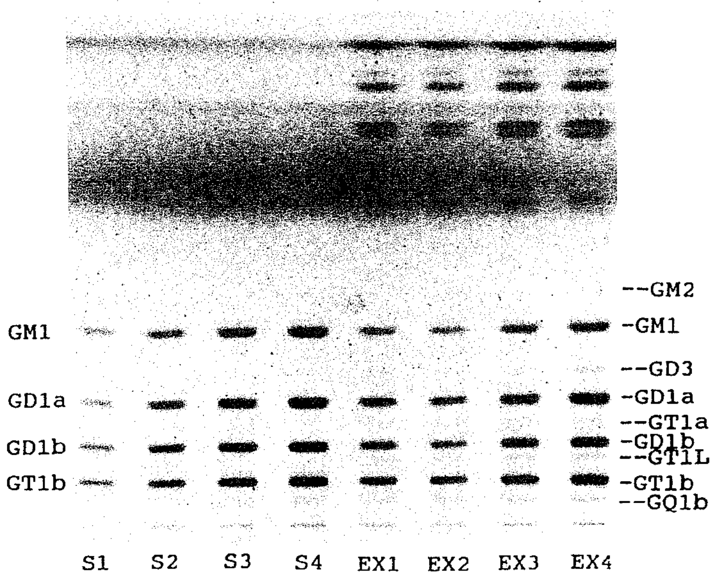

Fig. 1. Typical HPTLC plate, as scanned quantitatively after visualization with resorcinol–HCl reagent (*see* Methods). Authentic standards are as indicated on the left. The identity of the other (minor) components, as labeled on the right, is deduced from other publications. S1, S2, S3, S4: 0.25, 0.5, 0.75, and 1.0 μg, respectively, each of standards G_{T1b}, G_{D1b}, G_{D1a} and G_{M1}. EX1, EX2, EX3, EX4: extracts containing 1.0, 1.0, 1.5, and 2.0 μg, respectively, of total ganglioside NANA from a control frontal cortex. (G_{M3} and G_{M4}, buried in the nonganglioside material, were not scanned).

were not included in the quantitative analyses by HPTLC because of interfering material on the chromatograms (Fig. 1), but these are relatively minor components.

Assay of Choline Acetyltransferase (ChAT) Activity

The activity of ChAT was estimated in the brain tissue homogenates by the method of Fonnum (1975), modified by the use of [^{3}H]acetyl coenzyme-A (Amersham, 5.0 Ci/mmol) as labeled substrate, at 500 μ*M*. Activity was expressed as nmol acetylcholine formed per h/mg protein.

Protein was estimated by the Lowry method, and all ganglioside concentrations were expressed in terms of protein in order to allow for possible changes in water content of the tissue samples.

RESULTS

Correction for White Matter Contamination

Because there are substantial differences between gray matter and white matter in the concentration of gangliosides and in their relative proportions (e.g., Ando, 1983), it was necessary to normalize all values

for cerebral cortical and cerebellar tissue for variations (sometimes large) in the contamination of these supposed gray matter samples with white matter. The correction was made by reference to the uniform cholesterol concentration of carefully dissected control frontal cortex (nominally taken as 100% gray matter) and that of corpus callosum (taken as 100% white matter), as described fully in the accompanying paper (Brooksbank and Martinez, 1989).

Total Gangliosides (Lipid-Soluble NANA) and Ganglioside Composition in Corpus Callosum

In corpus callosum, the total lipid-soluble NANA in DS was not significantly different from that in controls, nor were the proportions of the individual gangliosides significantly different (Table 1). The concentrations of individual gangliosides (G_{Q1b}, G_{T1b}, G_{T1L}, G_{D1b}, G_{T1a}, G_{D1a}, G_{D3}, G_{M1}, and G_{M2}) are not given in the tables, since these can be calculated from the data presented, but they also showed no differences between DS and controls in corpus callosum.

