Sphingomyelin metabolism in rat liver after chronic dietary replacement of choline by N-aminodeanol

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Abstract Sphingomyelin (SM) is a structural element of cell membranes and lipoproteins, and participates in signal transduction. To determine whether a choline analog (N-amino-N,N-dimethylaminoethanol, N-aminodeanol, NADe) can be substituted for choline in the SM of liver, rats (male, Sprague-Dawley-derived) were fed a diet that was low in choline and methionine, and contained 35.5 mmol of NADe/kg. After 18 months, liver plasma membranes and microsomes contained 48.9 ± 3.6 and 93.6 ± 6.9 nmol/mg protein of phosphatidyl-NADe, respectively, and 3.2 ± 0.2 and 3.5 ± 0.1 nmol/mg protein of ceramide phospho-NADe. The SM content of microsomes from NADe-fed rats was about one-third lower than for the control, and phosphatidylcholine (PC) was reduced by <10%; there was also a small decrease in PC, but not SM, in plasma membranes. In vitro assays of enzymes involved in SM metabolism found no change in PC:ceramide cholinephosphotransferase, but the NADe-fed animals had higher phosphatidylethanolamine:ceramide ethanolaminephosphotransferase activity, greater incorporation of methyl groups from [methyl-^{3}H]-S-adenosyl methionine into SM, and a lower neutral sphingomyelinase activity. These results show that NADe-fed rats form considerable amounts of ceramide phospho- and phosphatidyl-NADe; however, liver plasma membranes retain relatively normal levels of PC and SM, perhaps due to increases in the de novo pathway for SM synthesis and decreases in SM turnover.—**Nikolova-Karakashian, M. N., R. W. Russell, R. A. Booth, D. J. Jenden, and A. H. Merrill, Jr.** Sphingomyelin metabolism in rat liver after chronic dietary replacement of choline by N-aminodeanol. *J. Lipid Res.* 1997. **38:** 1764–1770.

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Earlier research has described an experimental model with development of a hypocholinergic state in rats that mimics many of the features of human progressive degenerative dementias (1–3). The model state is induced by chronic administration of an analogue of choline, N-aminodeanol (NADe), that shares most of the physicochemical and biochemical characteristics of choline (4), but is utilized much less efficiently in both phospholipid metabolism and in the synthesis of acetylcholine. The present experiment was designed to study the effects of NADe on phospholipid levels and metabolism because large amounts of NADe have been found in the Folch extracts from liver, plasma, and cerebral cortex in rats fed an NADe-supplemented diet for a period of 4 months (3). NADe replaced 80% of the choline in its lipid-bound form (3); however, individual choline-containing phospholipid classes have not been analyzed nor has the effect of longer term feeding.

The lipid-bound forms of choline are phosphatidylcholine (PC) and sphingomyelin (SM), which are important components of cellular membranes and function in membrane assembly, organization (5), and signal transduction pathways for cytokines, growth factors, and diverse agonists (6, 7). These lipids are also thought to provide choline for the synthesis of acetylcholine in the CNS; therefore, substitution of the choline headgroup of PC and SM with NADe could contribute to some of the physiological and behavioral effects of this analog. SM is synthesized from ceramide and PC via the enzyme PC:ceramide cholinephosphotransferase which has been described in microsomes, Golgi, and plasma membranes (8–10). A de novo pathway of SM synthesis has also been described, which involves the formation of ceramide phosphoethanolamine (CPEth) via the transfer of phosphorylethanolamine from phosphatidylethanolamine (PE) to ceramide, followed by

Abbreviations: NADe, N-aminodeanol; SM, sphingomyelin; Cer, ceramide; CPNADe, ceramide phospho-NADe; CPEth, ceramide phosphoethanolamine; SAM, S-adenosylmethionine; SAH, S-adenosylhomocystein; PC, phosphatidylcholine; PE, phosphatidylethanolamine; DAG, diacylglycerol; PCho, phosphorylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol.

