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Original Article

Increase in sphingolipid catabolic enzyme activity during aging

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Aim: To understand the contribution of sphingolipid metabolism and its metabolites to development and aging.
Methods: A systemic analysis on the changes in activity of sphingolipid metabolic enzymes in kidney, liver and brain tissues during development and aging was conducted. The study was conducted using tissues from 1-day-old to 720-day-old rats.
Results: Catabolic enzyme activities as well as the level of sphingomyelinase (SMase) and ceramidase (CDase) were higher than that of anabolic enzyme activities, sphingomyelin synthase and ceramide synthase. This suggested an accumulation of ceramide and sphingosine during development and aging. The liver showed the highest neutral-SMase activity among the tested enzymes while the kidney and brain exhibited higher neutral-SMase and ceramidase activities, indicating a high production of ceramide in liver and ceramide/sphingosine in the kidney and brain. The activities of sphingolipid metabolic enzymes were significantly elevated in all tested tissues during development and aging, although the onset of significant increase in activity varied on the tissue and enzyme type. During aging, 18 out of 21 enzyme activities were further increased on day 720 compared to day 180.
Conclusion: Differential increases in sphingolipid metabolic enzyme activities suggest that sphingolipids including ceramide and sphingosine might play important and dynamic roles in proliferation, differentiation and apoptosis during development and aging.

Keywords: aging; sphingolipid; metabolism; sphingomyelinase; ceramidase; sphingomyelin synthase; ceramide synthase

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Introduction

Aging involves a progressive accumulation of diverse and deleterious changes in cells and tissues with time that increase the risk of disease and death[1]. Several molecular models of aging have evolved, including damage by reactive oxygen species (ROS) generated by metabolism, genome instability, genetically programmed extension mechanisms, cell death, and systemic aging[2]. Sphingolipid metabolism is considered to be a novel signal transduction pathway that generates multiple lipid messengers, such as ceramide, sphingosine, and sphingosine-1-phosphate[3–5]. Oxidative and metabolic stresses as well as other factors that alter the metabolism of sphingolipids increase the risk of development and progression of pathogenesis of several age-related diseases including cancers, diabetes, atherosclerosis, and neurodegenerative disorders[6]. Recent studies have shown that sphingolipids, including ceramide and sphingosine, accumulate in the liver and brain during aging[7, 8]. Neutral sphingomyelinase (N-SMase) was also shown to be activated eight fold in senescent fibroblasts[9].

The fact that sphingolipid signaling through ceramide and other metabolites can influence the proliferation, differentiation and survival of many different types of cells strongly suggests fundamental roles of bioactive sphingolipid products in development and aging[6]. Within the last two decades, considerable progress has been made in elucidating the roles of sphingolipids. But till date, the activity changes in sphingolipid metabolic enzymes in developing and aging tissues have not been systematically studied. Thus, in the present study we investigated the changes in activity of sphingolipid metabolic enzymes in kidney, liver and brain tissues from 1-day-old to 720-day-old rats. The investigated enzyme activities include sphingolipid catabolic enzymes (acidic and neutral sphingomyelinases: A-SMase & N-SMase; acidic, neutral, and alkaline ceramidases: Ac-CDase, N-CDase and Al-CDase) and sphingolipid anabolic enzymes (sphingomyelin synthase and ceramide synthase). The activities of the sphingolipid catabolic enzymes (SMase and

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ceramidases) were significantly higher and changed more robustly than that of the anabolic enzymes studied (SM synthase and ceramide synthase). The activites of the enzymes were elevated with development and aging, and these results differed with the various tissue and enzyme type studied. This indicates a differential accumulation of ceramide and sphingosine and their contribution toward development and aging.

Materials and methods

Study design

To compare sphingolipid metabolic enzyme activities from 1-day-old to 720-day-old rat tissues; rats were mated. Brain, kidney and liver tissues were collected and homogenized as described below. Each enzyme activity was measured as described below using radioactive substrates.

Materials

[N-methyl-^{14}C]-sphingomyelin was purchased from Sigma (St Louis, MO, USA), and [N-palmitoyl-1-^{14}C]-sphingosine from Moravek Biochemicals (Brea, CA, USA). D-*erythro*, [3-^{3}H]–sphingosine and E^{3} Enhancer were procured from PerkinElmer Las, Inc (Boston, MA, USA). D-*erythro*-sphingosine (synthetic) was purchased from Avanti Polar Lipids Inc (Alabaster, AL, USA). Silica gel HPTLC (60 F_{254}, 20×20) was obtained from Merck (Darmstadt, Germany) and Kodak Medical X-ray film from Eastman Kodak Company (NY, USA). All other materials were purchased from Sigma-Aldrich Korea (St Louis, MO, USA).