Total Gangliosides and Ganglioside Composition in Frontal Cortex

In the adult DS frontal cortex (Table 2), the total gangliosides were significantly reduced. The proportion of G_{T1b} was clearly reduced ($p < 0.01$) and that of G_{D1b} at $p < 0.05$; the fraction of total b-series gangliosides was 18% less than in age-matched controls ($p < 0.05$). The proportion of a-series gangliosides, G_{M1} and G_{M2}, was increased ($p < 0.01$), as was that of G_{T1a} and of G_{D3} (taken as a b-series coupound), at $p < 0.05$. The ratio of total b-series to total a-series gangliosides in DS was reduced by 31% ($p < 0.05$). These abnormalities in the DS frontal cortex gray matter were *not* present in AD frontal cortex, which showed only a slight and statistically insignificant reduction in total gangliosides, and no abnormalities in their composition, except for apparent decreases in the fractions of the minor components G_{Q1b} and G_{T1L}. The ratio of total b-series to total a-series gangliosides was totally unchanged in AD compared with their controls. The *concentrations* (μg/mg protein) of G_{D3}, G_{M1}, and G_{M2} were the same in the DS and control series; in the AD series, there were no significant differences in individual ganglioside concentrations (except those of G_{Q1b} and G_{T1L}) apparent in the fractional values. Almost all the above abnormalities were still discernible in the values not corrected for white matter (data not shown). No significant correlations between either age or ChAT activity could be elicited with any ganglioside parameter.

Table 1
Total Ganglioside and Ganglioside Composition in Corpus Callosum of Adult DS and Controls

	(Means ± SE)	
	Downs (n = 5) (56.2 ± 3.61 yr)	Controls (n = 5) (69.8 ± 6.06 yr)
Total Ganglioside N-acetyl-neuraminic acid, NANA (μg/mg protein)	3.9 ± 0.77	3.1 ± 0.11
	Percent of Total Ganglioside NANA	
Ganglioside		
G_{Q1b}	0.3 ± 0.14	0.5 ± 0.29
G_{T1b}	10.1 ± 0.42	11.7 ± 0.71
G_{T1L}	1.3 ± 0.48	0.8 ± 0.29
G_{D1b}	12.7 ± 0.56	12.1 ± 0.77
G_{T1a}	1.4 ± 0.56	0.8 ± 0.23
G_{D1a}	17.6 ± 1.32	17.0 ± 1.81
G_{D3}	14.7 ± 1.61	14.5 ± 2.16
G_{M1}	38.0 ± 1.95	39.7 ± 2.85
G_{M2}	3.8 ± 1.07	2.9 ± 0.53
Total b-series	39.2 ± 2.21	39.6 ± 1.78
Total a-series	60.8 ± 2.21	60.4 ± 1.78
Total b-series/ total a-series	0.66 ± 0.06	0.66 ± 0.05

None of the differences is significant (Mann-Whitney U-test, one tailed).

Total Gangliosides and Ganglioside Composition in Cerebellum

In the cerebellum (Table 3), total and individual ganglioside differences between DS and controls were in the same direction as those found in the frontal cerebral cortex although, since only three DS cerebella were analyzed, variances were wider. The decrease in the ratio of total b-series

Table 2
Total Ganglioside, Ganglioside Composition, and ChAT Activity in Frontal Cortex of Adult DS, AD, and Matched Controls

