Tay-Sachs disease, an inborn lysosomal disease featuring a buildup of G\textsubscript{M2} in the brain, is caused by a deficiency of \(\beta\)-hexosaminidase A (Hex A) or G\textsubscript{M2} activator. Of the two human lysosomal Hex isoforms, only Hex A, not Hex B, cleaves G\textsubscript{M2} in the presence of G\textsubscript{M2} activator. In contrast, mouse Hex B has been reported to be more active than Hex A in cleaving G\textsubscript{M2} (Burg, J., Banerjee, A., Conzelmann, E., and Sandhoff, K. (1983) Hoppe Seyler’s Z. Physiol. Chem. 364, 821–829). In two independent studies, mice with the targeted disruption of the \textit{Hex} gene did not display the severe buildup of brain G\textsubscript{M2} or the concomitant abnormal behavioral manifestations seen in human Tay-Sachs patients. The results of these two studies were suggested to be attributed to the reported G\textsubscript{M2} degrading activity of mouse Hex B. To clarify the specificity of mouse Hex A and Hex B and to better understand the observed results of the mouse model of Tay-Sachs disease, we have purified mouse liver Hex A and Hex B and also prepared the recombinant mouse G\textsubscript{M2} activator. Contrary to the findings of Burg et al., we found that the specificities of mouse Hex A and Hex B toward the catabolism of G\textsubscript{M2} were not different from the corresponding human Hex isoforms. Mouse Hex A, but not Hex B, hydrolyzes G\textsubscript{M2} in the presence of G\textsubscript{M2} activator, whereas G\textsubscript{M2} is refractory to mouse Hex B with or without G\textsubscript{M2} activator. Importantly, we found that, in contrast to human G\textsubscript{M2} activator, mouse G\textsubscript{M2} activator could effectively stimulate the hydrolysis of G\textsubscript{M2} by mouse Hex A and to a much lesser extent also by Hex B. These results provide clear evidence on the existence of an alternative pathway for G\textsubscript{M2} catabolism in mice by converting G\textsubscript{M2} to G\textsubscript{A2} and subsequently to lactosylceramide. They also provide the explanation for the lack of excessive G\textsubscript{M2} accumulation in the \textit{Hexa} gene-disrupted mice.

Human tissues contain two major isoforms of lysosomal \(\beta\)-hexosaminidase (Hex).\(^1\) Hex A, a heterodimeric protein composed of \(\alpha\)- and \(\beta\)-subunits, and Hex B, a \(\beta\)-subunit homodimer (1, 2). These two isoforms have also been reported to exist in other mammals (3). Human Hex A hydrolyzes the GalNAc from G\textsubscript{M2} in the presence of a specific protein cofactor, G\textsubscript{M2} activator (4–6). Human Hex B, on the other hand, is not able to hydrolyze G\textsubscript{M2} with or without G\textsubscript{M2} activator (7–10). A deficiency of Hex A or G\textsubscript{M2} activator causes Tay-Sachs disease in humans, a lysosomal storage disease characterized by an excessive buildup of G\textsubscript{M2} in the central nervous system (11). Burg et al. (3) reported that, in sharp contrast to human Hex isoforms, the partially purified Hex B prepared from several different mammalian tissues were able to degrade G\textsubscript{M2} and that rat Hex B degraded G\textsubscript{M2} more effectively than the Hex A. They also reported that the mouse activator preparation made from heat-treated mouse kidney extract was only slightly effective in stimulating the hydrolysis of G\textsubscript{M2} by mouse Hex A and inhibited mouse Hex B in the same reaction. Recently, in two independent studies, mice with the targeted disruption of the \textit{Hexa} gene were found to display neither the severe buildup of brain G\textsubscript{M2} nor the concomitant abnormal behavioral manifestations seen in human classical Tay-Sachs patients (12, 13). In both studies, the mild manifestations were attributed to the reported G\textsubscript{M2} degrading activity of mouse Hex B (3). Based on the fate of the radioactive G\textsubscript{M2} fed to embryonic fibroblasts derived from \textit{Hexa}−/− and \textit{Hexb}−/− mice, Sango et al. (14) proposed the presence of an alternative pathway in mice where sialidase acts upon G\textsubscript{M2} to produce G\textsubscript{A2} which can be hydrolyzed subsequently by Hex A or Hex B.