Tissue collection

Animal care and all experimental procedures were conducted in accordance with the Guide for Animal Experiments edited by the Korean Academy of Medical Sciences. All animals were sacrificed under ether anesthesia between 9~12 AM on respective days[10] and kidney, liver and brain tissues were isolated and used for homogenate preparation as described below. The kidney, liver and brain tissues of 180-day, 510-day, and 720-day-old rats were obtained from the aging tissue bank (Korea national research resource center).

Preparation of tissue homogenates

Rat tissue homogenates were prepared as described by others[11] with some modifications. Tissues including kidney, liver and brain were removed from Sprague-Dawley rats and washed in cold phosphate-buffered saline (PBS) separately. The tissues were placed in 20 mmol/L Tris-HCl (pH 7.5) and 2 mmol/L EDTA solution(10 mL, cold), homogenized by Tekmar homogenizer (OH, USA) at 4 °C. The homogenate was centrifuged at 500×*g* for 10 min to remove unbroken cell debris, and the supernatant was used as an enzyme source for the enzyme analysis that followed.

Sphingomyelinase (SMase) activity

The activity of N- and A-SMases was determined using a method described by Liu and Hannun[12] with slight modifications. Briefly, tissue homogenates were centrifuged at 1000×*g* for 10 min and the supernatant was used for further analysis. The activity of both SMases was measured using radiolabelled substrate, [N-methyl-^{14}C]-sphingomyelin. For N-SMase, the reaction mixture contained 100 nmol of sphingomyelin (1154 dpm/nmol) in 100 mmol/L Tris-HCl (pH 7.4), 5 mmol/L $MgCl_2$, 0.1% Triton X-100 and 5 mmol/L dithiothreitol in a final volume of 0.2 mL, which contained 5 mg of protein. In the case of A-SMase, the assay mixture contained 100 nmol of sphingomyelin (1154 dpm/nmol) in 100 mmol/L sodium acetate (pH 5.0), 0.1% Triton X-100 and 0.1 mmol/L EDTA, which contained 5 mg of protein. After incubation at 37 °C for 1 h the reaction was stopped by adding 1.5 mL of chloroform: methanol (2:1), followed by 0.2 mL of water. A portion of the aqueous phase was transferred to scintillation vials and counted in a liquid scintillation counter for the radioactivity of the reaction product, ^{14}C-choline phosphate.

Ceramidase (CDase) activity

The activity of Al-CDase, N-CDase and Ac-CDase ceramidases was determined using the method of Nikolove-Karakashian and Merrill[13] with slight modifications. The activity of the enzymes was measured using radiolabelled substrate [N-palmitoyl-1-^{14}C]-sphingosine. The reaction was initiated by addition of supernatant (5 mg of protein) to the tubes containing 20 μL of substrate mixture (50 nmol of ceramide – 2353 dpm/nmol, 2.5 mg Triton X-100, 1 mg Tween 20, 0.4 mg sodium cholate) and 130 μL of a reaction buffer. The reaction buffer contained 125 mmol/L sucrose, 0.01 mmol/L EDTA and 125 mmol/L sodium acetate (pH 4.5) or 100 mmol/L Tris-HCl (pH 7.2) or 125 mmol/L HEPES (pH 8.0) for Ac-CDase, N-CDase and Al-CDase activity assay, respectively. After incubation at 37 °C for 1 h, the reaction was stopped by adding 2 mL of basic Doyle's solution (isopropanol:heptane:1 mol/L NaOH, 40:10:1, *v/v/v*), 1.8 mL of heptane and 1.6 mL of water. Samples were then centrifuged and the upper phase was discarded. The lower phase was washed twice with 1.6 mL heptane and then 1 mL of 0.5 mol/L H_2SO_4 and 2.4 mL of heptane were added. After centrifugation, 1 mL aliquots from the upper phase were transferred to scintillation vials and analyzed for radioactivity of the reaction product, ^{14}C-palmitate.

