Cholesterol and sphingomyelin syntheses are regulated independently in cultured human intestinal cells, CaCo-2: role of membrane cholesterol and sphingomyelin content

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Abstract There is a presumed association between cellular cholesterol and sphingomyelin metabolism. To study this relationship in the intestine, the activity of the rate controlling enzyme of sphingolipid synthesis, serine palmitoyltransferase (SPT), and the biosynthesis of long-chain bases were characterized in cultured human intestinal cells, CaCo-2. Cells were then incubated with substances known to alter cholesterol biosynthesis, and the effect of these mediators on SPT activity and long-chain base synthesis was determined and compared with their effects on HMG-CoA reductase activity and cholesterol synthesis. The polar sterol, 25-hydroxycholesterol, the squalene epoxide inhibitor, U18666A, and the inhibitor of HMG-CoA reductase, lovastatin, all significantly inhibited the synthesis of cholesterol without altering either SPT activity or long-chain base synthesis. Mevalonate, which increased cholesterol production 3-fold, also had no affect on SPT activity or sphingoid base synthesis. Serine, which significantly increased the synthesis of long-chain bases, did not alter cholesterol biosynthesis. Moreover, the suicide inhibitors of SPT, β-chloroalanine and cycloserine, did not alter cholesterol synthesis while markedly decreasing long chain base synthesis. Cells were incubated with palmitic, oleic, linoleic, and eicosapentaenoic acids. Only palmitic acid, the preferred substrate for SPT, increased the production of long-chain bases. Both palmitic and oleic acids, however, increased the synthesis of cholesterol. Cells enriched in sphingomyelin had higher rates of synthesis of both cholesterol and long-chain bases compared to their controls. In contrast, cholesterol and long-chain base syntheses were significantly decreased in cells enriched in cholesterol. Control cells incubated with phospholipid liposomes alone had higher rates of synthesis of both lipids. The results suggest that SPT and HMG-CoA reductase activities and cholesterol and long-chain base syntheses are regulated independently when the synthesis of either cholesterol or sphingomyelin is altered acutely. However, when membrane cholesterol or sphingomyelin mass are altered, parallel changes occur in the rates of synthesis of these two lipids. SPT activity in human small intestinal mucosa is also documented.—**Chen, H., E. Born, S. N. Mathur, and F. J. Field.** Cholesterol and sphingomyelin syntheses are regulated independently in cultured human intestinal cells, CaCo-2: role of membrane cholesterol and sphingomyelin content. *J. Lipid Res.* 1993. **34:** 2159–2167.

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A close relationship between sphingomyelin and cholesterol has been recognized for many years. Within membranes of mammalian cells there exists a concentration gradient of unesterified cholesterol and sphingomyelin, such that plasma membranes contain the most and mitochondria contain the least of these two lipids (1, 2). In models of aging and cell proliferation, cholesterol and sphingomyelin content increase in parallel (3, 4). The accumulation of sphingomyelin and cholesterol, particularly cholesteryl esters, in lesions of atherosclerosis has been well documented (5). In lipoproteins of diet-induced hypercholesterolemia and in low density lipoproteins (LDL) of familial heterozygous and homozygous hypercholesterolemia, the percent of cholesterol and sphingomyelin within these particles is increased concomitantly (5–7). Moreover, in the hereditary disorder of Niemann-Pick disease Type C, normal cellular cholesterol trafficking is disrupted by the accumulation of sphingomyelin within lysosomes. Studies have suggested that sphingomyelin has a molecular attraction for cholesterol and it is postulated that this sphingolipid "traps" unesterified cholesterol within a particular membrane preventing its normal cellular movement and metabolism (8). Thus, after the uptake of LDL by fibroblasts from patients with Niemann-Pick disease type C, the normal down-regulation of HMG-CoA reductase and LDL receptor activities and the up-regulation of acyl

Abbreviations: LDL, low density lipoprotein; HMG, 3-hydroxy-3-methylglutaryl; SPT, serine palmitoyltransferase.

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CoA:cholesterol acyltransferase activity are markedly suppressed and/or delayed (9, 10). Although the above examples document a close association between cholesterol and sphingomyelin, the exact mechanisms to explain this association remain obscure. In a recent study, we observed that the amount of membrane sphingomyelin in cultured human intestinal cells regulated the amount of unesterified cholesterol that was absorbed (11). It is possible, therefore, that the synthesis of sphingomyelin by the intestine could play an important role in supplying the absorptive cell with sufficient amounts of this phospholipid for restructuring of cell membranes and providing a required surface lipid for the normal assembly and secretion of chylomicrons.