To clarify the role of the mouse Hex A and Hex B in the catabolism of G\textsubscript{M2} and also to understand better the observed results of the mouse models of classical Tay-Sachs disease (Type B G\textsubscript{M2} gangliosidoses), we have purified mouse liver Hex A and Hex B. We have also prepared the recombinant mouse G\textsubscript{M2} activator. Using the recombinant human and mouse G\textsubscript{M2} activators, we have studied the requirement of these two protein cofactors in the hydrolysis of G\textsubscript{M2} and G\textsubscript{A2} by mouse Hex A and inhibited mouse Hex B in the same reaction.

**EXPERIMENTAL PROCEDURES**

**Materials—** G\textsubscript{M2} was isolated from the brain of a Tay-Sachs patient (15). G\textsubscript{A2} was prepared from G\textsubscript{M2} by mild acid hydrolysis (16).

\[\text{Glc}_{\text{M2}}=\text{HexNAc}_{\text{M2}}=3\text{Gal}_{\text{M2}}=4\text{NeuAc}_{\text{M2}}\]

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3. The abbreviations used are: Hex, \(\beta\)-hexosaminidase; MU, 4-methylumbelliferyl; MUG, 4-methylumbelliferyl-\(\beta\)-GlcNAc; MUGS, 4-methylumbelliferyl-\(\beta\)-GlcNAc-6-SO\(_4\); \textit{G\textsubscript{M2}}, \textit{Galβ1–3GalNAc–} \(\beta\)-subunit homodimer; \(\beta\)-subunit homodimer
II'NeuAcGgOse₂ was prepared from G_m₂ using ceramide glycanase (17). Goat anti-human Hex A was a kind gift of Dr. Richard L. Prioa, Section of Biochemical Genetics, Genetics and Biochemistry Branch, NIDDK, National Institutes of Health, Bethesda, MD. The following were purchased from commercial sources: frozen mouse livers (SwissWebster strain; Pitman-Moore, Inc.), mouse poly(ADP-ribose) polymerase, Sigma; glyceraldehyde-3-phosphate dehydrogenase, Sigma; polyethylene glycol, Merck; amylase, Sigma; DNAse I, Sigma; MUGS, MUGS-cleaving activity; SDS, SDS-polyacrylamide gel electrophoresis. Purification of Mouse Liver Hex A and Hex B—Mouse liver crude enzyme preparation. Mouse liver crude enzyme preparation obtained after ammonium sulfate precipitation was applied onto a DEAE-Fractogel column (5 × 45 cm). Detailed conditions are described under "Experimental Procedures." Dotted line, absorbance at 280 nm; filled circles, MUGS-cleaving activity; empty circles, MUGS-cleaving activity; dashed line, NaCl gradient.

Molecular Mass Determination—The molecular masses of purified mouse Hex A and Hex B were determined using Superose 6 FPLC gel filtration in 50 mM sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl. The column was first calibrated under the same conditions using ferritin (440,000), catalase (232,000), aldolase (158,000), ovalbumin (45,000), and chymotrypsinogen A (25,000) as molecular weight standards.