(Means ± SE) $

	Downs (n = 6)	Controls (n = 6)	Alzheimer's (n = 6)	Controls (n = 6)
Age (yr)	55.8 ± 2.97	56.8 ± 2.88	75.0 ± 3.42	72.7 ± 4.20
Total ganglioside N-acetyl-neuraminic acid, NANA (µg/mg protein)	8.8 ± 0.71*	12.0 ± 0.68	8.7 ± 0.56	9.6 ± 0.39
Ganglioside	Percent of Total Ganglioside NANA			
G_{Q1b}	2.1 ± 0.27	2.8 ± 0.27	2.0 ± 0.10**	2.5 ± 0.14
G_{T1b}	18.4 ± 0.94**	25.3 ± 0.98	24.6 ± 1.48	25.6 ± 0.72
G_{T1L}	1.7 ± 0.22	1.8 ± 0.13	2.0 ± 0.20***	3.0 ± 0.08
G_{D1b}	18.8 ± 1.00*	21.8 ± 0.45	22.6 ± 1.26	20.2 ± 0.64
G_{T1a}	2.0 ± 0.15*	1.6 ± 0.14	1.9 ± 0.14	2.2 ± 0.17
G_{D1a}	27.6 ± 1.41	25.1 ± 0.97	22.6 ± 2.03	24.9 ± 1.07
G_{D3}	3.9 ± 0.15*	2.9 ± 0.42	2.7 ± 0.42	3.1 ± 0.08
G_{M1}	23.2 ± 1.05**	17.8 ± 0.92	19.2 ± 1.30	17.9 ± 0.56
G_{M2}	2.3 ± 0.50**	1.1 ± 0.07	1.3 ± 0.13	1.7 ± 0.18
Total b-series	44.9 ± 2.17*	54.5 ± 1.42	54.3 ± 2.48	54.0 ± 1.36
Total a-series	55.1 ± 2.17*	45.5 ± 1.42	46.0 ± 1.36	45.7 ± 2.48
Total b-series/ total a-series	0.84 ± 0.080*	1.21 ± 0.070	1.22 ± 0.112	1.19 ± 0.065
ChAT (nmol acetylcholine formed/h./mg protein)	1.1 ± 0.28**	3.3 ± 0.47	2.2 ± 0.32*	4.4 ± 0.76

$ Corrected for white matter contribution, by normalization from the values found for cholesterol concentration in corpus callosum (as described in the text).

Significant difference from matched control group: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ (Mann-Whitney U-test, one-tailed).

Table 3
Total Ganglioside, Ganglioside Composition, and ChAT Activity in Cerebellum of Adult DS and Matched Controls

	(Means ± SE) $	
	Downs (n = 3) (56.3 ± 6.56 yr)	Controls (n = 6) (56.7 ± 3.00 yr)
Total ganglioside N-acetyl-neuraminic acid NANA (μg/mg protein)	7.6 ± 0.30	7.0 ± 0.32
Ganglioside	Percent of Total Ganglioside NANA	
G_{Q1b}	4.4 ± 0.13*	7.7 ± 0.50
G_{T1b}	34.8 ± 1.0	37.5 ± 1.53
G_{T1c}	3.1 ± 0.13*	3.8 ± 0.17
G_{D1b}	19.5 ± 0.29	19.4 ± 1.19
G_{T1a}	2.5 ± 0.12	2.7 ± 0.17
G_{D1a}	18.2 ± 0.86*	11.6 ± 0.93
G_{D3}	6.7 ± 0.47	6.6 ± 0.32
G_{M1}	9.7 ± 0.38	9.6 ± 0.71
G_{M2}	1.2 ± 0.11	1.2 ± 0.03
Total b-series	68.5 ± 0.46*	74.9 ± 1.44
Total a-series	31.5 ± 0.46*	25.1 ± 1.44
Total b-series/ total a-series	2.17 ± 0.046*	3.06 ± 0.254
ChAT (nmol acetylcholine formed/h./mg protein)	1.9 ± 0.92	3.4 ± 0.75

$ Corrected for white matter contribution, by normalization from the values found for cholesterol concentration in corpus callosum (as described in the text).

Significant difference from controls; *$p < 0.05$ (Mann-Whitney U-test, one-tailed).

to total a-series gangliosides in DS was even greater in the cerebellum than in the cerebral cortex. The ChAT activity in cerebellum was lower in DS than in controls, but the difference did not reach statistical significance; the activity was not correlated with any ganglioside parameter.

DISCUSSION

The values for total gangliosides and for their individual components in corpus callosum are in general accord with published values for white matter (e.g., Urban et al., 1980; Kraĉun et al., 1984), although strict comparisons of composition are not possible because the minor components listed were not entirely the same in the different series.

