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Disialoganglioside G_{D1a} of Rat Brain Subcellular Particles during Development

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The increase observed in the amount of the disialoganglioside G_{D1a} of the rat cerebrum during development between 21 and 81 days of age accounted for nearly 40% of the overall increase in total ganglioside in the tissue during the same period. Subcellular fractionation showed the microsomal fraction to contribute by far the most towards this increase in cerebral ganglioside G_{D1a} . It is suggested that microsomal ganglioside G_{D1a} may serve as a marker for dendritic arborization in the rat cerebrum.

Developmental studies of the cerebral gangliosides of the rat (Suzuki, 1965; Vanier *et al.*, 1971; Merat & Dickerson, 1973) and man (Suzuki, 1965; Vanier *et al.*, 1971; Yusuf *et al.*, 1977) have shown the disialoganglioside G_{D1a} (nomenclature of Svennerholm, 1963) to be the largest fraction of total gangliosides in the tissue. The molar percentage proportion of this ganglioside relative to the other major species (gangliosides G_{M1} , G_{D1b} and G_{T1}) as well as its total amount in the brain increases with age, and it has been shown in man (Yusuf *et al.*, 1977) that the amount persists in increasing even when the other fractions begin to level off. This increase in cerebral ganglioside G_{D1a} during development has been thought (Vanier *et al.*, 1971; Yusuf *et al.*, 1977) to be indicative of the increase in dendritic arborization and neuronal interconnections in the tissue. The present study has been undertaken to investigate in the rat which subcellular fraction contributes most to this increase in cerebral ganglioside G_{D1a} during the developmental period between 21 and 81 days of age.

Materials and Methods

Male Wistar rats aged 21 (24 animals) and 81 (eight animals) days, fed on a stock pelleted diet (Spillers, Gainsborough, Lincs., U.K.), were used. The animals were killed by decapitation, the brains were quickly removed, and the cerebrum was separated from the rest of each brain by the method of Dickerson & Dobbing (1967). It was then rinsed in ice-cold 0.9% NaCl, blotted with a filter paper and weighed. Representative samples from each cerebrum were picked for direct analysis and the rest in a group were pooled for subcellular fractionation by differential and sucrose-density-gradient centrifugations as described by Eichberg *et al.* (1964). The brains

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of the rats aged 21 days were divided into three equal portions and each portion was subjected to the fractionation procedure. The separated fractions [nuclear, mitochondrial, myelin (combined 'large' and 'small'), synaptosomal and microsomal] were washed three or four times by suspension in water and re-sedimenting at 100000g for 30 min.

Gangliosides were extracted from the whole cerebrum samples by Suzuki's (1964) double-extraction procedure and those from the subcellular fractions by the procedure of Suzuki & Chen (1967). The gangliosides in each such preparation were purified by the re-partition technique of Suzuki & Chen (1967) and measured as *N*-acetylneuraminic acid by a modification (Miettinen & Takki-Luukkainen, 1959) of Svennerholm's (1957) resorcinol method. The major gangliosides were separated by t.l.c. and subsequently determined quantitatively (Merat & Dickerson, 1973; Yusuf *et al.*, 1977). The recovery of gangliosides during t.l.c. was $95 \pm 3\%$ (mean $\pm$ s.d. of eight determinations) of the independently assayed total.

Results

Between 21 and 81 days of age the cerebral weight increased by $21.5 \pm 4.6\%$ (mean $\pm$ s.d. for the number of determinations shown in the Tables) while the amount of total gangliosides in the tissue increased by $51.7 \pm 4.1\%$ (Table 1). Of this increase in total gangliosides, the increase in ganglioside in G_{D1a} , which at both 21 and 81 days of age was the most predominant fraction (about 40% of total), accounted for $38.6 \pm 1.4\%$, as against 17.7 ± 2.2 to $25.5 \pm 1.6\%$ contributed by one or the other of the remaining major species (Table 1).

Subcellular fractionation (Table 2) showed the microsomal fraction to be the richest in ganglioside content (57.1–51.3% of that in the whole homogenate) followed by the synaptosomal fraction

Table 1. *Increases in total and the individual major gangliosides of the rat cerebrum during growth between 21 and 81 days of age*

Total gangliosides were extracted from representative samples (in duplicate) of each brain and measured as *N*-acetylneuraminic acid. The gangliosides were separated by t.l.c. and the major fractions quantified similarly as the total ganglioside (see the text for the methods). The amounts of these major fractions were taken as 100% of the total. The molar proportions of the fractions were obtained by dividing the *N*-acetylneuraminic acid values of gangliosides G_{T1} by 3, of G_{D1a} and G_{D1b} each by 2, and of G_{M1} by 1. Each value is the mean $\pm$ s.d. for the number of determinations shown (*n*). Values in the parentheses are the molar percentages of total gangliosides present in the form of gangliosides G_{M1} , G_{D1a} etc. Ganglioside nomenclature is that of Svennerholm (1963).