Sphingomyelin synthase activity

The activity of sphingomyelin synthase was determined as reported by Luberto and Hannun[14] with slight modifications. In brief, the reaction mixture contained 50 mmol/L Tris-HCl (pH 7.4), 25 mmol/L KCl, 0.5 mmol/L EDTA[15] and homogenate containing ~5 mg of protein. The reaction was initiated by addition of [N-palmitoyl-1-^{14}C]-sphingosine (20 nmol) as an equimolar complex with fatty acid free bovine serum albumin (complex specific activity: ~9×10^{3} cpm/nmol) and allowed to proceed for 60 min. The reaction was stopped by addition of 3 mL chloroform: methanol (1:2); the mixture was vortexed and kept on ice. Lipids were extracted as indicated by the Bligh and Dyer method[16] and resolved by TLC in chloroform: methanol: 15 mmol/L anhydrous $CaCl_2$ (60:35:8).

The [N-palmitoyl-1-^{14}C]-SM produced was detected by autoradiography, scraped from the plates and quantitated by liquid scintillation counting. Values for blanks were subtracted from total values of [N-palmitoyl-1-^{14}C]-SM to yield the amount of [N-palmitoyl-1-^{14}C]-SM produced.

Ceramide synthase activity

The assay mixture for sphingosine N-acyltransferase contained 1 μmol/L [^{3}H]sphingosine, 25 mmol/L potassium phosphate buffer (pH 7.4), 0.5 mmol/L dithiothreitol, 200 μmol/L palmitoyl-CoA, and approximately 0.2 mg of microsomal protein in a total volume of 0.1 mL as previously reported[17]. The reaction was initiated by adding palmitoyl-CoA, and after incubation at 37 °C for 15 min, the products were extracted, resolved by TLC, and quantitated as described above. Background counts were subtracted using data from identical assays that omitted palmitoyl-CoA.

Statistical analysis

The results are expressed as mean±SE of 3–6 determinations. Statistical analysis was performed using GraphPad Prism 3 (GraphPad Software Inc, San Diego, CA, USA). Statistical significance of differences was determined using one-way ANOVA with Tukey test. Significance was accepted at $P<0.05$.

Results

Increase in SMase activity during development and aging

Since 180-day-old tissue was used as the representative young adult tissue in the present study, activity changes in each enzyme from 1-day to 180-day-old tissues were analyzed as developmental changes, while the changes from 180-day to 720-day-old-tissues were categorized as aging-dependent changes. N-SMase and A-SMase activities were measured in rat kidney, liver and brain tissue homogenates. In the liver, both activities increased significantly from day 42, in comparison to the 1-day-old tissue (Figure 1 and Table 1). N-SMase was further increased in the 720-day-old tissue compared to 180-day but no changes were observed in A-SMase from day 180 (Figure 1 and Table 2). N-SMase activity in liver was higher than that of the kidney and brain (Figure 1A). In brain, N-SMase increased significantly from day 42, while A-SMase from day 180 (Figure 1 and Table 1). In the kidney, both activities increased from day 180. A-SMase activities in the brain and kidney tissues from day 720 increased significantly, compared to the respective 180-day- and 520-day-old tissues (Figure 1B and Table 2).

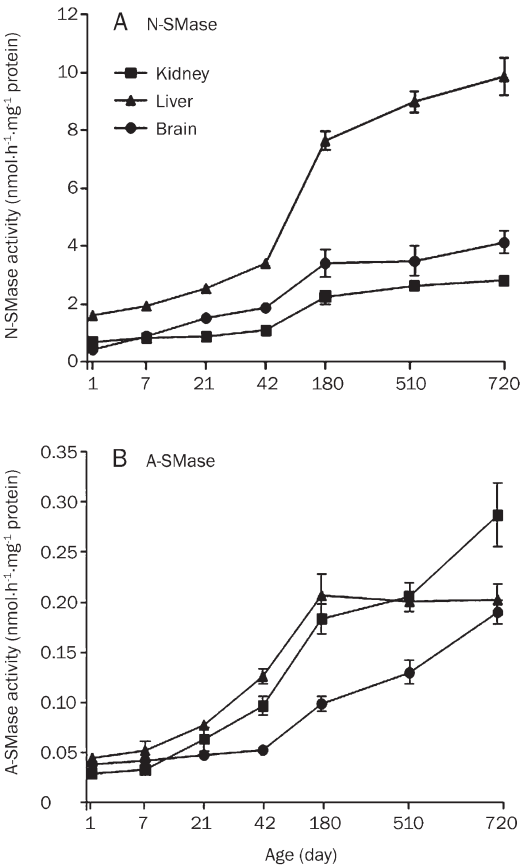

Figure 1. SMase activity in rat kidney, liver and brain tissues. N-SMase (A) and A-SMase (B) activities were measured from 7-day, 21-day, 42-day, 180-day, 510-day, and 720-day-old rat kidney, liver and brain tissues. Rat tissue homogenates were incubated with [N-methyl-^{14}C]–sphingomyelin. Lipids were extracted and radioactivity of reaction product ^{14}C-choline phosphate counted in a liquid scintillation counter. Acidic and neutral sphingomyelinases: A-SMase & N-SMase.

