Mouse model of G\textsubscript{M2} activator deficiency manifests cerebellar pathology and motor impairment

(\text{animal model}/G\textsubscript{M2} gangliosidosis/\text{gene targeting/lysosomal storage disease})

\textbf{YUJING LIU\textsuperscript{*}, ALEXANDER HOFFMANN\textsuperscript{†}, ALEXANDER GRINBERG\textsuperscript{‡}, HEINER WESTPHAL\textsuperscript{‡}, MICHAEL P. McDONALD\textsuperscript{§}, KATHERINE M. MILLER\textsuperscript{§}, JACQUELINE N. CRAWLEY\textsuperscript{§}, KONRAD SANDHOFF\textsuperscript{†}, KINUKO SUZUKI\textsuperscript{¶}, AND RICHARD L. PROA\textsuperscript{*}}

\textsuperscript{*}Section on Biochemical Genetics, Genetics and Biochemistry Branch, National Institute of Diabetes and Digestive and Kidney Diseases; \textsuperscript{†}Laboratory of Mammalian Genes and Development, National Institute of Child Health and Development; and \textsuperscript{‡}Section on Behavioral Neuropharmacology, Experimental Therapeutics Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892; \textsuperscript{§}Institut für Organische Chemie und Biochemie der Universität Bonn, Gerhard-Domagk-Straße 1, 53121 Bonn, Germany; and \textsuperscript{¶}Department of Pathology and Laboratory Medicine, and Neuroscience Center, University of North Carolina, Chapel Hill, NC 27599

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\textbf{ABSTRACT} The G\textsubscript{M2} activator deficiency (also known as the AB variant), Tay–Sachs disease, and Sandhoff disease are the major forms of the G\textsubscript{M2} gangliosidoses, disorders caused by defective degradation of G\textsubscript{M2} ganglioside. Tay–Sachs and Sandhoff diseases are caused by mutations in the genes (\textit{HEXA} and \textit{HEXB}) encoding the subunits of \textit{β}-hexosaminidase A. The G\textsubscript{M2} activator deficiency is caused by mutations in the G\textsubscript{M2A} gene encoding the G\textsubscript{M2} activator protein. For degradation of G\textsubscript{M2} ganglioside by \textit{β}-hexosaminidase A, the G\textsubscript{M2} activator protein must participate by forming a soluble complex with the ganglioside. In each of the disorders, G\textsubscript{M2} ganglioside and related lipids accumulate to pathologic levels in neuronal lysosomes, resulting in clinically similar disorders with an onset in the first year of life, progressive neurodegeneration, and death by early childhood. We previously have described mouse models of Tay–Sachs (\textit{Hexa} \textsuperscript{−}/\textsuperscript{−}) and Sandhoff (\textit{Hexb} \textsuperscript{−}/\textsuperscript{−}) diseases with vastly different clinical phenotypes. The \textit{Hexa} \textsuperscript{−}/\textsuperscript{−} mice were asymptomatic whereas the \textit{Hexb} \textsuperscript{−}/\textsuperscript{−} mice were severely affected. Through gene disruption in embryonic stem cells we now have established a mouse model of the G\textsubscript{M2} activator deficiency that manifests an intermediate phenotype. The \textit{Gm2a} \textsuperscript{−}/\textsuperscript{−} mice demonstrated neuronal storage but only in restricted regions of the brain (piriform, entorhinal cortex, amygdala, and hypothalamic nuclei) reminiscent of the asymptomatic Tay–Sachs model mice. However, unlike the Tay–Sachs mice, the \textit{Gm2a} \textsuperscript{−}/\textsuperscript{−} mice displayed significant storage in the cerebellum and defects in balance and coordination. The abnormal ganglioside storage in the \textit{Gm2a} \textsuperscript{−}/\textsuperscript{−} mice consisted of G\textsubscript{M2} with a low amount of G\textsubscript{M2}. The results demonstrate that the activator protein is required for G\textsubscript{M2} degradation and also may indicate a role for the G\textsubscript{M2} activator in G\textsubscript{M2} degradation.