The first committed step in sphingomyelin synthesis is catalyzed by serine palmitoyltransferase or SPT. SPT is a pyridoxal 5'-phosphate-dependent enzyme that catalyzes the condensation of palmitoyl-CoA and serine to form 3-ketosphinganine (12, 13). This enzyme has been studied in a variety of different tissues, but no information is available regarding its regulation in the intestine (14). As one of our goals is to gain an understanding of the importance of cellular sphingomyelin in the transport of lipids from the intestine into the lymph, it was important to study the relationship between sphingomyelin and cholesterol metabolism in the intestine. Because of the recognized association between these two lipids, it has been stated that the cellular synthesis of cholesterol and sphingomyelin is coordinately regulated (15, review).

The results of the present study demonstrate for the first time the presence of SPT activity in cultured human intestinal cells and in human intestinal mucosa. The activities of the two rate-controlling enzymes for cholesterol and sphingomyelin synthesis, HMG-CoA reductase and SPT, respectively, are regulated independently in intestinal cells as are the rates of cholesterol and sphingoid base synthesis. However, parallel changes in the rates of synthesis of these two lipids occur when the cholesterol/sphingomyelin ratio within membranes is disturbed.

MATERIALS AND METHODS

3-Hydroxy-3-methyl[3-^{14}C]glutaryl-CoA, $^{3}H_2O$, L-[^{3}H(G)]serine, and RS-[5-^{3}H]mevalonolactone were purchased from New England Nuclear (Boston, MA). Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, serine, palmitoyl CoA, β-chloroalanine, cycloserine, octadecylamine, cholesterol, sphingomyelin, egg phosphatidylcholine, phosphatidylserine, palmitic acid, oleic acid, linoleic acid, eicosapentaenoic acid, mevalonic acid and fatty acid-free bovine serum albumin were from Sigma (St. Louis, MO). The purity of the fatty acids was determined by gas–liquid chromatography. 3-Hydroxy-3-methylglutaryl-CoA was purchased from Pharmacia (Piscataway, NJ). 25-Hydroxycholesterol was purchased from Steraloids (Wilton, NH). All other reagents were reagent grade.

Cell culture

CaCo-2 cells were cultured for 14 days in 24-well plates in Dulbecco's modified Eagle's medium containing 20% fetal calf serum as previously described (16). On the day of the experiment, the cells were rinsed twice with 0.5 ml of medium-199/Earle's (M199; Gibco, Grand Island, NY), containing 10 mM HEPES, pH 7.4. The monolayers were then incubated for 18 h in M199 with the appropriate modulator as described for each experiment. Trypan blue exclusion and the amount of protein per dish were not altered by the treatments.

Measurement of lipid synthesis

Rates of cholesterol synthesis were estimated as previously described using $^{3}H_2O$ (2,000 dpm/nmol) as substrate (17). Sphingoid base synthesis was determined as described by Merrill and Wang (18) using [^{3}H]serine as substrate. Briefly, after the overnight incubation, [^{3}H]serine (sp act 10,000 dpm/nmol) was added to the monolayers and the incubation was continued for up to 4 h. The cells were rinsed twice with M199, harvested, and the total lipids were extracted with chloroform–methanol 2:1 (v/v) using 4 N HCl to separate the phases. Unlabeled sphingosine, sphinganine, ceramide, and sphingomyelin were added to the lipid extract as carriers. The chloroform phase was washed once with methanol–water 1:1, and the chloroform was completely removed under nitrogen. The total lipid extracts were then incubated for 18 h at 70°C in 0.5 ml of 12 N HCl–water–methanol 8.6:9.4:82 (v/v) to release long-chain bases from complex (amide-linked) sphingolipids (19). The mixture was neutralized and the lipids were again extracted with chloroform and methanol. The chloroform phase was evaporated under nitrogen and the long-chain bases were separated by thin-layer chromatography using chloroform–methanol–2 N NH_4OH 40:10:1 as the eluent (20). The appropriate bands were visualized by iodine vapor, scraped from the plates, and counted. Essentially all of the label resided in residues that migrated with sphingosine and sphinganine standards. If phospholipids were separated using chloroform–methanol–acetic acid–water 75:45:12:6 prior to acid hydrolysis, 65% of the label was found in phosphatidylserine and phosphatidylethanolamine, and 32% in sphingomyelin and ceramide.