N-SMase activities in the 710-day-old kidney tissue increased compared to the 180-day-old tissue but not in the brain (Figure 1A and Table 2). However, A-SMase activity was relatively lower than N-SMase in all the tested tissues. For example, N-SMase activities of kidney, liver and brain tissues at 710-days were about 9-fold, 49-fold and 20-fold higher than that of A-SMase activities from each respective tissue. Nevertheless, the increase in activity of A-SMase appeared to be dependent on aging after 180 days in the kidney and brain tissues. This

Table 1. The earliest day of significant increase observed for each enzyme activity compared to respective 1-day-old tissue during development.

	A-SMase	N-SMase	Ac-CDase	N-CDase	Al-CDase	SM synthase	CD synthase
Liver	42	42	7	7	7	180	21
Kidney	180	180	42	42	42	42	21
Brain	180	42	42	21	180	21	21

acidic and neutral sphingomyelinases: A-SMase & N-SMase; acidic, neutral, and alkaline ceramidases: Ac-CDase, N-CDase and Al-CDase.

Table 2. Significant increase observed for each enzyme activity compared to respective 180-day-old tissue during aging.

	A-SMase	N-SMase	Ac-CDase	N-CDase	Al-CDase	SM synthase	CD synthase
Liver	510 d$^-$	510 d$^-$	510 d^b	510 d$^-$	510 d$^-$	510 d$^-$	510 d^c
	720 d$^-$	720 d^c	720 d^c	720 d^c	720 d^b	720 d^c	720 d^c
Kidney	510 d$^-$	510 d$^-$	510 d^c	510 d^b	510 d$^-$	510 d$^-$	510 d$^-$
	720 d^c	720 d^b	720 d^c	720 d^c	720 d^b	720 d$^-$	720 d^b
Brain	510 d^b	510 d$^-$	510 d$^-$	510 d$^-$	510 d^c	510 d$^-$	510 d^c
	720 d^c	720 d$^-$	720 d^c	720 d^c	720 d^c	720 d^c	720 d^c

$^bP<0.05$, $^cP<0.01$ compared to 180-day-tissue activity. "–", not signifiant. acidic and neutral sphingomyelinases: A-SMase & N-SMase; acidic, neutral, and alkaline ceramidases: Ac-CDase, N-CDase and Al-CDase.

implied practical roles of A-SMase during aging in the kidney and brain. In the liver, however, N-SMase might play a more significant role during aging based on its relatively higher activity among the different tissues and its further increase during aging.

Increase in CDase activity during development and aging

CDases can regulate the cellular ceramide level by hydrolyzing ceramide to sphingosine and free fatty acids. This enzyme not only regulates the levels of ceramide, but also that of sphingosine. We measured the activity of CDases (Ac-CDase, N-CDase and Al-CDase) in rat kidney, liver and brain tissue homogenates to understand the relative activity changes of CDases during development and aging. It was found that the activities of all isoforms of CDases were increased in three rat tissues compared to 1-day-old tissues (Figure 2). The earliest changes were observed in the liver. The activity of Ac-CDase, N-CDase, and Al-CDase increased significantly from day 7 in the liver, and day 42 in the kidney (Table 1). In the brain, the activity of Al-CDase increased from day 180, Ac-CDase from day 42 and N-CDase from day 21 (Table 1).

On day 710, the CDase activities of kidney and brain were higher by almost 11-fold as compared to 710-day-old liver tissue (Figure 2). Collectively, the kidney and brain showed comparatively higher CDases activities, while the liver showed relatively lower CDase activities. Compared to the 180-day-old tissue, three CDase activities in the 710-day-old brain, kidney and liver tissues were significantly increased (Figure 2 and Table 2). In particular, the activity of Ac-CDase in the kidney and brain was further increased in 710-day-old tissues comparison to 180-day tissues and that of Al-CDase in the brain (Figure 2).