	21 days old (<i>n</i> = 24)	81 days old (<i>n</i> = 8)	Absolute increase	Percentage increase	Percentage of total increase
Brain wt. (g)	1.05 $\pm$ 0.02	1.26 $\pm$ 0.03	0.22 $\pm$ 0.07	21.5 $\pm$ 4.6	—
Ganglioside <i>N</i> -acetylneuraminic acid (μ g)					
Total	885 $\pm$ 28	1343 $\pm$ 48	457 $\pm$ 34	51.7 $\pm$ 4.1	100
G_{M1}	261 $\pm$ 20 (29.5 $\pm$ 3.1)	343 $\pm$ 36 (25.5 $\pm$ 2.7)	81 $\pm$ 16	30.9 $\pm$ 3.5	17.7 $\pm$ 2.2
G_{D1a}	372 $\pm$ 17 (42.1 $\pm$ 2.7)	550 $\pm$ 36 (40.9 $\pm$ 2.3)	177 $\pm$ 19	47.4 $\pm$ 3.0	38.6 $\pm$ 1.4
G_{D1b}	142 $\pm$ 13 (16.0 $\pm$ 1.9)	226 $\pm$ 27 (16.8 $\pm$ 2.1)	85 $\pm$ 15	59.3 $\pm$ 5.1	18.4 $\pm$ 1.9
G_{T1}	109 $\pm$ 10 (12.3 $\pm$ 1.6)	226 $\pm$ 26 (16.8 $\pm$ 2.0)	117 $\pm$ 16	107.3 $\pm$ 3.8	25.5 $\pm$ 1.6

Table 2. *Increases in total gangliosides and in ganglioside G_{D1a} of the subcellular particles of rat cerebrum during growth between 21 and 81 days of age*

The cerebrums of 24 rats aged 21 days were pooled, after removal of small samples from each for direct analysis, and divided into three equal portions. Each portion (6–7 g) was subjected to subcellular fractionation by the method of Eichberg *et al.* (1964). The separated fractions of the three portions were combined respectively. Similarly, the cerebrums of eight rats aged 81 days were pooled, and the whole portion was fractionated as above. The values shown for the subcellular fractions are the means of duplicate determinations made on a single extract from each and those for the whole homogenate are the means $\pm$ s.d. for the numbers of determinations shown (*n*). See the text for methods of determination of total and individual gangliosides. The values in parentheses are the percentages of mean total gangliosides in the whole homogenate recovered in the respective subcellular fractions.

Age (days)	...	Total ganglioside <i>N</i> -acetylneuraminic acid				Ganglioside G_{D1a} <i>N</i> -acetylneuraminic acid			
		Amount (μ g)		Absolute increase (μ g)	Percentage of cerebral increase	Amount (μ g)		Absolute increase (μ g)	Percentage of cerebral increase
		21	81			21	81		
	<i>n</i> ...	24	8			24	8		
Fraction									
Whole homogenate		885 $\pm$ 28	1343 $\pm$ 48	457 $\pm$ 34	100	372 $\pm$ 17	550 $\pm$ 36	177 $\pm$ 19	100
Nuclear		28 (3.2)	41 (3.1)	13	2.8	11 (2.9)	16 (2.9)	5	2.8
Mitochondrial		15 (1.7)	28 (2.1)	13	2.8	5 (1.3)	8 (1.5)	3	0.5
Myelin		7 (0.8)	16 (1.2)	9	2.0	Trace	Trace	—	—
Synaptosomal		124 (14.0)	280 (20.8)	156	34.1	53 (14.2)	80 (14.6)	27	15.3
Microsomal		505 (57.1)	689 (51.3)	184	40.2	221 (59.2)	346 (63.0)	125	71.0
Total in subcellular fractions		679	1054			290	450		
Recovery (%)		76.7	78.5			77.7	82.0		

(14.0–20.8%). The nuclear, mitochondrial and the myelin fractions contained small percentages of total gangliosides in the whole cerebrum. The recovery of gangliosides (both of total and of ganglioside G_{D1a}) in the subcellular fractions varied between

76.7 and 82.0% (Table 2). The amount of gangliosides in each fraction increased during the period studied, and the microsomal and the synaptosomal fractions contributed almost equally (the former 40.2%, and the latter 34.1%) to the total increase

in the whole cerebrum during the period. On the other hand, when the increases of ganglioside G_{D1a} in the various subcellular fractions were compared with its increase in the whole cerebrum (Table 2) it was found that the microsomal increase contributed as high as 71.0% as against only 15.3% contributed by the synaptosomal fraction. The nuclear and mitochondrial increases were almost insignificant, and the myelin fraction contained only traces of the ganglioside at any age.