The G\textsubscript{M2} gangliosidoses are severe neurodegenerative disorders caused by excessive accumulation of G\textsubscript{M2} ganglioside. Three genetic forms exist: Tay–Sachs disease, Sandhoff disease, and the G\textsubscript{M2} activator deficiency (also known as the AB variant) (reviewed in ref. 1). Tay–Sachs disease is caused by mutations in the \textit{HEXA} gene, Sandhoff disease by mutations in the \textit{HEXB} gene, and the G\textsubscript{M2} activator deficiency by mutations in the \textit{Gm2a} gene. The \textit{HEXA} and \textit{HEXB} genes encode the subunits of \textit{α}- and \textit{β}-subunits, respectively, of the lysosomal enzyme, \textit{β}-hexosaminidase. The subunits dimerize to form the three \textit{β}-hexosaminidase isozymes, A (\textit{β}A), B (\textit{β}B), and S (\textit{α}A). The \textit{Gm2a} gene encodes the G\textsubscript{M2} activator protein. In each disorder, the respective genetic lesion results in impairment of the degradation of G\textsubscript{M2} ganglioside and related substrates.

In humans, in \textit{vivo} G\textsubscript{M2} ganglioside degradation requires the G\textsubscript{M2} activator protein to form a complex with G\textsubscript{M2} ganglioside. \textit{β}-Hexosaminidase A then is able to interact with the activator–ganglioside complex and remove the terminal N-acetylgalactosamine residue from the oligosaccharide portion of the ganglioside. In both Tay–Sachs and Sandhoff diseases, ganglioside degradation is impaired due to an absence of \textit{β}-hexosaminidase A. The homodimeric \textit{β}-hexosaminidase isozymes, S and B, cannot effectively substitute for \textit{β}-hexosaminidase A in ganglioside degradation. It is believed that the function of the activator protein is to transform the hydrophobic, membrane-bound ganglioside into a substrate complex that is accessible to the watersoluble enzyme (2). Therefore, in the G\textsubscript{M2} activator deficiency, the ganglioside is refractory to enzymatic hydrolysis by \textit{β}-hexosaminidase A. In each disease, a massive accumulation of G\textsubscript{M2} ganglioside and related lipids occurs in neuronal lysosomes, resulting in severe cellular malfunction and damage. The clinical phenotype of each disease is nearly identical. In the most severe forms, the onset of the disease occurs during the first year of life. Rapidly progressing neurodegeneration culminates in demise usually by the age of 4. Disorders of lesser severity and later-onset result when mutations lead to a partial deficiency in ganglioside degradation capacity.

Through targeted gene disruption of the \textit{Hexa} and \textit{Hexb} genes in mouse embryonic stem (ES) cells, we previously have described the creation of mouse models corresponding to Tay–Sachs and Sandhoff diseases (3–5). Unlike the human diseases, the two mouse models were of dramatically different phenotypes as a result of differences in the ganglioside degradation pathway between mice and humans. The Tay–Sachs model developed ganglioside accumulation in restricted regions of the brain but was asymptomatic. The Sandhoff disease model exhibited much more extensive ganglioside accumulation and developed a severe neurologic phenotype.

We now have established mice with a disrupted \textit{Gm2a} gene as a model for the third form of the G\textsubscript{M2} gangliosidoses. Similar to the \textit{Hexa} \textsuperscript{−}/\textsuperscript{−} mice, the \textit{Gm2a} \textsuperscript{−}/\textsuperscript{−} mice demonstrated storage in restricted regions of the brain. However, these mice also displayed some significant differences. They exhibited storage in the cerebellum, a site of minimal pathology in the \textit{Hexa} \textsuperscript{−}/\textsuperscript{−} mice (4) and developed defects in balance and motor coordination. This mouse model of the G\textsubscript{M2} activator deficiency is, thus, of an intermediate phenotype when compared with the severely af-

\textbf{Abbreviations:} ES, embryonic stem; G\textsubscript{M1} ganglioside, Gal\textsubscript{1}β→3Gal\textsubscript{1}Nac\textsubscript{1}β1→4(NeuAc\textsubscript{2}→3)Gal\textsubscript{1}β→4Glc\textsubscript{1}β1→1Cer; G\textsubscript{M3} ganglioside, Gal\textsubscript{1}Nac\textsubscript{1}β1→4(NeuAc\textsubscript{2}→3)Gal\textsubscript{1}β→4Glc\textsubscript{1}β1→1Cer; G\textsubscript{M2} glycolipid, Gal\textsubscript{1}Nac\textsubscript{1}β1→4Gal\textsubscript{1}β→4Glc\textsubscript{1}β1→1Cer; G\textsubscript{M2} ganglioside, NeuAc\textsubscript{−}2→3Gal\textsubscript{1}β1→4Glc\textsubscript{1}β1→1Cer; Cer, ceramide.

