

Ganglioside Levels in Hypoxic Brains from Neonatal and Premature Infants

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ABSTRACT

In this study, 13 cases of newborn term-gestational infants and six cases of premature infants who died of hypoxia were selected for the determination of ganglioside levels in several regions of brains obtained at autopsy. Cases were divided into three groups according to the hypoxic interval and gestational age:

Group A, six cases of newborn infants. The average time of hypoxia was 6.4 h.

Group B, seven cases of newborn infants. The average time of hypoxia was about 71 h.

Group C, six cases of premature infants. The average hypoxia time was 34.7 h.

Frontal cortex, forebrain, hippocampus, and parahippocampal gyrus and cerebellum of each brain were examined. The method of Ladisch and Gillard (1985) was used to purify and quantify gangliosides.

The results showed that total gangliosides decreased significantly in three regions of cerebral hemispheres of group B and in four brain regions of group C, as compared with group A ($p < 0.01$). The amount of gangliosides in frontal cortex in group B was lower than in group C ($p < 0.01$). The four major gangliosides (GM1, GD1a, GD1b, and GT1b) were all reduced in cerebral hemispheres of group B and C. In hypoxic brains, the percentage of gangliosides also showed some alterations. There was less GD1a in the cerebral hemispheres of group B and the frontal cortex of group C. The amount of GD1b was

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also less in the frontal cortex and forebrain of group B than in group A or C. The results suggest that severe hypoxia might cause decreases in brain gangliosides that correlate to the severity of brain damage.

INTRODUCTION

Gangliosides are a family of acidic glycosphingolipids present in high concentrations in neuronal membranes (Nagai and Zwamori, 1984). They play important roles in cell-cell recognition (Yasuda et al. 1988), intercellular adhesion (Cheresh, 1986), synaptic transmission (Wieraszko and Seifert, 1986), antigenicity (Cheresh, 1985), and receptor function (Markwell et al. 1986), as well as in learning and memory mechanisms (Szekely et al., 1987).

Changes in the ganglioside compositions of nervous tissues are well documented for several disease states, including the gangliosidoses, which are attributable to deficiencies of specific catabolic enzymes (Suzuki, 1984). There are also changes in the gangliosides in brains from humans and animals with metachromatic leukodystrophy (Suzuki, 1966), mucopolysaccharidosis (Suzuki, 1966), multiple sclerosis (Yu et al., 1974), epilepsies (Seyfried et al., 1978), motor neuron disease (Rapport et al., 1985), Pick's disease (Kamp et al., 1986), and cerebellar ataxia (Seyfried et al., 1987).

However, studies on the changes of gangliosides in neonatal brains that suffered hypoxic or ischemic damage have not been reported. Because damage to the brains of newborns following hypoxic or ischemic events is a common clinical syndrome, we report in this paper the levels of gangliosides in different brain areas of premature and newborn infants who died of hypoxia. These data are then correlated to the histopathological alterations of these brains.

MATERIALS AND METHODS

Brain Specimens

Fresh brain tissues were obtained from the Departments of Pathology of Gynecologic and Obstetric Hospital in Beijing and of Beijing Friendship Hospital. There were 13 newborn infants with gestational ages ranging from 38–42 wk and six cases of premature infants who had gestational ages of 30–35 wk. All of them died from acute, severe hypoxia; those suspected to have congenital malformations of the nervous system were excluded. According to the time of hypoxia and gestational age, they were divided into three groups (see Table 1):

Group A, six cases of newborn infants. Diagnoses of death are congenital malformation, not of the nervous system, causing sudden death; and abruptio placenta and placenta previa caus-

Table 1
Case Studies

Groups	Number of cases	Gestational age, wk	Mean hypoxia time, h
A	6	38-42	6.43
B	7	38-42	71
C	6	30-35	34.7

ing late pregnancy hemorrhage. The time interval was less than 24 h from hypoxia to death. The average time of hypoxia was 6.4 h. Group B, seven cases of newborn infants. Diagnoses of death are fetal intrauterine delayed development with low birth weight; fetal intrauterine asphyxia and postnatal depression with Apgar score below 4; severe infant pneumonia; and digestive tract hemorrhage. Symptoms of hypoxia for the above cases lasted more than 24 h. The average time of hypoxia was about 71 h. Group C, six cases of premature infants. The causes of death were fetal edema and fetal respiratory distress syndrome. The average hypoxia time was about 34.7 h.