Change in SM synthase activity during development and aging

SM synthase could contribute to the accumulation of ceramide depending on its relative activity to SMase. SM synthase (phosphatidylcholine:ceramide phosphorylcholine transferase) catalyzes the transfer of the phosphocholine head group of phosphatidylcholine to ceramide, thus forming SM[7]. To determine whether development and aging affected the activity of this enzyme, we measured the SM synthase activity in the same set of tissues using [N-palmitoyl-1-^{14}C]-sphingosine as the substrate. We observed that the SM synthase activity increased with development in the brain from day 21, kidney from day 42 and liver from day 180 (Figure 3). During aging, the activity further increased in the 710-day-old liver and brain tissues compared to the 180-day-old tissues. However, no significant increase was observed in the kidney from day 180 (Figure 3 and Table 2). Though SM synthase activity was increased in developing and aging tissues, the rate of conversion of ceramide to SM was relatively lower than that of SMase, for example 0.610±0.022 versus 9.783±0.638

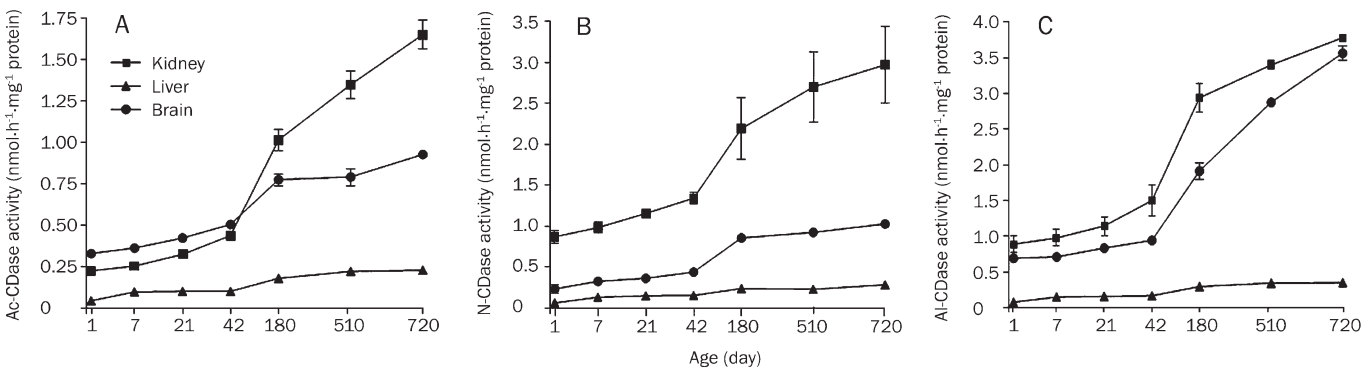

Figure 2. CDase activity in rat kidney, liver and brain tissues. Ac-CDase (A), N-CDase (B) and Al-CDase (C) activities were measured from 7-day, 21-day, 42-day, 180-day, 510-day, and 720-day-old rat kidney, liver and brain tissues. Rat tissue homogenates were incubated with [N-palmitoyl-1-^{14}C]–sphingosine. Lipids were extracted and radioactivity of reaction product ^{14}C-palmitate was counted in a liquid scintillation counter.

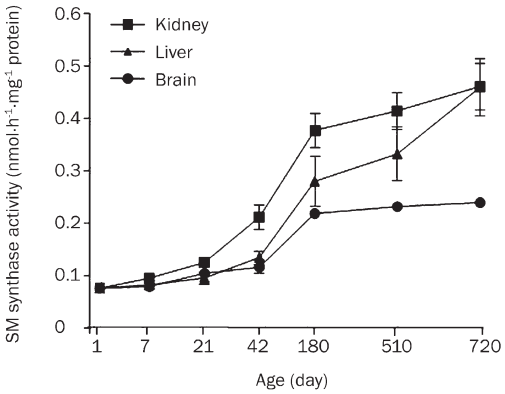

Figure 3. SM synthase activity in rat kidney, liver and brain tissues. SM synthase activities were measured from 7-day, 21-day, 42-day, 180-day, 510-day, and 720-day-old rat kidney, liver and brain tissues. Rat tissue homogenates were incubated with [N-palmitoyl-1-^{14}C]–sphingosine. Lipids were extracted and [N-palmitoyl-1-^{14}C]-SM isolated on TLC by autoradiography and quantitated using a liquid scintillation counter.

in the liver (Figures 1 and 3). This suggested that the level of accumulated cellular ceramide had increased during development and aging of the tissues due to the higher activity of SMase than SM synthase. Furthermore, the ceramide content in the kidney increaseed further during aging because SM synthase activity was not increased but A-SMase activity was continuously increased even after day 180 in the kidney (Figure 1B).