Purification of Mouse Liver Hex A and Hex B—All operations were performed at 0–5°C except the chromatographies on Con A-Sepharose and SP-Fractogel that were carried out at room temperature. Centrifugation was routinely carried out at 10,000 × g for 50 min using a Sorvall RC5C ultracentrifuge. Unless otherwise indicated, chromatography on SP-Fractogel was carried out with an Amicon stirred cell using a PM-10 membrane. Two hundred frozen mouse livers (391 g) were homogenized using a Polytron (Brinkmann) homogenizer with 5 volumes of cold phosphate-buffered saline (10 mM sodium phosphate, 138 mM NaCl, 2.7 mM KCl, pH 7.4) containing 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride as protease inhibitors, followed by centrifugation. The supernatant was brought to 30% saturation with solid ammonium sulfate. After 2 h, the precipitate was removed by centrifugation, and the supernatant was further brought to 65% saturation with solid ammonium sulfate. After standing overnight, the precipitate was collected by centrifugation and resuspended in 500 ml of 10 mM sodium phosphate buffer, pH 7.0 (buffer A). The suspension was placed into several dialysis bags and dialyzed against 10 liters of buffer A overnight, changing buffer every 4 h (4 changes). This crude enzyme preparation (780 ml) was centrifuged and applied to a DEAE-Fractogel column (5 × 45 cm) equilibrated with buffer A. The column was washed overnight with buffer A at 2 ml/min, and proteins were eluted with a linear gradient of NaCl from 0 to 0.5 M in the same buffer (total volume 4 liters), and 20-ml fractions were collected. Fractions were assayed for both MUG- and MUGS-cleaving activities. Hex B, which cleaves only MUG, was eluted in the nonadsorbed fractions (Fig. 1) and was concentrated by ultrafiltration. As shown in Fig. 1, MUG-cleaving activity eluted with NaCl as a main peak with a leading shoulder. The shoulder contained very low MUGS-cleaving activity, whereas the main peak contained both MUG- and MUGS-cleaving activities. Fractions in the main peak were pooled and concentrated to make a crude mouse Hex A preparation. This preparation was applied to a Sephacryl S-300 column (5 × 90 cm) equilibrated with 50 mM sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl. The column was eluted with the same buffer at 1 ml/min, and 20-ml fractions were collected. MUG- and MUGS-cleaving activities coeluted as a broad peak, and the entire peak was pooled (Fig. 2A) and concentrated to 25 ml by ultrafiltration. The concentrated Hex A was dialyzed thoroughly against buffer A overnight. The crude Hex B preparation (from DEAE-Fractogel column) was dialyzed against buffer A and applied to an SP-Fractogel column (2.5 × 17 cm) equilibrated with buffer A. The column was washed with buffer A at 2 ml/min and eluted with a linear gradient of NaCl from 0 to 0.5 M in buffer A (total volume, 500 ml) and 17-ml fractions were collected. Fractions were assayed for MUG-cleaving activity. No activity was detected in the nonadsorbed fractions. The Hex B activity eluted as a single peak starting at 0.1 M NaCl was pooled and concentrated to make an SP-Fractogel-purified mouse Hex B preparation (elution pattern not shown). This preparation was applied to a Sephacryl S-300 column and eluted under the same conditions as the DEAE-Fractogel purified Hex A (Fig. 2B).

The purified mouse Hex A and Hex B—Mouse liver crude enzyme preparation obtained after ammonium sulfate precipitation was applied onto a DEAE-Fractogel column (5 × 45 cm). Detailed conditions are described under "Experimental Procedures." Dotted line, absorbance at 280 nm; filled circles, MUGS-cleaving activity; empty circles, MUGS-cleaving activity; dashed line, NaCl gradient.
The two major Hex isozymes were resolved from the crude mouse liver extract by DEAE-Fractogel chromatography at pH 7.0 (Fig. 1). The acidic mouse Hex A was purified to near homogeneity using the scheme described under “Experimental Procedures.”

**Western Blotting**—The purified mouse liver Hex A after SP-Fractogel chromatography was analyzed by 15% SDS-PAGE (23). The gel was electrophoretically transferred onto a nitrocellulose membrane in 20 mM Tris/150 mM glycine buffer, pH 8.0, containing 20% methanol at 18 V for 4 h using a Bio-Rad transfer apparatus. Membranes were overlaid with goat anti-human Hex A (24, 25) as the primary antibody followed by horseradish peroxidase-conjugated rabbit anti-goat IgG as the secondary antibody. For visualization, the membrane was incubated with 8 nmol of 4-chloro-1-naphthol with 0.01% hydrogen peroxide to produce a purple color. The reaction was stopped by washing the membrane with water.

**RESULTS**

**Purification and Characterization of Mouse Liver Hex A and Hex B**—The two major Hex isozymes were resolved from the crude mouse liver extract by DEAE-Fractogel chromatography at pH 7.0 (Fig. 1). The acidic mouse Hex A was purified to near homogeneity using the scheme described under “Experimental Procedures.”

**Expression and Characterization of Mouse G_{M2} Activator**—
The enzymatic assays and purification were carried out as described under “Experimental Procedures.” Steps 1–5 show the preparation of Hex A and steps 1 and 6–9 describe the preparation of Hex B, starting from 200 frozen mouse livers.

**TABLE I**

**Purification of Hex A and Hex B from mouse liver**

The molecular mass of the recombinant mouse GM₂ activator determined by SDS-PAGE was 18.5 kDa, which is as expected from the cDNA sequence and is identical to that of the human GM₂ activator. By Western blot analysis, mouse GM₂ activator was recognized by the polyclonal antibodies against human GM₂ activator, indicating similarities in protein structure, although with a weaker interaction than that for the human GM₂ activator.