The bodies were immediately refrigerated (4°C), and autopsies were carried out during the first 24 h after death. The cerebral hemispheres and cerebellum were immediately frozen at -20°C until they were dissected, for biochemical studies, into the following regions: frontal cortex, forebrain (i.e., frontal pole containing gray and white matter), hippocampus and parahippocampal gyrus, and cerebellum. Each specimen for extraction was 3 g of wet tissue.

Lipid Extraction and Analysis

The wet samples were extracted twice with 20 vol of chloroform/methanol (1/1, v/v) according to the method of Ladisch and Gillard (1985). The total lipid extracts were clarified by centrifugation to remove particulate matter, and their original volumes were reduced by rotatory evaporation. They were then dried by evaporation under nitrogen gas.

The total lipid extracts were dissolved in diisopropyl ether/1-butanol/0.9% NaCl (60/40/50, v/v) and mixed by vortexing and sonicating for several minutes. The upper organic phase (containing neutral lipids and phospholipids) was then removed. The ganglioside-containing lower aqueous phase was reextracted with the original volume of fresh organic solvent mixture and centrifuged.

The clear aqueous phase was lyophilized, redissolved in a small volume of distilled water, applied to a Sephadex G50 column, and eluted with double-distilled water. The ganglioside content was monitored using a 751G UV spectrophotometer with the wavelength at 206 nm. The ganglioside-containing fraction was collected, lyophilized, and stored at -20°C until analysis.

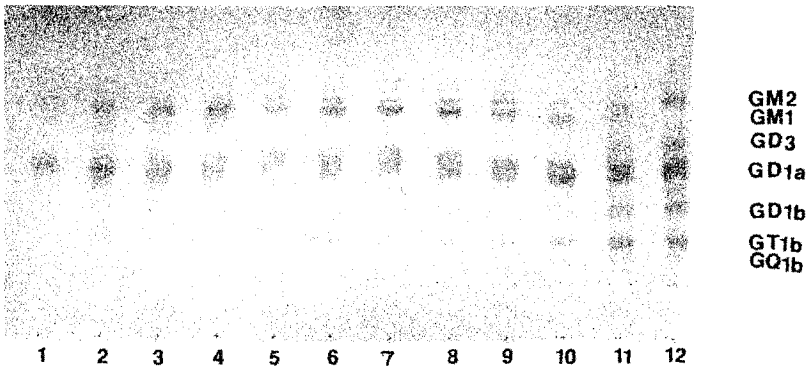


Fig. 1. TLC of ganglioside species separated on silica gel 60 in chloroform/methanol/0.25% KCl (5/4/1, v/v) and then visualized with resorcinol-HCl reagent.

Lanes 1–4: standard gangliosides from oX brain; Lane 5 (frontal cortex), Lane 7 (forebrain), Lane 9 (hippocampus and parahippocampal gyrus), and Lane 11 (cerebellum) are sample gangliosides from hypoxic neonatal brains; Lane 6 (frontal cortex), Lane 8 (forebrain), Lane 10 (hippocampus and parahippocampal gyrus), and Lane 12 (cerebellum) are sample gangliosides from hypoxic premature infant brains.

Thin-layer chromatography (TLC) was performed using 10- × 20-cm silica gel 60 TLC plates (Merck, West Germany), which were preactivated by heating at 90°C for 45 min. The plates were developed in chloroform/methanol/0.25% KCl (5/4/1, v/v). Gangliosides were visualized with resorcinol-HCl reagent (Svennerholm, 1957), and quantitated by scanning densitometry using a CS-910 densitometer (Shimadzu, Japan). Gangliosides were quantitated using known amounts of standard bovine gangliosides and linear regression. The results are expressed as mean ± SD. Differences among groups were examined by analysis of variance and Student's *t*-test.

RESULTS

Figure 1 shows a TLC of standard and sample ganglioside patterns in different brain regions. The total amounts of gangliosides in several brain regions of groups A, B, and C are shown in Table 2. The results show that the quantities of the total gangliosides were significantly less in cerebral hemispheres of group B and in four brain regions of group C than in group A ($p < 0.01$). The total amount of gangliosides in frontal cortex is lower in group B than group C ($p < 0.01$).

Concentrations of the four major gangliosides on a wet-weight basis for the same specimens are shown in Table 3. The four major gan-

Table 2
Distribution of Total Gangliosides in the Different Brain Regions^a

Brain region	Ganglioside-bound sialic acid ($\mu\text{g/g}$ wet tissue)		
	Group A	Group B	Group C
Frontal cortex	275 \pm 13	153 \pm 21 ^b	198 \pm 40
Forebrain	198 \pm 16	117 \pm 21 ^c	111 \pm 12
Hippocampus and parahippocampal gyrus	252 \pm 16	140 \pm 22	144 \pm 26
Cerebellum	88 \pm 19	70 \pm 15	56 \pm 18

^aValues are means \pm SEM, expressed as μg of ganglioside-bound sialic acid per gram wet tissue.