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fected Sandhoff model and the asymptomatic Tay–Sachs model. The basis for the distinct phenotypes of the human and mouse \( \text{GM}_2 \) gangliosidoses are species-specific differences in the ganglioside degradation pathway.

**MATERIALS AND METHODS**

**Targeting Vector Construction.** We previously have constructed a plasmid containing both the neomycin-resistance (\( \text{neo} \)) gene and the thymidine kinase gene in the pBluescript KS vector (3). Genomic clones from a 129/SV strain library (Stratagene; catalogue no. 946306) containing the mouse \( \text{Gm}2a \) gene were isolated and characterized. A 4.5-kb genomic fragment containing part of intron 2 was inserted between the \( \text{SalI} \) and \( \text{NotI} \) sites downstream of the \( \text{Neo} \) gene. Next, a 4.5-kb genomic fragment containing part of the exon 4 and downstream region was cloned into the \( \text{XhoI} \) site between the thymidine kinase and \( \text{Neo} \) genes. The organization of the targeting vector is illustrated in Fig. 1A. The design of the targeting vector would result in a 1-kb deletion that includes the entire exon 3, entire intron 3, and part of exon 4.

**Selection of Targeted ES Cells and Generation of Mutant Mice.** The targeting vector (50 \( \mu \)g) was linearized with \( \text{SspI} \) endonuclease and introduced into the J1 line (6) of ES cells (1.6 \( \times \) 10\(^7\) cells) by electroporation (400 V and 25 \( \mu \)F) in a Bio-Rad Gene Pulser. Targeted clones were isolated as described (3). Targeted ES cells lines Act-4 and Act-25 were injected into the blastocysts of 3.5-day C57BL/6J embryos. Highly chimeric male mice were obtained and bred to C57BL/6 females. The agouti offspring were tested for transmission of the disrupted allele by Southern blot analysis. Heterozygous matings were set up to generate homozygous mutant mice.

**Biochemical Analysis.** \( \beta \)-Hexosaminidase assays were performed as described (3). Methodology for the analysis of brain lipids from gray and white matter has been described (5).

**Pathology.** Six \( \text{Gm}2a^-/- \) and two \( \text{Gm}2a^+/- \) mice, ages ranging from 2 to 7 months, were studied. Mice were anesthetized and perfused with phosphate-buffered 4% paraformaldehyde (pH 7.4). The brain, spinal cord, and pieces of visceral organs from two of the \( \text{Gm}2a^-/- \) mice were processed for paraffin sections, cut and stained with hematoxylin-eosin, solochrome-cosin, Luxol-fast blue-periodic acid-Schiff, and Bielschowsky stains. In other mice the cerebrum, cerebellum, brainstem, and spinal cord were cryoprotected by immersion in 0.1 M phosphate buffer (pH 7.4) containing 30% sucrose, snap-frozen in a Hist Bath (Shandon, Pittsburgh), and serially sectioned at 7 \( \mu \)m thick with a Frigocut 2800 (Leica, Germany). Every fourth section was stained with periodic acid-Schiff and counterstained lightly with hematoxylin.

**Behavioral Methods.** Subjects were 24 mice (six wild type, six \( \text{Gm}2a^+/- \), and 12 \( \text{Gm}2a^-/- \)). All procedures were approved by the National Institute of Mental Health Animal Care and Use Committee and followed the National Institutes of Health guidelines, “Using Animals in Intramural Research.” Mice were 13 weeks old at the start of testing. Body weights were measured biweekly during the 20-week testing period. There were no significant differences in body weight across treatment groups. Male mice weighed significantly more than females across all groups (\( F_{1,18} = 32.59, P < 0.0001 \)). Repeated-measures analysis showed that there was a significant increase in weight over the 20-week testing period across all groups combined (\( F_{5,162} = 83.61, P < 0.0001 \)), but no difference in weight gain among groups.

**Rotord.** The ability to maintain balance on a rotating cylinder was measured with a standard rotord apparatus (model 7650, Ugo Basile, Varese, Italy). The cylinder was 3.2 cm in diameter and covered with textured rubber. Mice were confined to a section of the cylinder 6.0 cm long by gray Plexiglas dividers. Each mouse was placed on the cylinder, which increased rotation speed over a 5-min period from 5 to 40 revolutions per min. Rotational speed at which the mouse fell off the rotating cylinder was measured. Mice that fell in less than 15 s were given a second trial. Mice that did not fall during the 300-s trial period were removed and given a score of 40 rpm. Rotord testing was conducted once every 2 weeks for 20 weeks.