Discussion

The rate of accretion of gangliosides in the rat brain is highest during the first 3 weeks of life, and the major changes in the ganglioside pattern also occur during this period (Suzuki, 1965; Vanier *et al.*, 1971; Merat & Dickerson, 1973). However, it takes another 2–3 months for the gangliosides to attain adult concentration and composition (Merat & Dickerson, 1973). The same has been shown in the present study. During the period studied, total gangliosides in the cerebrum increased by more than 51% compared with about 22% increase in the weight of the tissue, indicating an increase in concentration of the gangliosides. Slight changes were also observed in the ganglioside pattern during the period. These changes in concentration and composition, although small in comparison with those occurring between birth and 21 days of age, are significant because of their possible vulnerability to chronic undernutrition (H. K. M. Yusuf & J. W. T. Dickerson, unpublished work).

The recovery (between 76 and 82%) of gangliosides during subcellular fractionation as obtained in the present study is fairly comparable with that obtained by others (Lapetina *et al.*, 1967). Two major findings emerged from the present study. Firstly, the increase in ganglioside G_{D1a} contributed to the highest degree to the overall increase in total gangliosides in the developing cerebrum, and, secondly, the microsomal fraction contributed by far the most towards the increase in cerebral ganglioside G_{D1a} , although the synaptosomes contributed almost equally with the microsomal fractions to the overall increase in total gangliosides in the whole cerebrum. It has been suggested (Vanier *et al.*, 1971; Yusuf *et al.*, 1977) that the increase in ganglioside G_{D1a} in the developing cerebrum might represent the

increase in dendritic arborization as the tissue matures. Evidence for this suggestion is provided by our findings (Merat, 1971; H. K. M. Yusuf, A. Merat & J. W. T. Dickerson, unpublished work) that in both man and the rat undernutrition during development decreases selectively the accretion of ganglioside of G_{D1a} in the cerebrum, and the histological studies by Eayrs & Horn (1955) and Cragg (1972) have shown decreased dendritic arborization in the brain of nutritionally deprived animals. If ganglioside G_{D1a} truly represents dendritic surface area, then the results of our present study may lead one to make the suggestion that, of all the ganglioside species of the various subcellular organelles of the cerebrum, the disialoganglioside G_{D1a} of the microsomal fraction may serve as a reliable marker of the degree of dendritic arborization and neuronal interconnections in the tissue. This seems very likely in the light of the notion (Wolfe & McIlwain, 1961) that the microsomal fraction is composed of membranous fragments that arise mainly from dendrites besides minor proportions of other cellular processes.

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References

- Cragg, B. G. (1972) *Brain* **95**, 143–150
- Dickerson, J. W. T. & Dobbing, J. (1967) *Proc. R. Soc. London Ser. B* **166**, 384–395
- Eayrs, J. T. & Horn, G. (1955) *Anat. Rec.* **121**, 53–61
- Eichberg, J. W., Whittaker, V. P. & Dawson, R. M. C. (1964) *Biochem. J.* **92**, 91–100
- Lapetina, E. G., Soto, E. F. & De Robertis, E. (1967) *Biochim. Biophys. Acta* **135**, 33–43
- Merat, A. (1971) Ph.D. Thesis, University of Surrey
- Merat, A. & Dickerson, J. W. T. (1973) *J. Neurochem.* **20**, 873–880
- Miettinen, T. & Takki-Luukkainen, I.-T. (1959) *Acta Chem. Scand.* **13**, 856–858
- Suzuki, K. (1964) *Life Sci.* **3**, 1227–1233
- Suzuki, K. (1965) *J. Neurochem.* **12**, 969–979
- Suzuki, K. & Chen, G. C. (1967) *J. Neurochem.* **14**, 911–915
- Svennerholm, L. (1957) *Biochim. Biophys. Acta* **24**, 604–611
- Svennerholm, L. (1963) *J. Neurochem.* **10**, 613–623
- Vanier, M.-T., Holm, M., Ohman, R. & Svennerholm, L. (1971) *J. Neurochem.* **18**, 581–592
- Wolfe, L. S. & McIlwain, H. (1961) *Biochem. J.* **78**, 33–40
- Yusuf, H. K. M., Merat, A. & Dickerson, J. W. T. (1977) *J. Neurochem.* **28**, 1299–1304