^b $p < 0.01$ vs corresponding Group A.

^c $p < 0.01$ vs corresponding Group C.

Table 3
Distribution of Individual Gangliosides in the Different Brain Regions^a

Brain region		Ganglioside-bound sialic acid ($\mu\text{g/g}$ wet tissue)		
		Group A	Group B	Group C
Frontal cortex	GM1	47 \pm 6	26 \pm 4 ^b	34 \pm 7
	GD1a	166 \pm 13	85 \pm 10 ^b	108 \pm 20
	GD1b	15 \pm 1	7 \pm 2	12 \pm 3
	GT1b	39 \pm 6	17 \pm 5 ^b	29 \pm 3
Forebrain	GM1	37 \pm 4	21 \pm 4 ^b	23 \pm 5
	GD1a	114 \pm 10	60 \pm 1 ^b	61 \pm 9
	GD1b	11 \pm 3	4 \pm 2	6 \pm 1
	GT1b	25 \pm 6	14 \pm 6 ^b	16 \pm 1
Hippocampus and parahippocampal gyrus	GM1	48 \pm 7	23 \pm 3 ^b	23 \pm 5
	GD1a	144 \pm 10	68 \pm 11 ^b	78 \pm 15
	GD1b	15 \pm 3	8 \pm 2	8 \pm 4
	GT1b	32 \pm 12	19 \pm 6 ^c	24 \pm 6
Cerebellum	GM1	9 \pm 2	6 \pm 1 ^c	5 \pm 2 ^a
	GD1a	23 \pm 5	18 \pm 4	18 \pm 5
	GD1b	10 \pm 2	9 \pm 3	7 \pm 2
	GT1b	22 \pm 7	20 \pm 4	15 \pm 5 ^b

^aValues are means \pm SEM, expressed as μg of ganglioside-bound sialic acid per gram wet tissue.

^b $p < 0.01$ vs corresponding Group A.

^c $p < 0.05$ vs corresponding Group A.

gliosides (GM1, GD1a, GD1b, and GT1b) are significantly less concentrated in cerebral hemispheres of groups B and C ($p < 0.01$ and 0.05 , respectively). In cerebellum, GM1 in group B, and GM1 and GT1b in group C are lower than in group A ($p < 0.01$ and 0.05 , respectively).

Percentage distributions of the major gangliosides in four brain regions are shown in Figs. 2A-2C. In each group examined, all cerebral