**Open field behavior.** Exploratory locomotor activity was measured in an open field as described (3, 5). Locomotor activity was measured once every 2 weeks for 20 weeks.

**Passive avoidance.** Passive avoidance learning was tested in a mouse shuttle box as described (3, 5).

**Shock threshold.** Testing was performed as described (7).

**Tail-flick nociception.** Each mouse was placed within a restraining tube on a tail-flick monitor (Omnitech Electronics, Columbus, Ohio). The mouse’s tail protruded from a hole in the restraining tube and was allowed to move freely. The mouse’s tail was placed in a tail guide, which contained a photobeam for precise measurement of tail-flick latencies. A coil, offset in the floor of the monitor, was used to apply a heat stimulus to the mouse’s tail. The temperature of the heating coil rose 15.0°C from room temperature in 15 s. The time it took for the mouse to flick its tail away from the heat was measured automatically by connecting the photobeam upon tail flick.

**Hot-plate nociception.** Each mouse was placed on a hot plate maintained at 55°C (Nuova II; Thermolyne, Dubuque, Iowa). A plastic cylinder 15 cm in diameter and 12.5 cm high confined the mouse to the surface of the hot plate. The time it took the mouse to jump or lick its paw was measured with a stop watch. Mice were then immediately removed from the hot plate. Mice that did not jump or lick a paw within 10 s were removed from the hot plate and given a score of 10 s.
Startle response/prepulse inhibition. Startle response and pre-pulse inhibition were measured using two SR-Lab startle chambers (San Diego Instruments, San Diego, CA). Each chamber contained a Plexiglas cylinder 5.1 cm in diameter, resting on a Plexiglas frame (20.4 cm length × 12.7 cm width × 0.4 cm thick) within a ventilated enclosure. The startle session was immediately preceded by a 5-min acclimation period in the startle chambers. The mice were then tested with a series of white noise bursts in the chamber, presented through a loudspeaker mounted 28 cm above the animal. Each startle session consisted of 42 trials over a 10.5-min period. There were seven trial types: six trials on which no stimulus was presented, six trials on which only the startle stimulus was presented, and 30 trials on which the startle stimulus was preceded by one of five “prepulse” stimuli, by 100 ms. The startle stimulus was a 40-ms, 120-dB burst of white noise. The prepulse stimuli were 20-ms bursts of 74, 78, 82, 86, or 90-dB white noise; each presented on six trials. The trials on which no stimulus was presented were used to measure baseline movement in the cylinders. The seven trial types were presented in pseudorandom order such that each trial type was presented once within a block of seven trials. The intertrial interval ranged from 10–20 s with an order such that each trial type was presented once within a block.

Statistical analysis. Because there were no differences on any measure between wild types and heterozygotes, these two groups were combined into a single control group for statistical analysis. Two-group comparisons were made using unpaired Student's t tests. Analyses of performance over time were made using two-factor repeated-measures ANOVA.

RESULTS

Targeted Disruption of the Mouse Gm2a Gene. Using a mouse Gm2a cDNA (8) as probe, we isolated genomic DNA fragments covering the entire mouse Gm2a gene from a 129/sv strain library. Like the human GM2A gene (9), the mouse Gm2a gene has four exons (Fig. 1A). The exon-intron borders are completely conserved between human and mouse (Y.L. and R.L.P., unpublished results).

To disrupt the Gm2a gene in mouse ES cells, a replacement-type of targeting vector was created with these genomic segments. A 1-kb region covering exon 3 and the entire coding region of exon 4 was deleted and replaced with the MC1NeopolyA cassette. The targeting vector had a total of 9 kb of homologous genomic sequence with 4.5 kb of sequence flanking each side of the Neo cassette. Additionally, the thymidine kinase gene was positioned outside the homologous sequences to enable selection against nonhomologous integrants.