Sphingomyelin and cholesterol enrichment

CaCo-2 cells were enriched in sphingomyelin as described for fibroblasts by Gott and Bierman (21). Appropriate amounts of chloroform containing sphingomyelin and/or octadecylamine were mixed and evaporated under nitrogen. M199 medium was added to make the final

concentrations of sphingomyelin and octadecylamine 200 and 32 μM, respectively. Control liposomes were prepared containing octadecylamine alone. The solutions were warmed to 45°C and sonicated continuously for 1 min. This process was repeated to ensure complete dispersion of the lipids. Cholesterol liposomes were prepared by taking appropriate amounts of chloroform containing cholesterol, phosphatidylcholine, or phosphatidylserine and drying the mixture under a stream of nitrogen. M199 medium was added to obtain final concentrations of 500 μM, 175 μM, and 75 μM for cholesterol, phosphatidylcholine, and phosphatidylserine, respectively. Control liposomes contained the two phospholipids minus the cholesterol. Cells were then incubated for 18 h at 37°C with 0.5 ml of the liposomal solution, and on the following day they were washed thoroughly with warm M199 prior to use.

Enzyme, lipid, and protein assays

HMG-CoA reductase activity was measured as described (22). The specific activity of HMG-CoA was 22,000 dpm/nmol and the reaction was performed over 120 min. The production of labeled mevalonate was linear over this time. SPT activity was estimated by the methodology of Williams, Wang, and Merrill (23) as described by Holleran et al. (24) in cultured human keratinocytes. Briefly, the total assay mixture of 0.1 ml contained the enzyme source, either total membranes (CaCo-2 cells) or whole homogenates (intestinal mucosal biopsies) in a buffer consisting of 100 mM HEPES (pH 8.3), 5 mM DTT, 2.5 mM EDTA, and 50 μM pyridoxal phosphate. This mixture was incubated for 10 min at 37°C before adding 150 μM palmitoyl-coenzyme A and 1 mM [^{3}H]serine. The reaction was stopped by placing the mixture on ice and adding 0.2 ml of 0.5 N NH_4OH. Products soluble in the organic phase were isolated by adding 1.5 ml of chloroform–methanol 1:2, 2 ml of 0.5 N NH_4OH, and 25 μg of sphingosine as carrier. After vortexing and centrifugation to separate the phases, the aqueous phase was removed. The organic phase was washed twice with 2 ml water, and dried completely under a stream of nitrogen. Radioactivity was determined by adding 10 ml Budget-Solve (Research Products International Corp, Mount Prospect, IL) and counting. The specific activity of the labeled serine was 40,000 dpm/nmol and the reaction time was up to 30 min. To correct for radioactivity that may not have been due to SPT activity, assays were routinely performed with heated (80°C for 10 min) protein and in the absence of palmitoyl-CoA. Cholesterol was measured by gas–liquid chromatography as previously described (25). Phospholipid mass was determined by the method of Chalvardjian and Rudnicki (26). Protein was estimated by the method of Lowry et al. (27).

Statistical analysis

One-way ANOVA and Dunnett's test were used to determine significant differences from control of treatments presented in Table 1. The unpaired Student's *t*-test was used in all other statistical analyses.

RESULTS

SPT activity in CaCo-2 cells and human intestinal mucosa

Because there was no demonstrable SPT activity in the cell cytosol of CaCo-2 cells (data not shown), the assay for SPT was characterized in total cell membrane preparations. **Fig. 1** shows data demonstrating the effect of time, protein, and serine concentration on membrane SPT activity. Under the experimental conditions, the assay was linear for 30 min and protein concentrations up to 0.2 mg. Maximal enzyme activity occurred at a serine concentration of 1.0 mM. The concentration of palmitoyl-CoA was maintained at 150 μM as higher concentrations inhibited the activity of the enzyme. The inclusion in the assay of the suicide inhibitor of SPT, β-chloroalanine, inhibited SPT activity by 90% at a concentration of 1 mM.

To compare SPT activity in CaCo-2 cells with human intestinal mucosa, endoscopic biopsies were obtained from patients undergoing small intestinal biopsies as part of their evaluation. All biopsies were from the third part of the duodenum and were considered to be normal small intestinal mucosa by H-and-E staining. Whole homogenates were used as an enzyme source as material was limiting. In four individual samples done in triplicate, the activities were 10.3, 12.1, 36.4, and 25.5 pmol/mg per min.

Long-chain base synthesis in CaCo-2 cells

To address possible relationships between the rates of cellular cholesterol and sphingomyelin synthesis, the synthesis of sphingomyelin was estimated by measuring the incorporation of labeled serine into sphingoid bases. The incorporation of serine into long-chain bases was linear for up to 4 h and was maximal at a serine concentration in the medium of 2 mM (**Fig. 2**). Within 15 min after adding β-chloroalanine, sphingoid base synthesis was maximally inhibited at a concentration of 1 mM.