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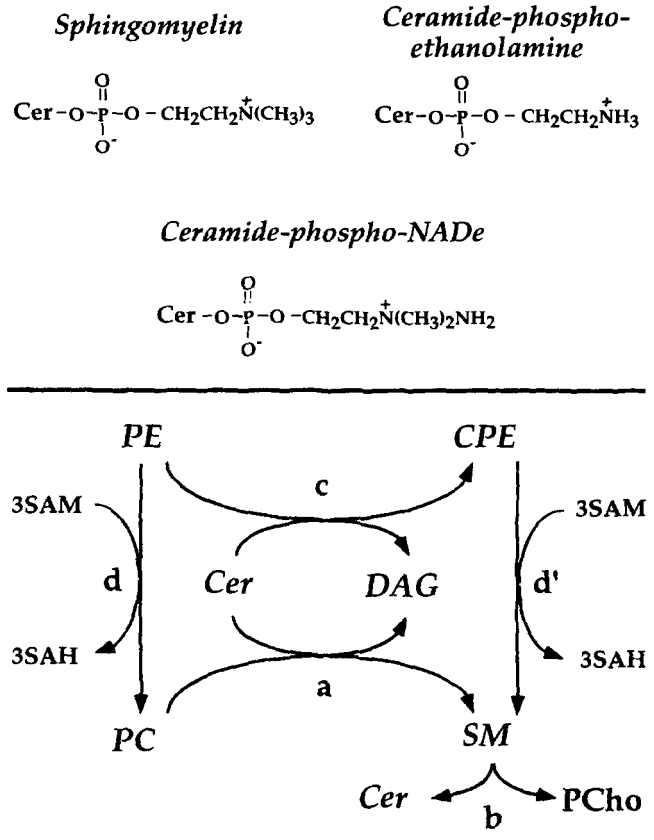

Fig. 1. Structures of sphingomyelin, ceramide-phosphoethanolamine, and ceramide-phospho-NADe analog (upper panel) and enzymes of SM and CPEth metabolism (lower panel). a) phosphatidylcholine-ceramide: cholinephosphotransferase; b) sphingomyelinase; c) phosphatidylethanolamine-ceramide:ethanolaminephosphotransferase; d) N-methyltransferases. Abbreviations: SAM, S-adenosylmethionine; SAH, S-adenosylhomocystein; PC, phosphatidylcholine; PE, phosphatidylethanolamine, Cer, ceramide, DAG, diacylglycerol, PCho, phosphorylcholine, CPE, ceramide phosphoethanolamine.

methylation of CPEth to SM in analogy to de novo synthesis of PC (8, 11). SM turnover involves both neutral and acidic sphingomyelinases (12, 13) (**Fig. 1**).

Liver is one of the key organs in lipid synthesis and degradation, and is the primary organ of de novo choline biosynthesis (via PC and SM) for potential use by other organs, e.g., brain. Thus, the goal of the present study was to analyze the livers of animals maintained on NADe for 18 months to determine how the phospholipid compositions of the microsomes and plasma membranes have been affected by substitution of this analog for choline. Our experiments found that the NADe-analogs of both PC and SM are formed; however, the amounts of PC and SM are surprisingly well maintained. The possible enzymatic basis for this was explored for SM, and may involve an increased capacity for SM synthesis de novo and a reduced activity of the neutral sphingomyelinase.

MATERIALS AND METHODS

Materials

The [methyl-^{3}H]-S-adenosyl methionine, [ethanolamine-^{14}C]phosphatidylethanolamine and [choline-^{14}C]sphingomyelin were purchased from Amersham; [choline-^{14}C]phosphatidylcholine was isolated by preparative thin-layer chromatography (TLC) from a macrophage cell line (J774 cells) after labeling the cells with [^{14}C]choline chloride (sp. activity of 55 mCi/mmol, 0.5 μCi/ml of culture medium) for 48 h. N-acetyl-D-*erythro*-C_{20}-sphinganine was synthesized as described previously (14). Unlabeled lipids, and most other biochemicals, were from Sigma. The high performance thin-layer chromatography (HPTLC) plates were purchased from Merck.

Animals, diet, and membrane preparation

Male rat pups (Sprague-Dawley derived) were weaned at the age of 29 days and fed the experimental and control diets ad libitum. The diets were essentially the same as previously described (3): dextrose (69%), casein (12%), soya oil (10%), mineral mix (5%), alphacel (ground cellulose, 2%), vitamin mix (1.5%) and were tested to be choline free (<14 μmol/kg). Either choline chloride (control) or NADe chloride (experimental) was added at a concentration at 35.5 mmol/kg. The L-methionine content was reduced to the minimum recommended for weight maintenance (0.23%, (2)).