Change in ceramide synthase activity during development and aging

Next, we measured the ceramide synthase activity in the same set of tissues. We found that the enzyme activity was increased with development from day 21 in the rat kidney, liver and brain (Figure 4). We also observed that ceramide

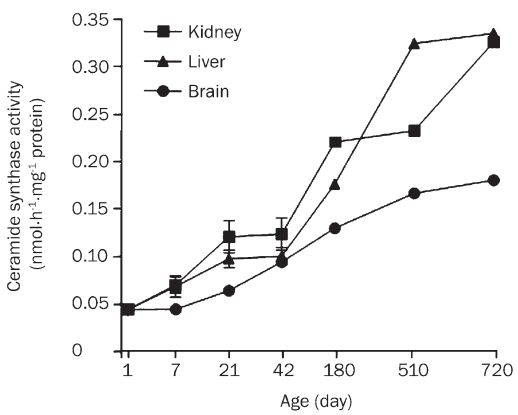

Figure 4. Ceramide synthase activity in rat kidney, liver and brain tissues. Ceramide synthase activities were measured from 1-day, 7-day, 21-day, 42-day, 180-day, 510-day, and 720-day-old rat kidney, liver and brain tissues. Rat tissue homogenates were incubated with [^{3}H]–sphingosine. Lipids were extracted and [^{3}H]–ceramide isolated on TLC by autoradiography and quantitated using a liquid scintillation counter.

synthase activity in those tissues was drastically lower compared to other sphingolipid catabolic enzyme activities like SMase and CDase; however, a further increase in ceramide synthase was observed in three tissues from day 180 (Figures 4 and 5). Thus, the rate of ceramide generation from sphingosine might be low and comparable to the rate of sphingomyelin generation from ceramide.

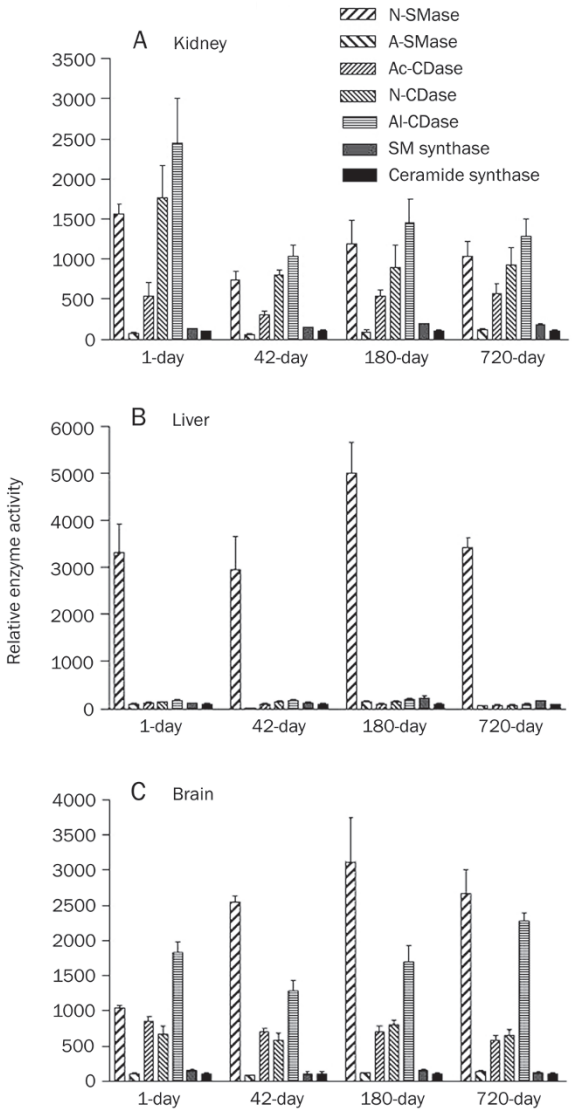

Figure 5. Relative sphingolipid metabolic enzyme activities in 1-day-old, 42-day-old, 180-day-old, and 720-day-old rat tissues of kidney (A), liver (B) and brain (C). Sphingolipid metabolic enzyme activities were compared by assuming activity of ceramide synthase as 100%.

Comparison of sphingolipid metabolic enzyme activities during development and aging

We studied the relative enzyme activities of the 1-day-old, 42-day-old 180-day-old and 710-day-old tissues of rat kidney, liver and brain, because all the tested enzyme activities

increased during development and/or aging. For this analysis we assumed ceramide synthase activity as 100% because this activity amounted to very low as compared to other measured activities; we compared the enzyme activity to that of the ceramide synthase activity of the respective aged tissue (Figure 5). We found that the activity of N-SMase in all liver tissues was higher than that of the other enzymes (Figure 5B). N-SMase activity in the liver was higher than that of the kidney and brain tissues (Figure 1). On the other hand, N-SMase, N-CDase and Al-CDase activities in kidney tissues were higher than that of other enzymes (Figure 5A). In the brain, the activities of N-SMase and Al-CDase were relatively higher than that of other enzymes (Figure 5C). SM synthase activity was relatively lower and similar to ceramide synthase activity in any of the tissues tested.