Cholesterol and sphingoid base synthesis

To address whether cholesterol and sphingomyelin syntheses were coordinately regulated in cultured human intestinal cells, monolayers were incubated for 18 h with a variety of modulators known to alter the synthesis of either of these two membrane lipids. Their effects on HMG-CoA reductase activity and ^3H_2O incorporation into cholesterol were then contrasted with their effects on

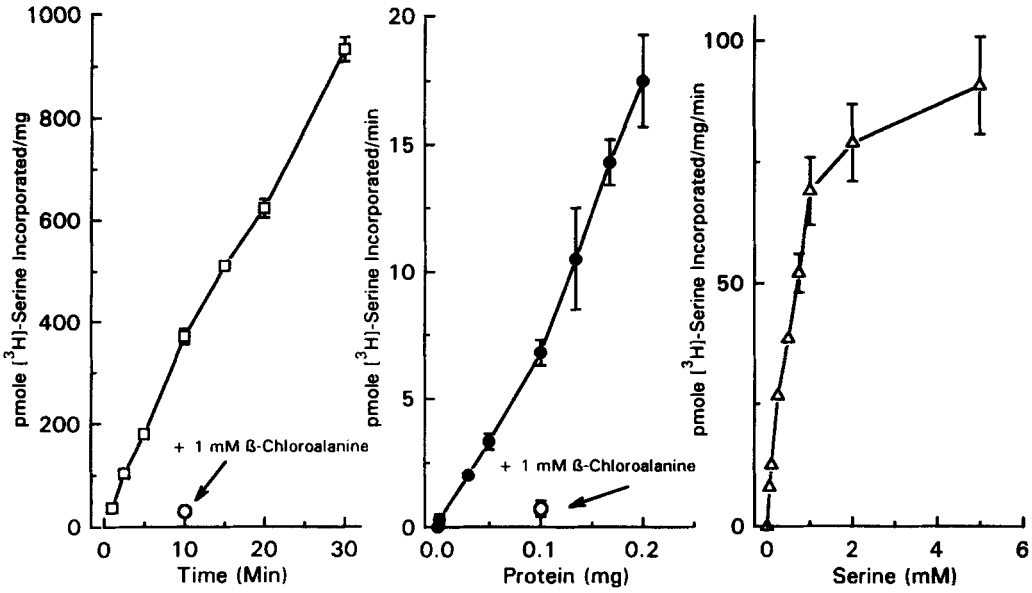

Fig. 1. SPT activity. Total membranes from CaCo-2 cells were prepared by centrifuging whole homogenates for 1 h at 105,000 *g*. SPT activity was determined as described in Methods. In some assays, 1 mM β-chloroalanine (○) was added just prior to adding the labeled substrate. The data are expressed as the mean of triplicate assays ± SE.

SPT activity and [^{3}H]serine incorporation into sphingoid bases. The results of these experiments are shown in **Table 1.** As expected, the polar sterol, 25-hydroxycholesterol, caused a profound decrease in both HMG-CoA reductase activity and the rate of cholesterol synthesis. SPT activity and the rate of sphingoid base synthesis, however, were not affected by the sterol. The squalene epoxide inhibitor, U18666A, suppressed the rate of cholesterol synthesis by 75% without altering the activity of reductase. U18666A, however, did not significantly affect either SPT activity or sphingoid base synthesis. In cells incubated with mevalonate, the rate of cholesterol synthesis was increased more than 3-fold while HMG-CoA reductase activity decreased 83%. Despite these rather marked alterations