The rats (six animals per group) were fed these diets for 18 months, then killed; the livers were removed, pooled together, and homogenized. These homogenates were used for microsome (15), and plasma membrane (16) isolation and the fractions were stored at −80°C until analysis. The purity of membrane fractions was checked by marker enzymes (17) and found to be within the parameters reported previously (18). The yield of plasma membranes was similar for the two different dietary feedings.

Lipid analysis

Lipids were extracted by a Bligh and Dyer procedure as modified by Williams et al. (19). Individual lipids were separated by TLC, using chloroform–methanol–triethylamine–2-propanol–0.25% potassium chloride 30:9:18:25:5 (by vol) as developing solvents. For analysis of sphingolipids, glycerophospholipids were degraded by a mild alkaline hydrolysis in 0.6 N NaOH in methanol, for 1 h at 37°C. The phases were separated by adding 1 ml each of chloroform and water, and the organic phase was dried through an anhydrous (granular) sodium sulfate column. The alkali-stable lipids were separated on HPTLC plates using chloroform–metha-

nol–acetic acid–water 25:15:4:2 (by vol) as the developing solvent. Lipids were visualized under I_2 and identified by comparison with standards, and by exposing the HPTLC plates to Dragendorff's reagent (positive reaction for a choline group) and ninhydrin (positive reaction for primary amines). Regions of the TLC plates representing individual phospholipids were quantitated by phosphate assay (20). For further analysis, ceramide, SM, CPEth, and the unknown alkali-stable compound in the experimental group were scraped off the plate and the lipid material was eluted from the silica with chloroform–methanol 1:2 (by vol). The solvent was evaporated under reduced pressure, the lipids were acid hydrolyzed in 0.5 M HCl in methanol for 15 h at 65°C, and the amounts of sphingosine and sphinganine were estimated by HPLC, as previously described (21), with correction for recovery by adding N-acetyl-C_{20}-sphinganine as an internal standard.

Assay of microsomal N-methyl transferases

N-methyltransferase activity was assayed by the method of Ridgway and Vance (15), developed for measuring the methylation of PE. The incubation mixture contained 125 mM Tris-HCl, (pH 9.2, at 22.5°C), 5 mM dithiothreitol, and 1.0 mM Triton X-100 in a final volume of 0.15 ml. After the addition of 25 μg of membrane protein, the mixture was placed on ice for 10 min. [Methyl-^{3}H]-S-adenosyl methionine (sp act of 33 mCi/mmol) was added to a final concentration of 200 μM, and the mixture was incubated at 37°C for 30 min. The reaction was stopped with chloroform–methanol 2:1 (by vol.) and the amount of radiolabel in the chloroform phase was measured by scintillation counting with correction for quenching. The amount of radiolabel in SM was quantitated after separation of the SM from the other lipids by TLC. Approximately 25 μg of unlabeled SM was used as a carrier during the extraction procedure and chromatographic analysis.

Assay of SM synthase, CPEth synthase, and neutral sphingomyelinase activities

SM synthase and CPEth synthase were assayed according to the procedure described by Malgat, Maurice and Baraud (11). The reaction medium contained 132 nmol of dioleoylphosphatidyl [^{14}C]ethanolamine (sp act of 0.5 mCi/mmol) or 100 nmol of [^{14}C]choline-labeled phosphatidylcholine (sp. activity of 0.5 mCi/mmol), 50 mM Tris-HCl, (pH 7.4), 0.25 M sucrose, 0.15 mM KCl, Triton X-100 (20 μg/ml), and 0.25 mg of membrane protein in a total volume of 0.35 ml. When PE was used as a precursor, 5 mM β-hydroxyethylhydrazine (14) was added to prevent methylation of PE or CPEth. After incubation in a shaking water bath for 3 h at 37°C, the reactions were stopped by addition of 2 ml of chloroform–methanol 2:1 (by vol). The labeled glycerolipid substrates were cleaved by mild alkaline hydrolysis as described above, then the sphingolipid products were extracted with chloroform and quantitated with a scintillation counter.