Discussion

Cutler and Mattson suggested a major role for sphingolipid metabolism in regulating the rate of development and lifespan, based on several lines of evidence[6]. For example, stress conditions are related to both aging and sphingolipid metabolism. Calorie restriction, the representative model of lifespan expanding animal model, decreases sphingolipid synthesis[18]. Alterations in sphingolipid metabolism increase the risk and progression of age-related disease[6]. Finally, sphingolipids, including ceramide and sphingosine, accumulate in several tissues (*eg* liver and brain) during aging[7, 19]. Several lines of evidence support the postulation that age-related neurodegenerative diseases like Alzheimer's disease are related to sphingolipid metabolism: (1) β-amyloid protein ($A\beta_{1-42}$) and human immunodeficient virus type 1 gp120 activates N-SMase in human primary neurons, resulting in apoptosis[20–23]. (2) Ceramides significantly accumulate in the brain of Alzheimer's disease patients[24]. (3) Sphingomyelin inhibits γ-secretase activity[23]. However, changes in sphingolipid metabolic enzyme activities during development and aging have been poorly studied. In the present study, we conducted a systemic analysis of sphingolipid metabolic enzyme activities from 1-day-old to 710-day-old rat tissues.

In this study, 180-day-old rats were supposed to be young adult rats. Therefore, the changes up to day 180 were interpreted as developmental, while the changes in 520-day- and 710-day-old rats were considered to be aging-dependent changes. We report that N-SMase activity was significantly increased in rat kidney, liver and brain developing tissues. Among the tissues, interestingly, the liver showed higher N-SMase activity than the others. Petkova *et al* reported an age-induced increase in the activity of rat liver plasma membrane-bound N-SMase[25]. N-SMase was also shown to be activated eight fold in senescent fibroblasts[9] as well as being linked to cell cycle arrest[26]. In the liver, N-SMase was further increased during aging (Figure 1). Among the 7 enzyme activities tested, only N-SMase showed a 30-fold higher activity than others (Figure 5). This implied that N-SMase played a significant role in liver development and aging. In the kidney, N-SMase and A-SMase increased significantly during aging; in the brain, A-SMase increased during aging. Thus, N-SMase was suggested to be a good candidate for maintenance of aging in the liver. A-SMase in the brain and both N-SMase and A-SMase in the kidney might contribute to aging in these organs, although it must be noted that the relative activity of A-SMase was relatively lower than that of N-SMase.

Ceramidases are key enzymes regulating cellular levels of apoptosis-related sphingolipids, ceramide and sphingosine. Activation of Ac-CDase has been linked to resistance of ceramide-mediated death pathways. For instance, overexpression of Ac-CDase was shown to protect cells from TNF-α-induced apoptosis[27]. We observed that ceramidase (Ac-CDase, N-CDase and Al-CDase) activities increased specifically in 710-day and/or 520-day-old rat kidney, liver and brain tissues as compared to 180-day-old tissues (Figure 2 and Table 2). Among the isoforms of ceramidases, Al-CDase showed the highest activity and was higher in the kidney and brain than the liver. Al-CDase activity in the 710-day-old kidney was 2.2-fold and 1.4-fold higher than Ac-CDase and N-CDase activities in kidney. Thus, the activities of ceramidases decreased in the following order: Al-CDase > N-CDase > Ac-CDase. Spence *et al* proposed that N-CDase activity was higher than Ac-CDase activity and that both activities decreased in the following order: kidney > brain > cardiac muscle > cerebellum > liver > spleen > cardiac muscle > lung > psoas muscle[28]. Activities of the isoforms of ceramidases were found to increase in the following order: kidney > brain > liver, supporting the previous study (Figures 2 and 5). Increase in the ceramidase activity in kidney and brain suggests increased sphingosine generation with aging in the tissues. The lower level of CDase activity in the liver suggests the decreased conversion of ceramide to sphingosine, leading to ceramide accumulation in aging liver tissues.