**Hydrolysis of GM₂ by Mouse Hex A and Hex B**—The purified mouse Hex A and Hex B were examined for their ability to hydrolyze GM₂. As shown in Fig. 5A, the specificities of the mouse Hex A and Hex B toward GM₂ are the same as their human counterparts. Under the same conditions, mouse Hex A was found to slowly hydrolyze GA₂ in the absence of GM₂ activator (Fig. 5B, lane 8). Hex A was found to slowly hydrolyze GA₂ in the absence of mouse GM₂ activator (Fig. 5B, lane 3). We found that even though GA₂ is refractory to human Hex A in the presence of human GM₂ activator (19), mouse Hex A was able to effectively hydrolyze GA₂, the amount of which increased with incubation time (Fig. 5B, lane 5), and no detectable hydrolysis was observed in the presence of mouse GM₂ activator (Fig. 5B, lane 6) after 30 min of incubation. However, after extended incubation (6 h of incubation), mouse Hex B was found to be able to slowly hydrolyze GA₂ in the presence of mouse GM₂ activator (Fig. 5B, lane 8).

It has been shown previously that the ceramide portion of the glycolipids (3 mmol) were incubated with 20 milliunits of Hex at 37 °C for 30 min or 6 h for the extended incubation. The detailed assay conditions are described under “Experimental Procedures.” The plates were developed with chloroform/methanol/water, 60:35:8 (v/v/v), and stained with di-phenylamine reagent. A: 1, GM₃ standard; 2, GM₂ + mouse GM₂ activator; 3, GM₂ + mouse Hex A; 4, GA₂ + mouse Hex A; 5, GM₂ + mouse Hex B; 6, GM₂ + mouse Hex B + mouse GM₂ activator; 7, GM₂ + mouse Hex B, 6 h of incubation; 8, GA₂ + mouse Hex B + mouse GM₂ activator, 6 h of incubation; 9, LacCer; 2', GA₂ + mouse GM₂ activator; 3', GA₂ + mouse Hex A; 4', GA₂ + mouse Hex A + mouse GM₂ activator; 5', GA₂ + mouse Hex B; 6', GA₂ + mouse Hex B + mouse GM₂ activator; 7', GA₂ + mouse Hex B, 6 h of incubation; 8', GA₂ + mouse Hex B + mouse GM₂ activator, 6 h of incubation.

**FIG. 4.** Analysis of mouse liver Hex A by SDS-PAGE (A) and Western blotting (B). A, purified mouse liver Hex A was analyzed by 15% SDS-PAGE according to the conditions described under “Experimental Procedures.” Protein bands were visualized by Coomassie Brilliant Blue staining: molecular weight standards (lane 1); purified mouse liver Hex A, reduced with 2-mercaptoethanol (lane 2); purified mouse liver Hex A, not reduced (lane 3). B, Western blot analysis of purified mouse liver Hex A after 15% SDS-PAGE: purified mouse liver Hex A, reduced with 2-mercaptoethanol (lane 3); purified mouse liver Hex A, not reduced (lane 3). Detailed conditions are described under “Experimental Procedures.”

**FIG. 5.** Thin layer chromatography showing the hydrolysis of GM₂ (A) and GA₂ (B) by mouse Hex A and Hex B. The glycolipids (3 mmol) were incubated with 20 milliunits of Hex at 37 °C for 30 min or 6 h for the extended incubation. The detailed assay conditions are described under “Experimental Procedures.” The plates were developed with chloroform/methanol/water, 60:35:8 (v/v/v), and stained with di-phenylamine reagent. A: 1, GM₃ standard; 2, GM₂ + mouse GM₂ activator; 3, GM₂ + mouse Hex A; 4, GA₂ + mouse Hex A; 5, GM₂ + mouse Hex B; 6, GM₂ + mouse Hex B + mouse GM₂ activator; 7, GM₂ + mouse Hex B, 6 h of incubation; 8, GA₂ + mouse Hex B + mouse GM₂ activator, 6 h of incubation; 9, LacCer; 2', GA₂ + mouse GM₂ activator; 3', GA₂ + mouse Hex A; 4', GA₂ + mouse Hex A + mouse GM₂ activator; 5', GA₂ + mouse Hex B; 6', GA₂ + mouse Hex B + mouse GM₂ activator; 7', GA₂ + mouse Hex B, 6 h of incubation; 8', GA₂ + mouse Hex B + mouse GM₂ activator, 6 h of incubation.
Catabolism of G_{M2} in Mouse