The targeting vector was linearized and electroporated into the J1 ES cell line. The ganciclovir counter selection resulted in a 7-fold enrichment of homologous recombinants. Genomic DNA from G418- and ganciclovir-resistant clones was analyzed by Southern analysis. Of the 113 colonies examined, DNA from 13 contained the 6.5-kb EcoRI band diagnostic of a homologous recombination event (Fig. 1B). Cells from two of the targeted ES clones were injected into C57BL/6 mouse blastocysts, and one of the clones produced highly chimeric male mice that transmitted the targeted allele through the germ line. The heterozygotes were intercrossed to obtain mice homozygous for the disrupted gene (Fig. 1B).

The Gm2a Homozygous Mutant Mouse Has a Total Gm2 Activator Deficiency with Normal Hexosaminidase Activity. The targeting event resulted in a deletion of the C-terminal 112 amino acids of the 162 total amino acids contained within the mature Gm2 activator protein. The deletion was evidenced by genomic Southern blot analysis using a cDNA probe composed of the coding sequence presumed removed during homologous recombination. With this probe, no hybridization signal was detected from the homozygous mutant mice (data not shown). The total RNA of tests and kidney from wild-type and Gm2a −/− mice was examined by Northern blot analysis (Fig. 1C). A ~2.3-kb mRNA was detected in wild-type mice tissues. No Gm2a-related transcript was present in the Gm2a −/− mice tissues, demonstrating that the targeting event resulted in a null allele.

Like humans with GM2 activator deficiency, the Gm2a −/− mice had normal levels of β-hexosaminidase activity. Liver extracts from wild-type, Hexa −/−, Hexb −/−, and Gm2a −/− were chromatographed on a Mono Q column under conditions that separate β-hexosaminidase A and B (3). Different from the Hexa −/− and Hexb −/− mice that lacked one or both of the two isozymes, the Gm2a −/− mice had normal levels of β-hexosaminidase isozyme activity (data not shown).

Glycolipid Accumulation in Gm2a −/− Mice. Sphingolipids were isolated from gray matter and white matter of brains from wild-type, Hexa −/−, Hexb −/−, and Gm2a −/− mice at 4 months of age. The glycolipid fraction was then separated by high-performance thin-layer chromatography, and the Gm2a and Gm2A genes were quantified (Fig. 2). The Gm2a −/− mice accumulated GM2 ganglioside in their brains at a level comparable to the Hexa −/− mice but less than the Hexb −/− mice. As previously described (5) the Hexb −/− mice also accumulated a large amount of Gm2a. The Gm2a −/− mice did not accumulate large amounts of Gm2. However, a closely migrating band made it difficult to determine if a small amount of Gm2A was present in the Gm2a −/− mice by this analysis.

FIG. 2. Glycolipid accumulation in the brains of 4-month-old Hexa −/−, Hexb −/−, and Gm2a −/− mice. (A) The sphingolipid fraction of brain tissues was separated by thin-layer chromatography. Gm2A and Gm2A standards are indicated. g, gray matter; w, white matter. (B) The sphingolipid fraction of gray and white matter was quantified, respectively (μmol/g wet weight).
Therefore the gray and white matter glycolipids were separated in a two-dimensional performance-high-performance thin-layer chromatography system (first solvent: CHCl₃/MEOH/7 M NH₄OH (65:25.4 vol/vol), second solvent system: CHCl₃/MEOH/H₂O, 0.22% CaCl₂ (60:35:8 vol/vol)). In this analysis, a heavy GA₂ accumulation was found in Hexb −/− mice, no GA₂ was detected in Hexa −/− mice and, most importantly, a slight accumulation was identified in the Gm2a −/− mice.

The ganglioside degradation pathway of the Gm2a −/− mice was analyzed by administration of Gm2a ganglioside radiolabeled in GA₂ (5, 10). As described previously, Hexa −/− fibroblasts degraded GM₁ to ceramide, demonstrating that the catabolic pathway in these mice was not completely blocked. However, the Gm2a −/− fibroblasts accumulated a higher quantity of GA₂ than that found in the Hexa −/− fibroblasts.