Sphingolipid anabolic enzyme (SM synthase and ceramide synthase) activities were increased in kidney, liver and brain tissues during development and aging. The activity of SM synthase in those tissues, however, was relatively lower than the catabolic enzyme activities (Figure 5). Lightle *et al*, reported that aging led to a slight decrease in SM synthase in the microsomes, however, the difference did not reach statistical significance in young rats versus old animals[7]. Our results showed that SM synthase activity increased during development and aging, but the activity itself was lower than that of catabolic enzyme activities. This suggested that a decreased conversion of ceramide to SM might also contribute to age-related increases in ceramide levels. It was also an indication that aging resulted in a slower synthesis of SM and/or a slower synthesis of SM resulted in aging[7].

Similar to SM synthase, ceramide synthase activity was also very low. Thus, ceramide generation by ceramide synthase in aging tissues was supposed to be very low, suggesting a minor contribution of ceramide synthesis to ceramide accumulation.

In the present study, we observed that the activities of sphingolipid metabolic enzymes increased differentially in three tested tissues during development and aging. We

measured 7 enzyme activities from 3 tissues. Thus 21 enzyme activities were monitored from day 1 to 710. All 21 enzyme activities were increased during development, although significant increases appeared from different time points depending on tissue and enzyme type. On day 710, 18 out of 21 enzyme activities were further increased compared to day 180, suggesting important roles of sphingolipid metabolic enzyme activities in aging.

In the present study, N-SMase activity was higher in the liver than in the kidney and brain, while A-SMase and ceramidases (Ac-CDase, N-CDase and Al-CDase) activities were higher in the kidney. We compared these activities in kidney, liver and brain by assuming ceramide synthase activity 100% (Figure 5). We report that (1) N-SMase showed highest activity in the liver, while N-SMase and CDases (especially alkaline and neutral) showed higher activities in kidney and brain tissues and (2) the sphingomyelin synthase and ceramide synthase activities were absolutely and relatively lower than SMase and ceramidases activities in developing and aging tissues. Thus, in the liver N-SMase is postulated to actively produce ceramide, while N-SMase and CDases might produce ceramide and sphingosine in the kidney and brain during aging, thereby leading to an accumulation of ceramide and/or sphingosine. Stress conditions in aged tissues may contribute to the higher activities and the accumulated ceramide/sphingosine induces apoptosis of the cells in aged tissues (Figure 6).

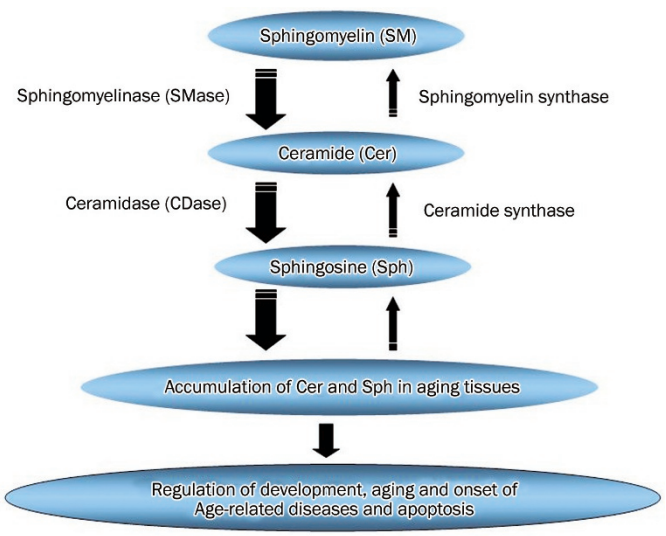

Figure 6. Biosynthesis and catabolism of sphingolipids.

In summary, on the basis of our results we propose that (1) an accumulation of ceramide/sphingosine occurs with development and aging and plays important roles in regulating an array of physiological processes, including cell proliferation, differentiation, and apoptosis. This increase in the level of ceramide/sphingosine could result from (i) an increased generation from sphingomyelin via mainly N-SMase and minorly A-SMase and ceramidases and (ii) relatively lower activities of SM synthase and ceramide synthase. (2) A differential increase in the sphingolipid metabolic rate in the liver, kidney and brain tissues via differential increased sphingolipid metabolic enzyme activities (SMase, CDase, SM synthase and ceramide synthase) during development and aging may contribute to the varying accumulation of ceramide/sphingosine in the liver, kidney and brain (Figure 6).

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Author contribution

Dong-soon IM designed research; Santosh J SACKET performed research; Santosh J SACKET, Hae-young CHUNG, Fumikazu OKAJIMA and Dong-soon IM analyzed data; Santosh J SACKET and Dong-soon IM wrote the paper.

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