Discussion

To understand the catabolism of G_{M2} in mouse, we have purified and characterized mouse liver Hex A and Hex B and compared their properties with human Hex A and Hex B. As seen with the recombinantly expressed α- and β-chains (25), the purified mouse liver Hex A was recognized by goat anti-human Hex A. Purified mouse Hex A was determined to be composed of 57- and 59-kDa subunits by SDS-PAGE under nonreducing conditions, and smaller polypeptides were observed in the presence of 2-mercaptoethanol or dithiothreitol (Fig. 4A, lane 2). Therefore, mouse Hex A has a similar subunit composition to human Hex A, with noncovalently linked α- and β-subunits (2). This is also the first direct evidence that one of the subunits is composed of nonidentical cystine-linked polypeptide chains, which, by comparison with the human enzyme, is probably the β-subunit (29).

While the isoelectric points of purified mouse Hex A and Hex B are similar to the isoelectric points of their human counterparts, the presence of mouse Hex A distributed in a wide range of isoelectric points has important consequences for purification. In the past, the separation of the mouse Hex A and Hex B isozymes has been routinely accomplished by passing a preparation over an anion exchange column equilibrated with 10 mM sodium phosphate buffer, pH 6.0–6.5. Hex B is collected in the pass-through fractions, while the retained Hex A is eluted by an NaCl gradient (3, 32). However, these reports followed the method that was originally optimized for the human Hex isozymes (1). We found that in following the previously reported methods (3), the mouse Hex B preparation that was not adsorbed to the DEAE column at pH 6.0–6.5 still contained a small amount of MUGS-cleaving activity. MUGS-cleaving activity has been correlated with the ability to hydrolyze G_{M2} (33). As reported by Burg et al. (3), we also found that mouse Hex B prepared by this method did contain some G_{M2}-cleaving activity. To ascertain whether this G_{M2}-cleaving activity was inherent in the mouse Hex B or due to contamination by Hex A, we increased the pH of the buffer solution to 7.0 for the separation of the two isozymes by anion exchange chromatography. Interestingly, the amount of MUGS-cleaving activity, as compared with the MUG-cleaving activity, decreased significantly and the resulting Hex B preparation became extremely weak in hydrolyzing G_{M2} (Fig. 5A), as was observed with human Hex B (11).

From the binding behavior of mouse Hex A to DEAE-Fractogel and also because of its acidic pI, the retention of the enzyme by the SP-Fractogel column at pH 7.0 (Fig. 3) was totally unexpected. This suggests that interactions other than ionic may be involved. This chromatography step was very effective for removing contaminating proteins. Because the Hex B preparation contained other proteins not adsorbed to DEAE-Fractogel at pH 7.0, it is not surprising that the SP-Fractogel chromatography was not as effective for purifying Hex B as for Hex A. Based on the DEAE-Fractogel chromatography, we estimated that approximately 90% of the total MUGS-cleaving activity present in the crude mouse liver extract was Hex A and 7% was Hex B. This is in agreement with previous reports of the level of the two isozymes in mouse liver tissues (32). Because the amount of Hex B in mouse liver is very low compared with Hex A it was not practical to purify Hex B to homogeneity as done for Hex A. However, the final Hex B preparation is free from contaminating glycosidases and proved to be suitable for the studies presented.

The recombinant human and mouse G_{M2} activators were expressed using the shortened version of cDNAs which encode...
only the mature activator proteins. The cDNA for human GM2 activator encodes for a protein of 193 amino acids that consists of a signal peptide (23 amino acids), a propeptide (8 amino acids), and a mature protein (162 amino acids). The signal and the propeptides are excised proteolytically to form the mature GM2 activator protein (5). In the full-length cDNA encoding for the mouse GM2 activator, the predicted cleavage site is between positions 19 and 20 of the deduced amino acid sequence (34). This site is very close to the cleavage site (positions 23 and 24) of the human sequence (5). Although there is no direct evidence that the first 31 amino acids in the mouse sequence contains a signal peptide and a propeptide, the mouse sequence shows a hydrophathy profile similar to that of the human sequence (18). In addition, the recombinant mouse GM2 activator and the native human protein were found to have the same specific activity toward the hydrolysis of GM2, indicating that the mature form of mouse GM2 activator is very likely to start from amino acid 32 as in the case of humans.