**Gm2a −/− Mice Show Restricted Neuronal Storage.** Gm2a −/− mice aged 2 to 7 months were examined. Although the central nervous system pathology progressively worsened with age, the overall distribution of the pathology was the same regardless of the age of the animal. Unlike noted otherwise, the description here is given for 5-month-old mice. Examination was carried out using periodic acid-Schiff staining on frozen sections, which results in the ganglioside storage appearing red. As previously described, the Hexa −/− mice showed a pattern of storage restricted to certain regions of the brain whereas the Hexb −/− mice exhibited storage throughout the brain. In the cerebral cortex, the pattern of neuronal storage in Gm2a −/− mice was very similar to that of the Hexa −/− mice (4). The storage was predominately in large pyramidal neurons in the middle layer (Fig. 4 A and B). In contrast, almost all neurons in the cerebral cortex from the Hexb −/− mice contained storage material (Fig. 4 C). Also like the Hexa −/− mice (4), storage in the Gm2a −/− mice was most pronounced in certain areas such as in the piriform, entorhinal cortex, and amygdala as well as in the hypothalamic nuclei (data not shown). In both the Gm2a −/− and Hexa −/− mice little storage was observed in spinal cord and visceral organs (refs. 4 and 5; data not shown).

The major difference between the neuropathology of the Gm2a −/− and Hexa −/− mice was the presence of storage cells in the cerebellum in the former. In Gm2a −/− mice, prominent storage material was present in the glial cells in the molecular and granular cell layers, and Purkinje and granular cell neurons (Fig. 4 D and E). A similar distribution of cerebellar storage also was observed in the youngest Gm2a −/− mice examined (2 months old). Very little storage was observed in comparable areas of the Hexa −/− cerebellum at any age. In the Hexb −/− mice, storage was abundant in the Purkinje cells as well as in the granular cells and molecular layer of the cerebellum (Fig. 4 F).

**Gm2a −/− Mice Are Viable but Have Neurologic Disturbances.** Heterozygous matings yield Gm2a −/− progeny in a Mendelian fashion, indicating that there was no embryonic lethality. Gm2a −/− mice grow normally and have survived for more than 1 year. Both males and females are fertile. They can be bred to each other and give litters of normal size.

The Gm2a −/− mice were tested to determine their neurologic function. Motor coordination and balance was measured with a standard rotarod apparatus. A total of 24 mice (six wild type, six Gm2a +/+ and 12 Gm2a −/−) were tested beginning at 13 and ending at 31 weeks of age. As shown in Fig. 5 A, overall rotarod performance was significantly impaired in the knockouts, compared with the controls (F₁,₂₂ = 5.18, P = 0.033). Analysis of the groups individually demonstrated that the controls displayed a significant improvement in rotarod performance over the 20-week testing period (F₀,₉₉ = 4.36, P < .0001), while the performance of the knockouts remained stable (F₀,₉₉ = 0.89, P = 0.534). Using open field testing, there was no significant difference between Gm2a −/− and controls in horizontal activity (F₁,₂₂ = 3.99, P = 0.058) or vertical activity (F₁,₂₂ = 0.215, P = 0.647), indicating the poor performance of the Gm2a −/− mice on the rotarod was not due to a generalized lack of mobility.

The passive-avoidance task was used as a test for learning and memory. Fig. 5 B illustrates passive-avoidance performance on the training and testing days for Gm2a −/− and control mice. There was no difference between groups on latency to enter the dark chamber on the training trial (t₂₀ = 0.20, P = 0.657). However, there was a small, but significant, difference in latency to enter the dark chamber 24 hr after a foot shock in that location (t₂₀ = 2.42, P = 0.025), indicating a possible memory deficit in the Gm2a −/− mice. No significant differences were detected among genotypes with the shock threshold test or with the tail-flick and hot-plate tests for nociception, suggesting that the deficit in passive-avoidance learning was not due to shock or pain insensitivity. No significant differences were detected among genotypes on prepulse inhibition, indicating that sensorimotor gating was normal in the Gm2a −/− mice.

**DISCUSSION**

We have established mouse models for the three major forms of the Gm₂ gangliosidoses: Tay–Sachs disease, Sandhoff disease, and, as described here, the Gm₂ activator deficiency (AB variant). Although the three human disorders are very similar in clinical phenotype, each of the mouse models displays a distinct phenotype. The Sandhoff mice (Hexb −/−), lacking β-hexosaminidase A and B, are the most severely affected with an early onset of severe, progressing motor dysfunction and a shortened life span. In contrast, the Tay–Sachs mice (Hexa