Neutral sphingomyelinase activity was assayed according to the procedure of Hostetler and Yasaki (22). The reaction mixture contained 267 nmol of [methyl-^{14}C]-labeled SM as a substrate (sp. activity of 0.5 mCi/mmol), 2.5 mg/ml Triton X-100, 40 mM $MgCl_2$, 0.2 mg of plasma membrane protein and 10 mM Tris-HCl, pH 7.2, in a total volume of 0.2 ml. After incubation for 1 h at 37°C, the reaction was stopped by 0.1 ml of 100 mM EDTA, the lipids were extracted with chloroform–methanol 2:1 (by vol), and the aqueous phase, which contained the liberated phosphoryl[methyl-^{14}C]choline, was counted.

RESULTS

Overall effects of NADe feeding

The animals on NADe gained significantly less weight than controls; after 18 months the mean weights (± SD) were 814 ± 33 g and 955 ± 30 g, respectively ($P < 0.001$); liver weights (23.4 ± 2.8 g and 27.5 ± 2.5 g) were also significantly ($P < 0.05$) lower, but not on a g liver per 100 g body weight basis. Otherwise, there were few grossly observable differences between animals on the choline and NADe diets. These observations are consistent with those previously reported for animals fed NADe (2, 23). The NADe replacement of choline in the diet did not cause apparent tumor formation in the liver, as has been reported in rats fed a choline-deficient diet (24).

Amounts of phospholipids in liver microsomes and plasma membranes

NADe caused a significant elevation of the total phospholipid content (nmol/mg protein) in both microsomes and plasma membranes (**Table 1**). This agrees with the previous finding of an elevation in the total lipids in liver homogenates (3). The major difference in phospholipid amounts was due to the presence of a number of new species that were presumed to reflect the NADe analogs of PC and SM based on their R_f and alkaline lability or stability, respectively. When the amounts of these "NADe-containing" phospholipids were subtracted from the total, the amounts of the remaining phospholipids were similar for the NADe-fed rats and the controls. Therefore, the difference in total phospholipid content is due mainly to the generation

TABLE 1. Phospholipid composition of microsomal and plasma membranes from livers of rats fed diets containing choline or N-aminodeanol

Lipid	R_f	Plasma Membranes		Microsomes	
		Choline	NADe	Choline	NADe
Lyso-PC	0.05	24.2 ± 1.8	23.9 ± 2.2	5.6 ± 0.4	8.4 ± 0.9[b]
SM	0.07	22.9 ± 2.0	24.1 ± 0.8	16.9 ± 3.4	9.9 ± 3.1[a]
PC	0.10	65.4 ± 2.3	56.8 ± 2.8[a]	166.8 ± 5.6	152.9 ± 4.9[a]
PtdNADe	0.15	—	48.9 ± 3.6	—	93.6 ± 6.9
CPNADe		—	(3.2 ± 0.2)[d]	—	(3.5 ± 0.1)[d]
PS	0.19	20.9 ± 2.1	23.7 ± 2.2	10.4 ± 0.9	8.0 ± 0.4[a]
PI + PA		18.0 ± 2.0	14.8 ± 1.2	46.2 ± 1.3	45.1 ± 2.0
PE	0.33	34.6 ± 3.1	36.6 ± 5.5	76.7 ± 9.6	64.1 ± 9.2
PG	0.49	12.0 ± 2.8	22.9 ± 2.7[b]	10.3 ± 2.0	11.2 ± 1.4
DPG	0.55	11.0 ± 1.8	14.3 ± 2.6	3.3 ± 0.2	4.1 ± 1.0
Total phospholipids		226 ± 13	297 ± 27[a]	339 ± 0.4	395 ± 1.6[c]
Total phospholipids minus NADe-containing lipids			245 ± 27		297 ± 7.1[a]

The total lipids were extracted from microsomal or plasma membrane fractions. The amounts of individual lipids were determined by TLC and phosphate analysis. The abbreviations represent: NADe, N-aminodeanol; PC, phosphatidylcholine; SM, sphingomyelin; PtdNADe, phosphatidyl-NADe; CPNADe, ceramidephospho-NADe; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol. Data are presented as nmol/mg of protein and are mean ± SD, (n = 3, where n are replicates for the TLC analysis.

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$, groups that are significantly different from the controls.

[d] See Table 2 for an explanation of the analysis of the CPNADe.

of new phospholipid species in the experimental group. Most of the other phospholipids were not affected by the NADe feeding, with the exception of phosphatidylglycerol in plasma membrane.