As seen with the human hex isozymes, mouse Hex A hydrolyzes GM2, with the requirement of the GM2 activator, whereas mouse Hex B has only a trace of activity to cleave GM2 with or without GM2 activator. To our surprise, in contrast to human Hex isozymes, mouse Hex A was also able to effectively hydrolyze Gb3a in the presence of mouse GM2 activator (Fig. 5B, lane 4). We were not able to detect the hydrolysis of GA2 by Hex B without GM2 activator, but when the activator is present, some hydrolysis of Gb3a could be seen after extended incubation (Fig. 5B, lane 6). These results provide the explanation for the observations made in mice with disrupted α-subunit gene. Mice defective in Hex A but not Hex B, because of the disrupted α-subunit were found to show relatively little buildup of GM2 or GA2 with no behavioral abnormalities, as compared with humans with defective α-subunits. (12, 13). The fact that mouse Hex B cannot hydrolyze GM2 but can act on GA2 suggests that in mice GM2 can be converted to GA2 that serves as a substrate for mouse Hex B. We have shown previously that clostridial sialidase can effectively convert GM2 to GA2 in the presence of human GM2 activator (35). Our results complement the recent pathobiological findings of the three mouse models of human Tay-Sachs disease, types B, O, and AB of GM2 gangliosidosis. The mouse models of type B (Hexa−/−) and O (Hexb−/−) were generated by targeted disruption of Hex A (α-subunit) (12, 13) or Hex B (subunit) (36) genes encoding Hex A (αβ) and Hex B (ββ). The model of type AB GM2 gangliosidosis (Gm2a−/−/Gm2b−/−) (GM2 activator deficiency) was produced by targeted disruption of Gm2a gene (37). Unlike human type B GM2 gangliosidosis, the Hexa−/− mice were asymptomatic (12, 13), while Hexb−/− mice (36) were severely affected as in the case of human type O GM2 gangliosidosis. The Hexb−/− mice accumulated more GM2 and GA2 in the brain than the Hexa−/− mice. The Gm2a−/− mice (37) showed a phenotype which is intermediate to those of Hexa−/− (12, 13) and Hexb−/− (36) with storage of an excess amount of GM2 and a low amount of GA2. From these three murine models of Tay-Sachs disease, it has been proposed that Hexa−/− mice escape the disease through partial catabolism of GM2 via GA2 by the combined action of sialidase and Hex B (14). The pathogenesis of Gm2a−/− mice also suggested a role for the GM2 activator in GA2 degradation in mice (37).

Our results provide the explanation for the results generated by the above three mouse models. We have demonstrated the ability of mouse Hex A to participate in the catabolism of GA2 and a very weak activity of Hex B toward the degradation of GM2. We have also shown the ability of mouse GM2 activator to stimulate the hydrolysis of GA2 by mouse Hex A and to a lesser extent by mouse Hex B. We have also examined the species specificity of the interactions between the mouse and human Hex isozymes and the activators. Previously, crude activator preparations from other mammalian species (3) and purified mullet roe GM2 activator (38) were found to activate the hydrolysis of GM2 by human Hex A. We have shown here that purified recombinant mouse GM2 activator can effectively stimulate the hydrolysis of GM2 and GA2 by human Hex A. In reverse, human GM2 activator was not effective in stimulating the hydrolysis of GM2 or GA2 by mouse Hex A.

Although the mouse GM2 activator is 73.5% identical to the human protein, it also appears that the mouse activator does not share the specificity to the characteristic branched trisaccharide epitope of GM2 (19) but assists Hex A to hydrolyze GA2 as well. The observation that mouse GM2 activator can stimulate the hydrolysis of GM2 by both human and mouse Hex A, while human GM2 activator can only stimulate the hydrolysis of GM2 by human Hex A but not mouse Hex A, provides strong evidence that the GM2 activator proteins must somehow interact with Hex A. Similarly, the observation that the mouse GM2 activator can stimulate the hydrolysis of both GM2 and GA2 by human Hex A, but the human GM2 activator can only stimulate the hydrolysis of GM2 by mouse Hex A, shows that the GM2 activators of these two species may have different specificities for the two glycolipids.

Biochemical analysis of enzyme systems is an important complement to molecular and genetic studies in the effort to fully understand the roles of Hex isozymes in mouse. Despite the biochemical similarities between human and mouse Hex isozymes and GM2 activator proteins, the catabolic pathways for GM2 in mouse and human are clearly not identical. Therefore, the murine model for type B Tay-Sachs disease does not truly reflect its counterpart in man.

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