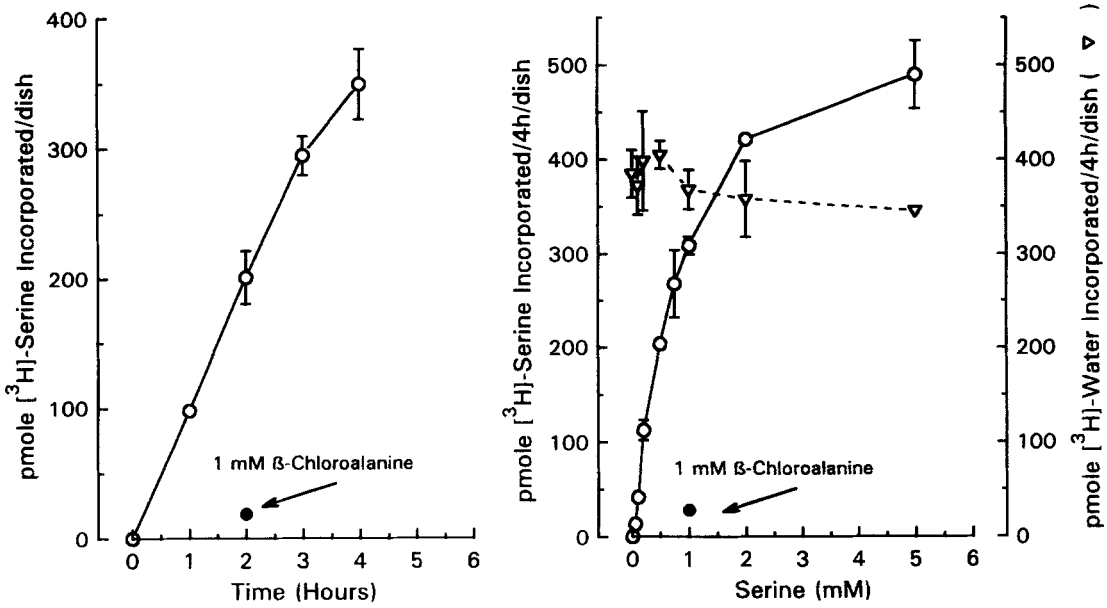

Fig. 2. Long-chain base synthesis. Sphingoid base synthesis was estimated by measuring the incorporation of [^{3}H]serine into long-chain bases (○) as described in Methods. In some dishes, 1 mM β-chloroalanine was added 15 min prior to the addition of labeled substrate (●). In the figure on the right, the rate of cholesterol synthesis at different serine concentrations was estimated by measuring the incorporation of ^3H_2O into cholesterol (▽) as described in Methods. The data are expressed as the mean of 4–6 dishes ± SE.

TABLE 1. Regulation of cholesterol and long-chain base syntheses

Treatment	HMG-CoA Reductase	SPT	HMG-CoAR/SPT	^3H_2O Incorporation into Cholesterol	[^{3}H]Serine Incorporation into Long-Chain Bases	^3H_2O/[^{3}H]Serine
	pmol/mg/120 min	*pmol/mg/15 min*		*pmol/4 h/dish*	*pmol/4 h/dish*	
Control (6)[a]	1515 ± 44	234 ± 30	6.5	413 ± 13	360 ± 24	1.1
25-OH (6)	388 ± 12[b]	174 ± 20	2.2	70 ± 14[b]	452 ± 12	0.2
Mevalonate (6)	263 ± 11[b]	171 ± 7	1.5	1342 ± 69[b]	440 ± 16	3.1
U18666A (6)	1316 ± 41	171 ± 20	7.6	101 ± 6[b]	324 ± 20	0.3
Lovastatin (6)	nd	nd		165 ± 16[b]	324 ± 20	0.5
β-Chloroalanine (6)	nd	nd		469 ± 17	60 ± 8[b]	7.8
Cycloserine (3)				319 ± 44	80 ± 8[b]	4.0

CaCo-2 cells were incubated for 18 h with 25-hydroxycholesterol (25-OH, 1 μg/ml), mevalonate (10 mM), U18666A (15 μM), lovastatin (1 μg/ml), β-chloroalanine (1 mM), or cycloserine (1 mM). After the incubation, total membrane HMG-CoA reductase and SPT activities or incorporation of ^3H_2O into cholesterol and [^{3}H]serine incorporation into long-chain bases were determined as described in Methods. Results are expressed as means ± SE; nd, not determined.

[a] Number of dishes in parentheses.

[b] $P < 0.01$ versus control.

in cellular cholesterol metabolism, SPT activity and sphingoid base synthesis remained unchanged. Increasing the concentration of serine in the medium, which significantly increased long chain base synthesis, had no effect on the rate of cholesterol synthesis (Fig. 2). Lovastatin, a competitive inhibitor of HMG-CoA reductase, decreased cholesterol synthesis by 60% but did not alter long-chain base synthesis. Moreover, β-chloroalanine and cycloserine, which decreased the rate of sphingoid base synthesis by 84% and 78%, respectively, did not alter the rate of cholesterol synthesis. If cholesterol and sphingomyelin syntheses were coordinately regulated, the ratios of either HMG-CoA reductase to SPT activities or ^3H_2O incorporation into cholesterol to [^{3}H]serine incorporation into long-chain bases would not vary greatly from controls. The data suggest, however, that the syntheses of these two lipids are regulated independently as the ratios varied as much as 6- to 7-fold.