Change in the amounts of sphingolipids

To characterize the putative NADe analog of SM (and to quantitate SM and CPEth with less interference from glycerophospholipids), the lipid extracts were subjected to mild alkaline hydrolysis (**Table 2**). As predicted, base hydrolysis fully cleaved the presumed NADe analog of PC but did not affect the ceramide-phospho-NADe.

To confirm that these compounds are sphingolipids, they were recovered from the HPTLC plate and acid hydrolyzed to liberate the long-chain base backbone(s), which were analyzed by HPLC (**Table 3**). These analyses proved that the compound identified as "ceramide-phospho-NADe" contained comparable amounts of sphingosine (i.e., 3.2 nmol/mg protein, Table 3) and phosphate (i.e., 3.5 nmol/mg protein, Table 2). The presence of a small amount of sphingosine (0.24 nmol/mg protein) in the respective samples from the choline-fed controls could be a result of "spill over" from CPEth and/or SM on the TLC plate. As further confirmation that this new compound was the NADe analog of SM, the HPTLC plates were analyzed with Dragendorff's reagent, which gave a positive response for the choline group of PC and SM and no response for ceramide phospho-NADe. This compound was also ninhydrin positive, that could be expected because of the primary amino group.

The data shown in both Tables 2 and 3 indicate that the NADe analog constitutes about 25% of the phosphosphingolipids in microsomes and 15% in the plasma membranes. It is also evident that there are small decreases in the amounts of SM in the NADe-fed rats, especially in microsomes. Furthermore, there was statistically significant elevation in CPEth when quantitated by HPLC analyses of the backbone (Table 3).

TABLE 2. Amounts of sphingolipids in microsomal and plasma membranes from livers of rats fed diets containing choline or N-aminodeanol

Lipid	R_f	Plasma Membranes		Microsomes	
		Choline	NADe	Choline	NADe
SM	0.15	18.8 ± 0.9	17.3 ± 1.3	11.5 ± 1.5	7.9 ± 0.8[a]
CPNADe	0.33	—	3.2 ± 0.2	—	3.5 ± 0.1
CPEth	0.38	2.6 ± 0.9	4.1 ± 0.6	2.6 ± 0.4	3.3 ± 0.8
Ceramide	0.90	7.3 ± 1.7	6.3 ± 0.9	5.8 ± 0.1	6.0 ± 0.6

The total lipids were extracted from microsomal or plasma membrane fractions and glycerophospholipids were hydrolyzed by mild alkaline hydrolysis. The alkali-stable lipids were analyzed by TLC and quantitated by phosphate analysis except for ceramide, which was quantitated by HPLC as described in the text. Data are presented as nmol/mg of protein and are means ± SD, (n = 3, where n are replicates for the TLC analysis. Abbreviations: NADe, N-aminodeanol; SM, sphingomyelin; CPNADe, ceramidephospho-NADe; CPEth, ceramide-phosphoethanolamine.

[a] $P < 0.05$, NADe groups that were significantly different from the corresponding control.

TABLE 3. Sphingosine released after acid hydrolysis of microsomal sphingophospholipids from livers of rats fed diets containing choline or N-aminodeanol

Lipid	R_f	Diet Components	
		Choline	NADe
		nmol/mg protein	
SM	0.15	10.35 ± 0.31	9.15 ± 0.15[a]
CPNADe	0.33	0.24 ± 0.13	3.15 ± 1.25[a]
CPEth	0.38	1.21 ± 0.04	2.30 ± 0.09[b]

The total lipids were extracted from microsomal or plasma membrane fractions and glycerophospholipids were hydrolyzed by mild alkaline hydrolysis. The alkali-stable lipids were analyzed by TLC and the sphingolipids were quantitated by HPLC as described in the Materials and Methods. The data are presented as means ± SD (n = 3, where n are replicates for the TLC analysis. Abbreviations: NADe, N-aminodeanol; SM, sphingomyelin; CPNADe, ceramidephospho-NADe; CPEth, ceramide-phosphoethanolamine.

[a] $P < 0.01$; [b] $P < 0.001$, groups that are significantly different from controls.