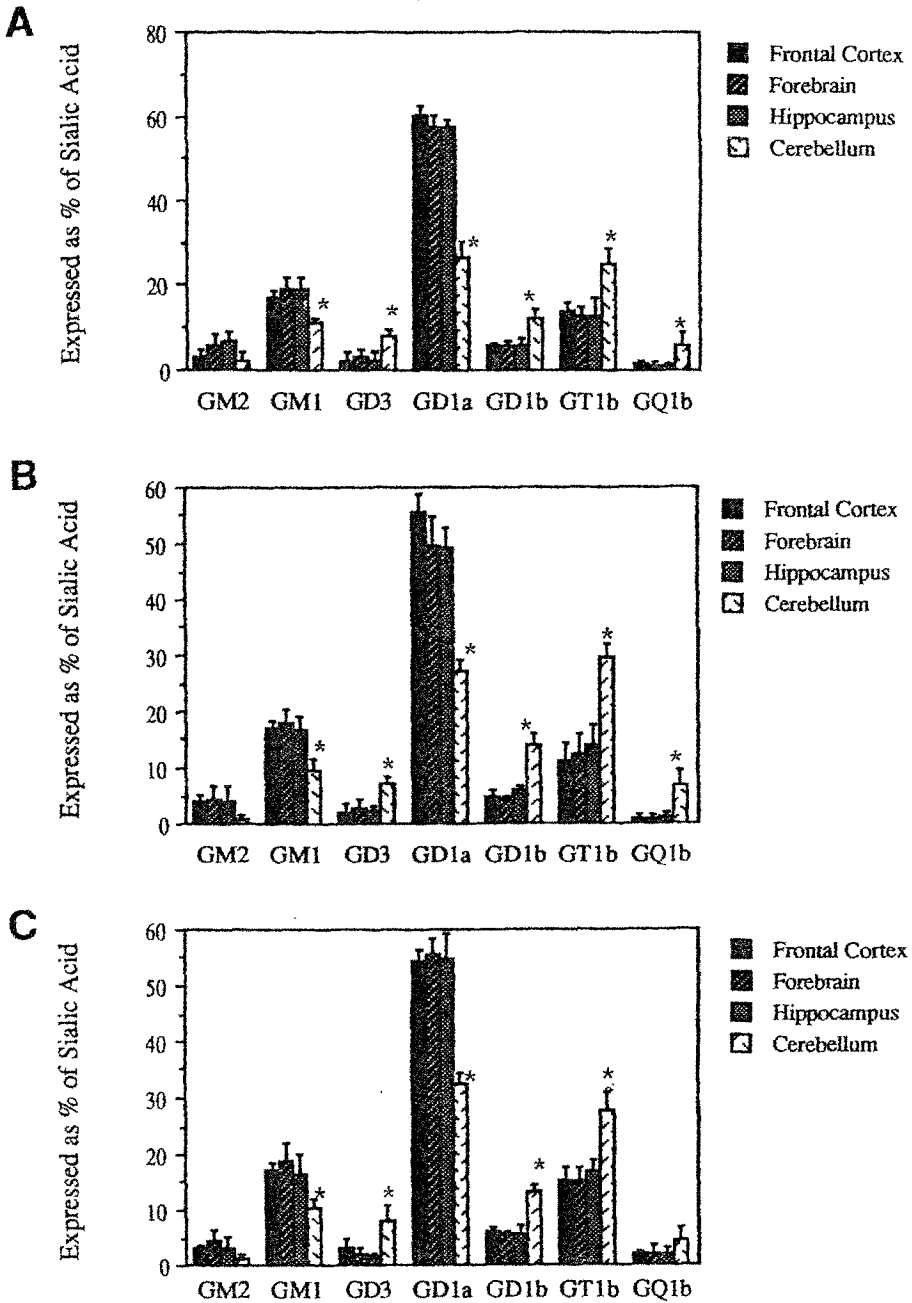


Fig. 2. Percentage distribution of individual gangliosides in different brain regions (■, frontal cortex; ▨, forebrain; ▩, hippocampus; ▪, cerebellum) was examined by TLC resorcinol staining and densitometry, expressed as percentages of total sialic acid. A) group A; B) group B; C) group C. Values indicated by the bars and vertical lines are means \pm SEM (for number per group, see Table 1). * $p < 0.01$ vs corresponding cerebral hemisphere (frontal cortex, forebrain, and hippocampus).

regions contain the same relative amounts of individual gangliosides, but the ganglioside patterns in cerebellum differ from those in the cerebral hemispheres. The former has less GM2, GM1, and GD1a, and more GD3, GD1b, GT1b, and GQ1b, GD1a and GM1 are the major gangliosides in cerebral hemispheres of both prenatal and postnatal infants, consisting of 50–60 and 16–19% of total gangliosides, respectively. In different groups, the proportions of the individual ganglioside are altered: GD1a is lower in the cerebral hemispheres of group B and in the frontal cortex of group C; GD1b is lower in the frontal cortices and forebrains of group B than in those of groups A and C ($p < 0.01$ and 0.05 , respectively). In addition, GD1a in cerebella of group C is significantly higher than in those of group A and B ($p < 0.01$).

Histopathologically, there are ischemic and homogeneous cell changes of pyramidal cells in neocortex, in h1 and h3 segments of the hippocampus, and in the cerebellar Purkinje cells within the three groups. In the frontal lobe and cerebellar cortex there are reactive proliferations of astrocytes. In this study we made no attempt to quantitate the degrees of hypoxia using morphological techniques.

DISCUSSION

Cerebral hypoxia is a common clinical problem in the perinatal period. With acute hypoxia there is a series of biochemical changes, such as a five- to 10-fold increase of glycolysis, lactic acid elevation in brain tissue, and decreases of creatine phosphate and acidosis. Recently, decreases of noradrenaline, 5-hydroxytryptamine, and acetylcholine, and increases of cAMP and cGMP, were found (Yang, 1987). However, changes in the ganglioside composition as a consequence of hypoxia and ischemia in premature and neonatal brains have not been reported previously.