Effect of fatty acids

In hepatocytes, certain fatty acids have been shown to alter both cholesterol and long-chain base synthesis. To investigate whether fatty acids coordinately regulate these two synthetic pathways in intestinal cells, monolayers were incubated for 18 h in serum-free medium containing 1 mM palmitic, oleic, linoleic, or eicosapentaenoic acids attached to albumin. The results are shown in **Fig. 3.** Only palmitic acid, the preferred substrate of SPT, increased the rate of synthesis of long chain bases. Both palmitic and oleic acids, however, increased cholesterol

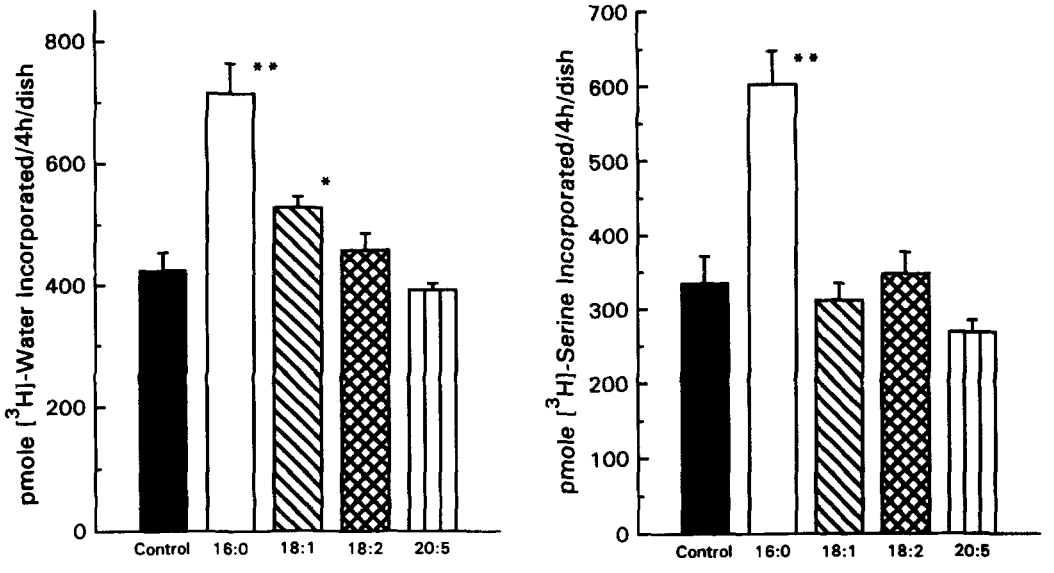

Fig. 3. Effect of fatty acids on cholesterol and long-chain base syntheses. After an 18-h incubation of monolayers with 1 mM of the respective fatty acids attached to albumin, the rates of cholesterol (left panel) and sphingoid base (right panel) synthesis were determined as described in Methods. The data are expressed as the mean of 6 dishes ± SE; *$P < 0.05$ vs. control; **$P < 0.01$ vs. control.

synthesis, although palmitic acid was slightly more potent. Linoleic and eicosapentaenoic acids did not alter cholesterol or sphingoid base synthesis significantly.

Sphingomyelin and cholesterol enrichment

Because the synthesis of cholesterol and sphingomyelin did not appear to be coordinately regulated by modulators that altered the synthetic rates of one of the lipids, it was asked whether changes in the amount of mass of the two lipids within cells would affect their synthesis. To address this, cells were enriched in sphingomyelin or cholesterol by incubating them for 16 h with liposomes containing the appropriate lipid. Control cells were incubated in medium alone or in medium containing the liposomes but without cholesterol or sphingomyelin. After the incubation, the rates of cholesterol and long-chain base synthesis were estimated. **Table 2** shows these results. In cells that were enriched 4-fold in sphingomyelin, i.e., cells incubated with octadecylamine plus sphingomyelin, the rates of synthesis of both cholesterol and long-chain bases were significantly increased compared to the rates observed in cells incubated with octadecylamine alone. In contrast, in cells enriched in cholesterol, the rates of cholesterol and long-chain base synthesis were decreased compared to their controls incubated with liposomes alone. The cells exposed to phosphatidylcholine-phosphatidylserine liposomes without cholesterol had higher rates of cholesterol synthesis compared to the rates observed in cells incubated in medium alone. Sphingoid base synthesis was also significantly higher in these cells. Although there was some variability in the ratios of ^3H_2O to [^{3}H]serine incorporation after lipid enrichment, the ratios approximated the controls more so than those seen under acute conditions listed in Table 1. Thus, changes in mass of cholesterol or sphingomyelin appear to cause a coordinate regulation of the synthesis of these two lipids.