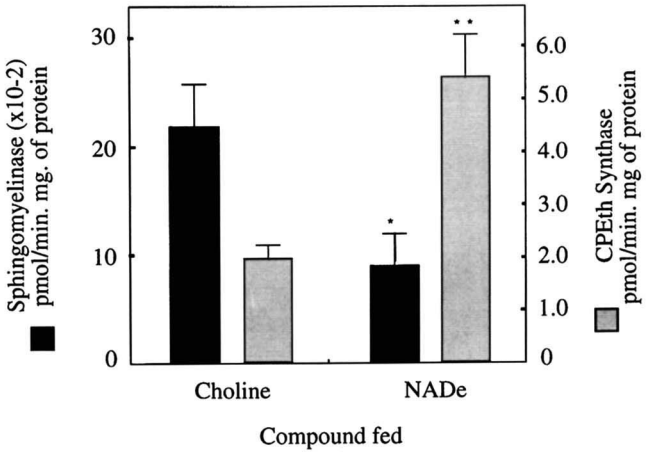

Fig. 2. Effect of dietary choline replacement by NADe on the activity of rat liver plasma membrane sphingomyelinase (dark bars) and microsomal phosphatidylethanolamine:ceramide ethanolaminephosphotransferase (light bars). CPEth synthase were assayed in microsomes from control and NADe-fed animals using 132 nmol of dioleoylphosphatidyl [^{14}C]ethanolamine (sp act of 0.5 mCi/mmol) in 50 mM Tris-HCl, (pH 7.4), 0.25 M sucrose, 0.15 mM KCl, Triton X-100 (20 μg/ml), 50 μM β-hydroxyethylhydrazine, and 0.25 mg of membrane protein in a total volume of 0.35 ml. The reaction was stopped with chloroform–methanol 2:1 (by vol) and, after mild alkaline hydrolysis, the labeled product was recovered and counted in the organic phase. Neutral sphingomyelinase activity was assayed in the plasma membrane fraction using 10 mM Tris-HCl, 2.5 mg/ml Triton X-100, 40 mM $MgCl_2$, 0.2 mg of plasma membrane protein, and in a total volume of 0.2 ml. [Methyl-^{14}C]-labeled SM (267 nmol) was used as a substrate (sp act of 0.5 mCi/mmol). The release of phosphoryl[methyl-^{14}C]choline was measured in the aqueous phase after standard extraction. The activities are presented as the means ± SD, n = 5, where n is the number of replicates for the enzyme assay.

Synthesis and hydrolysis of SM

The lack of a more significant reduction in SM is somewhat surprising given the large amount of phosphatidyl(al)NADe that was made. To determine whether this might involve changes in the activities of the enzymes that are thought to participate in SM homeostasis, we assayed SM synthase(s) and the neutral sphingomyelinase in vitro. The neutral sphingomyelinase activity was lower (by 60%) in plasma membranes from animals fed choline-replaced diet, as compared to that in membranes from control rats (**Fig. 2**). Therefore, it is possible that SM turnover at the plasma membranes is reduced in the NADe-fed rats, which could preserve the SM. The activity of PC: ceramide cholinephosphotransferase did not differ between control and NADe-fed rats (data not shown), however, the microsomal phosphatidylethanolamine: ceramide-ethanolaminephosphotransferase was elevated about 150% in the rats fed NADe (Fig. 2). Significant changes were also found in the microsomal PE and CPEth N-methyltransferase activity (**Fig. 3**). The incorporation of methyl groups from S-adenosyl methionine to total lipids (Fig. 3, light bars), or specifically into SM (Fig. 3, dark bars) was augmented in the NADe-fed animals.

DISCUSSION

This study has confirmed that replacing dietary choline with the choline analog NADe for 18 months results in significant accumulation of lipid-bound NADe. This study analyzes the classes of NADe-containing lipids formed as a result of this diet and shows that ceramide phospho-NADe has been formed as well as phosphatidyl(al)NADe (in the latter case, however, this is only presumed based on its chromatographic mobility and base lability). Even though substantial amounts of these SM and PC analogs were formed, there were only small changes in the amounts of choline-containing SM and PC. This was somewhat unexpected because a previous study (3) observed that after 4 months of feeding, 80% of the total lipid-bound choline was replaced by NADe. It is possible that the animals in this much longer term study have partially compensated for the deficiency of choline.