2y2y), lacking β-hexosaminidase A, appear to be phenotypically normal. The Gm2a−/− mice, which do not express the activator protein, are of an intermediate phenotype with motor function abnormalities but with a normal life span. The basis for the phenotypic differences between the human and mouse gangliosidoses is in large measure the result of different degradative pathways. Based on the biochemical differences between the Hexa−/− and Hexb−/− mice, we have proposed two independent catabolic pathways for G_{M2} in mice (5). In one, G_{M2} is degraded to G_{M3} primarily by β-hexosaminidase A with the G_{M2} activator protein. This is the major and almost exclusive pathway in humans. In a second pathway specific to the mouse, G_{M2} is degraded by sialidase first to G_{A2} and then by β-hexosaminidase B or A to lactosylceramide (Fig. 6). Consequently, the Hexa−/− mice lacking β-hexosaminidase A accumulate G_{M2}, but not G_{A2}, and are asymptomatic. The Hexb−/− mice lacking both β-hexosaminidase A and B accumulate both G_{M2} and G_{A2} due to blocks in both pathways and show a severe phenotype. In the brain of the Gm2a−/− mice there is accumulation of G_{M2} with a small amount of G_{A2}. The G_{M2} accumulation proves that the activator protein is essential for the degradation of G_{M2} ganglioside via the formation of G_{M3} and

**Fig. 4.** Gm2a−/− and Hexa−/− mice show restricted storage in the brain compared with Hexb−/− mice. All panels are photomicrographs of frozen sections stained by periodic acid-Schiff. B displays cerebral cortex from the Gm2a−/− mouse with storage predominant in large pyramidal neurons in the middle layer (bracketed) similar to the Hexa−/− mouse in A, while nearly all neurons contain storage in the Hexb−/− mouse (C). (×300) (E) The cerebellum of the Gm2a−/− mouse shows storage in some Purkinje cells (arrows), glial cells in the molecular cell layer, and some granular cell neurons (may be also glial cells) in the granular cell layers. (D) In a similar section from a Hexa−/− mouse minimal storage is seen in a few glial cells in the molecular layer but not in Purkinje cells (arrows). (F) The Hexb−/− mouse shows more storage in Purkinje cells (arrows), granular cells, and molecular layer. (×300.)
demonstrates, together with the results from the Hexa --/-- mice, that both the activator protein and β-hexosaminidase A are needed for this reaction in mice as in humans (Fig. 6).

The low level of G₄₃₂ storage in the Gm2a --/-- mice indicates that the hexosaminidase-mediated degradation of G₄₃₂ can proceed in the absence of the activator protein. However, evidence also suggests that the activator protein is likely required for this reaction to proceed at an optimal rate. In the ganglioside feeding experiment Gm2a --/-- fibroblasts contained a higher quantity G₄₃₂ than Hexa --/-- or wild-type fibroblasts. This result is consistent with the G₂₃₂ activator protein increasing the rate of G₄₃₂ degradation by hexosaminidase A and/or B. Indeed, administration of recombinant human GM₂ activator protein to mouse Gm2a --/-- fibroblasts enhances the degradation of radiolabeled G₄₃₂ (A.H. and K. Sandhoff, unpublished results).

The pathologic differences between the Hexa --/-- and Gm2a --/-- mice also indicate that ganglioside catabolism pathway is not equivalent in the two types of mice. For the most part, the regions of the brain affected by storage were very similar in the two knockout mice indicative of a common degradative block at GM₂ to GM₃ (Fig. 6). A major difference between the Gm2a --/-- and the Hexa --/-- mice was the presence of storage in the cerebellar neurons and glial cells of the Gm2a --/-- mice. The impaired motor coordination detected in the Gm2a --/-- mice was consistent with significant storage in this portion of the brain. With minimal storage in this cerebellar region, the Hexa --/-- mice were asymptomatic throughout their normal life span. Cerebellar storage in the Gm2a --/--, but not the Hexa --/--, mice must be due to the inability of the activator-independent degradative pathway to handle the level of ganglioside substrate produced in this region of the brain. This accumulation in the Gm2a --/-- cerebellum could be explained by a lower rate of GM₂ degradation in the absence of activator relative to when activator is present in the Hexa --/-- cerebellum. As illustrated by the late-onset forms of the GM₂ gangliosidoses, very low levels of degrading activity can drastically alter the degree of ganglioside storage (11, 12).

The three gangliosidoses mice provide models for the human disorders, allowing evaluation of potential therapies including protein and gene replacement, stem cell transplantation, and substrate degradative strategies (13). In addition, the degradative pathways in the mildly affected mice suggest possible therapies through manipulation of the human degradative pathway.

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