DISCUSSION

In a recent excellent review article on sphingomyelin metabolism, it was noted that alterations in sphingomyelin metabolism affect cholesterol metabolism and vice versa (15). The basis for this assumption is derived from studies demonstrating parallel changes in cellular cholesterol and sphingomyelin mass under varying conditions (3–5). In addition, it has been shown that cholesterol and sphingoid base syntheses are coordinately regulated by LDL in cultured fibroblasts and proximal tubular cells (28, 29). It is clear from the results of the present study, however, that this generality regarding cholesterol and sphingomyelin metabolism is not entirely correct. The two rate-controlling enzymes of cholesterol and sphingomyelin synthesis, HMG-CoA reductase and SPT, respectively, and the synthetic pathways of these lipids, were regulated independently in cultured human intestinal cells. This was demonstrated by several experiments that addressed the regulation of long-chain base synthesis by factors that are known to alter the rates of cholesterol synthesis. For example, 25-hydroxycholesterol is a potent regulator of cellular cholesterol metabolism. The oxygenated sterol decreased HMG-CoA reductase activity by 74% and the rate of cholesterol synthesis by 83%. If sphingomyelin and cholesterol syntheses were coordinately regulated, a significant decrease in either SPT activity or long-chain base synthesis would have been expected. This did not occur. Similarly, U18666A, by inhibiting a step in cholesterol synthesis distal to HMG-CoA reductase, decreased the production of cholesterol by 75%; yet, sphingomyelin synthesis was not affected. Moreover, when the amount of cholesterol produced was increased 3.2-fold by the addition of mevalonate, causing a 5.8-fold reduction in HMG-CoA reductase activity, SPT activity and sphingoid base synthesis were not

TABLE 2. Effect of cholesterol or sphingomyelin enrichment on cholesterol and long-chain base syntheses

Treatment	Cholesterol	Sphingomyelin	^3H_2O Incorporation	[^{3}H]Serine Incorporation	^3H_2O/[^{3}H]Serine
	μg/mg	*nmol/mg*	*pmol/4 h/dish*	*pmol/4 h/dish*	
Control	14.4 ± 0.3	5.55 ± 0.2	463 ± 30	320 ± 17	1.4
Octadecylamine	nd	5.40 ± 0.3	403 ± 19	232 ± 17	1.7
Octadecylamine + sphingomyelin	nd	21.5 ± 5.7[a]	1096 ± 53[b]	330 ± 13[c]	3.3
PS + PC liposomes	15.3 ± 0.9	nd	1019 ± 48[d]	487 ± 62[e]	2.1
PS + PC + cholesterol	18.2 ± 0.5[g]	nd	424 ± 42[f]	275 ± 20[g]	1.5

CaCo-2 cells were enriched in either cholesterol or sphingomyelin as described in Methods. The incorporation of ^3H_2O into cholesterol or [^{3}H]serine into long-chain bases was then estimated as described in Methods. Results are expressed as means ± SE; n = 6; nd, not determined. PS, phosphatidylserine; PC, phosphatidylcholine.

[a] $P < 0.02$ versus control and octadecylamine.
[b] $P < 0.001$ versus control and octadecylamine.
[c] $P < 0.02$ versus octadecylamine.
[d] $P < 0.001$ versus control.
[e] $P < 0.02$ versus control.
[f] $P < 0.001$ versus PS + PC liposomes.
[g] $P < 0.01$ versus PS + PC liposomes.

significantly altered. Inhibitors of reductase (lovastatin) or SPT (β-chloroalanine and cycloserine) also did not affect the synthetic pathways of the corresponding lipid. These data suggest, therefore, that in CaCo-2 cells, cholesterol and sphingomyelin syntheses are not coordinately regulated. Although the regulation of cholesterol metabolism in CaCo-2 cells is similar to what others have observed in non-neoplastic cells, caution should be used in interpreting the present data as differences in the regulation of sterol and sphingoid base synthesis between normal enterocytes and CaCo-2 cells may exist.