Under conditions of limiting choline and methionine, there are three main sources for choline that might contribute to such compensation: *1*) the choline that was already present when the diet started (at the 29th day after birth), which might be conserved by shifts in the utilization of choline by choline kinase and choline dehydrogenase (25, 26), and reduction in the production of betaine (27); *2*) the choline that is made de novo by methylation of PE and CPEth with S-adenosyl methionine, which might increase; and *3*) choline

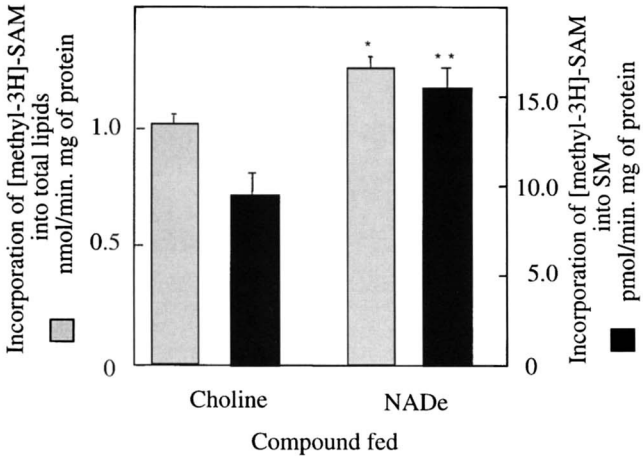

Fig. 3. Effect of dietary choline replacement by NADe on the incorporation of methyl groups from S-adenosyl-(^{3}H-methyl)-methionine into total lipids (light bars) and sphingomyelin (dark bars). N-methyl transferase activity was assayed in the microsomes from control and NADe-fed animals. The incubation mixture contained 125 mM Tris-HCl, (pH 9.2, at 22.5°C), 5 mM dithiothreitol, and 1.0 mM Triton X-100, 25 μg of membrane protein, and 200 μM [methyl-^{3}H]-S-adenosyl methionine (sp. act of 33 mCi/mmol) in a final volume of 0.15 ml. The reaction was conducted for 30 min and stopped with chloroform–methanol 2:1 (by vol). The amount of radiolabel in the total chloroform phase or in SM (after separation by TLC) was measured by scintillation counting with correction for quenching. The data are means ± SD, n = 5, where n is the number of replicates for the enzyme assay.

might be synthesized by methylation of free ethanolamine (28) using methyl groups donated from NADe because it has been shown in feeding studies with rats that deuterated methyl groups from dietary NADe appear in choline (23).

The changes in the enzyme activities involved in SM metabolism additionally suggest that the liver may adapt to the dietary choline deficiency by reducing SM turnover and, perhaps, by increasing synthesis of CPEth and conversion of CPEth to SM (thereby, reducing the requirement for choline from other sources). This study found an increased incorporation of [^{3}H]methyl groups into SM in vitro, however, it is not possible to identify the step that is elevated as this could arise from direct methylation of CPEth to SM or incorporation of methyl groups into PC, then SM. Nonetheless, under the incubation conditions used (i.e., pH 9.2) PC: ceramide cholinephosphotransferase activity is strongly inhibited) (8); therefore, it is most likely that SM was made from CPEth, a reaction that was first noted in crude fractions from rat liver (29) and has been demonstrated in isolated plasma membranes and microsomes (11). Enhanced SM synthesis from the CPEth pathway during choline deficiency is supported by the observations that: PE:ceramide ethanolaminephosphotransferase is more active in microsomes from the NADe-fed rats than from the controls; there are higher amounts of CPEth in microsomes from NADe-fed rats; and, the incorporation of [^{3}H]methyl groups into SM is higher (as noted above).

The levels of SM may also be maintained by a reduction in turnover because there is a reduction in the activity of the neutral sphingomyelinase in plasma membranes of NADe-fed animals. There was no change in the level of endogenous ceramide; however, this intermediate is involved in other metabolic reactions (such as incorporation into more complex sphingolipids or degradation to sphingosine) and, given the important roles of ceramides in cell regulation, it is sensible that the cells would have mechanism(s) to maintain the levels at as near normal as possible. It is intriguing to speculate that the NADe analog of SM might serve as an inhibitor of SMase activity, thereby helping to sustain the levels of SM. If so, chronic inhibition of the brain SMase during NADe-feeding might impair the production of ceramide in response to key agonists (such as nerve growth factor) (30). This certainly warrants further study given the importance of SM in cell regulation and, for brain, its suggested role in membrane aging (31) and regulation of neuron growth (32). Future research to test this possibility might help to advance our knowledge about the mechanism(s) underlying changes in cognitive behavior during progressive degenerative dementia.

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