In the gray matter of frontal cortex and cerebellum of controls, after normalization to a constant low white matter content, the values for total gangliosides and for the fractional distribution of individual components agree broadly with those previously reported (e.g., Suzuki, 1965; Urban et al., 1980; Kraĉun et al., 1984), insofar as comparisons can be made between the present and earlier series, bearing in mind the inherent uncertainties about identification implicit in most published data on ganglioside patterns. There were no differences between our slightly younger (mean age 56 yr) and older (mean age 73 yr) controls, but the age range was probably too narrow to permit the revelation of the decrease of total gangliosides across the adult age span and the shift towards b-series gangliosides reported to occur in human whole brain by Segler-Stahl et al. (1983).

The present findings demonstrate that there are significant abnormalities in both the total concentration of gray matter gangliosides and in their composition in DS in late adulthood. These differences from controls with no neuropathology were essentially absent in the frontal cortex of AD, whereas they were present in both cerebral and cerebellar cortex of DS. They can therefore hardly be attributed to Alzheimer-type neurodegeneration in DS; this conclusion is borne out by the absence of any correlation between ganglioside abnormalities and the reduction of ChAT activity that is a regular feature of AD pathology (for reviews, *see* Hardy et al., 1985; Candy et al., 1986; Collerton, 1986).

The decreased total ganglioside and the higher proportion of G_{M1}, G_{M2}, and G_{D3} in DS cortical gray matter might be a reflection of gliosis, since the gangliosides of astroglia differ from those of neurons in this manner (Ando et al., 1978; Urban et al., 1980; Ando, 1983). Moreover, in the DS fetal cerebral cortex, the gangliosides were found to be completely normal (Brooksbank et al., 1989). However, it is unlikely that the degree of gliosis in the adult DS frontal cortex is any greater than in AD, and the alterations in the fatty acyl composition of phosphoglycerides observed in the same series of DS brains, but not in the AD series (Brooksbank and Martinez, 1989) cannot be explained in terms of gliosis.

A question arises as to whether the differences in ganglioside concentration and composition observed in adult DS brain tissue reflect the late results of defective neuronal differentiation during development. Neuronal differentiation is associated with major changes in the amount and relative proportions of gangliosides, and gangliosides are suggested to play a role in the processes of neuritogenesis and synapse formation Yavin and Yavin, 1979; Hakomori, 1981; Ando, 1983; Ledeen, 1983, 1984; Gorio, 1986; Yates, 1986). During postnatal development in humans, in frontal cortex gray matter there is a decrease in the relative proportion of G_{D1a} and a moderate increase in that of G_{D1b} and G_{T1b} (Vanier et al., 1971). This trend is reported (in the whole brain) to continue through adult life into old age, with a reduction in the relative proportion of G_{M1} as well (Segler-Stahl et al., 1983). Thus the ganglioside pattern in the frontal cortex gray matter of adult DS suggests a relatively underdeveloped pattern of ganglioside formation in DS. If this is so, it seems to occur after the midgestational period of fetal development, for at that time the ganglioside composition of DS cerebral cortex was quite normal (Brooksbank et al., 1989).

A relative paucity of polysialogangliosides and of b-series gangliosides in the DS brain gray matter could well be associated with alterations in synaptic plasticity and in the function of nerve cell membranes, which no doubt underly the mental retardation. The suggested actions of gangliosides in modifying the lipid environment of such membrane enzymes as Na^+K^+ -ATPase (Leon et al., 1981), which well may function abnormally in DS (McCoy and Sneddon, 1983), or in modulating protein phosphorylation (Chan,1987), and the capacity of gangliosides to affect neuronal differentiation (Ledeen, 1984; Yates, 1986; Baker, 1988), are relevant.

Previous studies of the gangliosides in the AD brain appear to be limited. Loss of total ganglioside NANA has been reported (Suzuki et al., 1965; Cherayil, 1969; Bowen et al., 1977; Crino et al., 1989), and this seems to be most marked in laminae III to VI of the cerebral cortex (DeKosky and Bass, 1982; Sorbi et al., 1987). In recent studies, neither Crino et al. (1989) nor Majocha et al. (1989) found changes in ganglioside composition. In the present study, the concentration of total gangliosides in AD frontal cortex was possibly decreased, but the difference from age-matched controls was not statistically significant, and the composition of the gangliosides was essentially normal.

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