Previous studies have suggested that if the mass of either lipid within a membrane is altered, there is a compensatory change in the mass of the other. For example, Slotte and Bierman (30) demonstrated that the depletion of plasma membrane sphingomyelin in fibroblasts caused a similar loss of cholesterol from the membrane. This cholesterol entered the cell and expanded existing pools of cellular cholesterol, causing an inhibition of cholesterol synthesis and an increase in cholesterol esterification. This observation has been reproduced by Gupta and Rudney (31) in IEC cells and by our laboratory in CaCo-2 cells (11). Conversely, when fibroblast membranes were enriched in sphingomyelin, cholesterol synthesis was significantly increased (21). It was postulated that the accumulation of sphingomyelin "trapped" unesterified cholesterol within a cellular compartment, thus not allowing the sterol to regulate its own synthesis. These results, however, do not necessarily imply that sphingomyelin and cholesterol metabolisms are coordinately regulated. Rather, they suggest that the two lipids coexist within the membrane in close association (1, 2, 15). If this association or balance is disrupted, as occurs in atherosclerosis, neoplasia, Niemann-Pick disease type C, or in cells incubated with LDL, cholesterol or sphingomyelin metabolism will be altered because of the abnormal accumulation of either lipid within the cell (9, 10, 28, 29).

The present results support and extend these observations. In cells enriched in sphingomyelin, the rate of cholesterol synthesis was increased 2.4-fold. Whether this relates to the trapping of cholesterol within a particular membrane as postulated, or whether it represents a homeostatic mechanism of the cell to rectify an abnormal membrane sphingomyelin:cholesterol ratio through an increase in cholesterol synthesis, is not certain. If the latter reasoning were correct, however, an increase in cellular sphingomyelin mass might be expected to cause a compensatory decrease in the rate of sphingoid base synthesis. This did not occur. In fact, sphingoid base synthesis was increased compared to its control. The rates of cholesterol and long-chain base synthesis were significantly increased in cells incubated with phospholipid liposomes. It is well recognized that a small amount of cellular cholesterol will efflux into the medium in the presence of unilamellar phospholipid vesicles (32). As we and others have observed previously (33), this can occur without measurable changes in cellular cholesterol mass. Thus, under conditions of cholesterol efflux, both cholesterol and long-chain base synthesis increase together. Moreover, when cells were enriched in cholesterol, the rates of synthesis of both lipids decreased in parallel. These results would suggest, therefore, that a relationship does exist between the metabolism of cholesterol and sphingomyelin. It differs from the acute experiments discussed above in that significant alterations in the mass of these two lipids were required to observe the coordinated regulation.

In vitro studies performed in a variety of tissues, including rat intestine, have demonstrated that palmitoyl-CoA is the preferred substrate of SPT (14, 24, 34). Similar to previous observations made in rat hepatocytes (35), only the addition of palmitic acid to cultured intestinal cells increased the flux of serine into long-chain bases. Thus, the absorption of palmitic acid by the intestine would likely lead to an increase in the synthesis of sphingomyelin. In intestinal cells incubated with palmitic acid, the rate of cholesterol synthesis was also increased. Oleic acid, however, which did not increase the synthesis of sphingomyelin, similarly increased cholesterol synthesis. Palmitic acid, like oleic acid (36), promotes triglyceride-rich lipoprotein secretion in CaCo-2 cells (S. Murthy and F. J. Field, unpublished observations), a process that requires unesterified cholesterol. Thus, the coordinate regulation of sphingomyelin and cholesterol synthesis after the addition of palmitate may be unrelated to its effect on SPT activity and sphingoid base synthesis. Palmitic acid could have a direct effect on the cholesterol synthetic pathway or it could, like oleic acid, indirectly increase cholesterol synthesis by stimulating lipoprotein secretion. The disparity observed between the effects of palmitic and oleic acids on cholesterol and sphingomyelin syntheses lend further support for the independent nature of their acute regulation.

This is the first report that demonstrates the presence of SPT activity in human small intestine. Merrill, Nixon, and Williams (14) reported an SPT activity of 32.7 $\pm$ 17.2 pmol/mg per min in rat intestinal microsomes. Considering the absence of activity in cytosol, this is not significantly different from our activity of 21.1 $\pm$ 6.1 pmol/mg per min in whole homogenates of human small intestine. The importance of sphingomyelin synthesis, and thus SPT activity, in the intestine is unknown. Most dietary sphingomyelin is thought to be hydrolyzed in the lumen of the small intestine, either by a pancreatic lipase or by a neutral sphingomyelinase present in the brush-border membrane (11, 37). It is likely, therefore, that de novo synthesis of the sphingolipid may be necessary to maintain sufficient amounts of sphingomyelin for the synthesis and assembly of chylomicrons. Thus, SPT could be

playing a role in the absorption and transport of lipids by the intestine. Studies are presently underway to address this.

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