Gangliosides are enriched in neuronal membranes and considered as markers for the development of nerve endings and dendritic arborizations (Dimpfel et al. 1981; Tettamanti et al., 1980). Seglar-Stahl et al. (1983) found that the amount of gangliosides gradually decreased in adult brain during the aging process. Therefore, they proposed that changes in the gangliosides corresponded with disappearance of the neurons and synapse, and degeneration of myelin sheaths. In this study we found that the concentration of total gangliosides on a wet-weight basis decreased significantly in cerebral hemispheres of group B and in four brain regions of group C, as compared with group A, possibly because of severe hypoxia suffered by these patients. Therefore, it is possible that once acute cerebral hypoxia occurs, the gangliosides in the neuronal and synaptic membranes are involved in the pathological changes that occur in cortical neurons. Perhaps they disappeared as a result of disintegration of neuronal structures. On the other hand, biosynthesis of gangliosides is a process that requires energy and is cata-

lyzed by multiple enzyme complexes (Ledeen, 1983). Perhaps synthesis of gangliosides is reduced because of decreased energy production.

It has been reported that neuronal tolerance to hypoxia is different among various brain regions. There is also a relation between cellular maturation and sensitivity to hypoxia (Huang, 1965). Our finding that the decrease in ganglioside concentration is greater in the cerebral hemispheres of groups B and C than in the cerebella indicates that the effect of hypoxia on ganglioside concentration is different in different brain regions. Furthermore, the longer the average hypoxic interval, the lower are the levels of gangliosides. We suggest that the decrease in ganglioside concentration might correlate to the severity of neuronal degeneration and cellular necrosis. Our results demonstrate that the amounts of four gangliosides in cerebral cortices of groups B and C are generally less than those of group A. Hence, the changes in gangliosides resulting from hypoxia are nonspecific.

Suzuki (1965) pointed out that GM1 and GD1a are relatively predominant in brain tissue during the first decade of life. Gangliosides gradually reach the adult pattern during the third decade. In the aged cerebrum, GM1 and GD1a progressively decrease, but the proportions of GD1b, GT1b, and GQ1b increase (Seglar-Stahl et al., 1983). We also observed that the percentage of GM1 and GD1a in the cerebrum of postnatal and premature infants is higher than in adult brain, but GD1b and GT1b is rather low. GD1a is a marker of dendrites and synaptosomes, and thus may participate in connectivity and interactions between nerve cells (Yusuf and Dickerson, 1978). GM1 is enriched in myelin and neuronal membranes (Ledeen et al., 1980). Possibly in fetal and newborn brain there exists a period of rapid development for neurons, synaptic formation, and myelination that requires much more GM1 and GD1a, so their synthesis is relatively increased.

Previous authors have found that patterns of gangliosides differ among anatomical regions of the brain. For example, GD1a is predominant in cerebrum, but in cerebellum and brain stem there is more GT1 and GQ (Suzuki, 1965). The results that we obtained are consistent with the above literature. Cerebellum contains less GM2, GM1, and GD1a, and more GD3, GD1b, GT1b, and GQ1b, than cerebral structures. However, all three areas of the cerebrum have the same percentage of the individual gangliosides. This regional difference seems to result from physiological factors and not pathological events. The reason is yet unclear, but it is presumed that the brain regions that contain the same patterns of gangliosides probably play similar biological roles or have a close relationship in structure and function.

In this study, we have examined the effects of hypoxia on the patterns of gangliosides. The results show that the percentage of GD1a decreased in the cerebral hemispheres of group B and frontal cortices of group C; GD1b also decreased in frontal cortices and forebrains of group B. This phenomenon has not yet been well explained. As we know,

GD1a is one of the main components of glycosphingolipids in brains of children, composing approx. 50–60% of gangliosides (Svennerholm, 1963). Perhaps alteration of the amount of this ganglioside is more severe during hypoxia. On the other hand, its reduction might directly affect neuronal and axonal development, cellular contact, and recognition and formation of neuronal networks. In recent years, it has been reported that glutamate-related *N*-methyl-D-aspartate (NMDA) receptors correlate to the formation of long-term memory. Impairment of hippocampal neurons induced by glutamate could be prevented by ganglioside, so GD1b might be involved in the mechanism of learning and memory in hippocampal areas (Szekely et al., 1987). Therefore, it should be considered a possibility that reduction of gangliosides in the brain, especially GD1b, might affect the development of intelligence in children.

Among cerebral-palsy patients less than one year old, 25–50% had hypoxic or ischemic events in the perinatal period (Pu, 1981). We suggest that decreases of ganglioside levels in these brains might be one of the important biochemical consequences of those events causing subsequent impairment of the central nervous system.

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