Sphingomyelin content of intestinal cell membranes regulates cholesterol absorption

Evidence for pancreatic and intestinal cell sphingomyelinase activity

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Micellar cholesterol uptake and secretion were investigated in the human intestinal cell line CaCo-2 following depletion of apical membrane sphingomyelin. The addition of exogenous sphingomyelinase, which hydrolysed 60 % of prelabelled sphingomyelin, resulted in a 50 % decrease in the uptake of cholesterol from bile salt micelles. The flux of membrane cholesterol into the cell by the hydrolysis of membrane sphingomyelin decreased the rate of cholesterol synthesis by 43 % and inhibited hydroxymethylglutaryl-CoA reductase activity by 54 %. Moreover, the rate of cholesterol esterification was increased 4-fold. Total cellular cholesterol mass was unchanged by the addition of sphingomyelinase; however, cholesteryl esters increased by 50 % and the amount of unesterified cholesterol decreased significantly. The basolateral secretion of cholesterol mass was also decreased following sphingomyelin hydrolysis. Human pancreatic juice was found to contain neutral sphingomyelinase activity which required taurocholate for full expression. The presence of neutral sphingomyelinase activity was also documented in membranes prepared from CaCo-2 cells and in whole homogenates from human duodenal biopsies. The data suggest that the amount of sphingomyelin present in the apical membrane of the intestinal absorptive cell regulates cholesterol uptake from bile salt micelles. Sphingomyelinase activity within intestinal cells and in pancreatic juice could alter the sphingomyelin content of brush-border membranes of small intestinal absorptive cells and thus regulate the amount of cholesterol absorbed by the gut.

INTRODUCTION

The intestine plays a major role in regulating cholesterol homeostasis within the body (for a review, see [1]). In this regard, its exclusive and perhaps most important function is the absorption of dietary cholesterol. Despite its importance in controlling the amount of exogenous cholesterol which enters the body daily, little is known about endogenous factors which might regulate cholesterol absorption by the small-intestinal absorptive cell. Earlier data have suggested that the uptake of luminal cholesterol by the enterocyte is a passive process, depending solely upon the solubility of the sterol in the bulk water phase and the passive permeability coefficient of the molecule across the membrane [2,3]. Bosner et al. [4], however, have provided data which suggest that cholesterol absorption may be a facilitated process. These investigators demonstrated that pancreatic enzymes bind to heparin on intestinal cell membranes, which then facilitate the absorption of the subsequently hydrolysed lipids. More recently, Thurnhofer and co-workers [5–7] have postulated the involvement of a cholesterol-transfer protein in the intestinal cell brush-border membrane which mediates the uptake of cholesterol. They found that proteinase K treatment of brush-border membranes reduced the rate of cholesterol absorption and changed cholesterol absorption from a second-order to a first-order reaction. The uptake of micellar cholesterol could also be dependent upon the capacity of the brush-border membrane of the small-intestinal absorptive cell to solubilize the micellar cholesterol. Sphingomyelin, a phospholipid which is found in the plasma membrane of all eukaryotic cells, has a high affinity for cholesterol and is strongly correlated with the amount of cholesterol which is present in membranes [8]. In a variety of cultured cells, Slotte & Bierman [9] and Gupta & Rudney [10] have demonstrated that the depletion

of plasma membrane sphingomyelin causes a rapid flux of plasma membrane cholesterol into the cell. The resulting expansion of the intracellular pools of cholesterol leads to an inhibition of cholesterol synthesis and the stimulation of cholesterol esterification.

In view of these previous studies, we have postulated that the amount of sphingomyelin present in the brush-border membrane of the small-intestinal absorptive cell might regulate the uptake and secretion of dietary cholesterol by altering the solubility of cholesterol within the membrane. We further postulated that human intestine and pancreatic juice might contain sphingomyelinase activity. By the hydrolysis of brush-border membrane sphingomyelin, pancreatic and intestinal sphingomyelinase could contribute to the regulation of cholesterol absorption.

The present study confirms that human pancreatic juice and human intestine contain neutral sphingomyelinase activity. The pancreatic sphingomyelinase requires taurocholate for full expression of the activity. Moreover, the hydrolysis of apical membrane sphingomyelin inhibits the cellular uptake of micellar cholesterol and decreases the secretion of unesterified cholesterol.

MATERIALS AND METHODS

Materials

[9,10-³H]Oleic acid, [2-¹⁴C]acetate, [4-¹⁴C]cholesterol, [5-³H]mevalonic acid, 3-hydroxy-3-methyl[3-¹⁴C]glutaryl-CoA, [methyl-³H]choline chloride and [choline-methyl-¹⁴C]sphingomyelin were purchased from New England Nuclear (Boston, MA, U.S.A.). Oleic acid, glucose 6-phosphate, glucose-6-phosphate dehydrogenase, cholesterol, sodium taurocholate, ADP, cutscum, sphingomyelin, sphingomyelinase and mono-olein were purchased from Sigma (St. Louis, MO, U.S.A.). Hydroxymethylglutaryl (HMG)-CoA was from P. L. Biochem-

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icals (Milwaukee, WI, U.S.A.). All other chemicals were reagent grade.

Cell culture

CaCo-2 cells were cultured as previously described, with the exception that some experiments were performed on 14-day-old monolayers grown in 24- or 96-well plates [11].

Sphingomyelin hydrolysis

CaCo-2 cells were cultured for 10 days in 24-well plates. The cells were then incubated for 48 h in Dulbecco's modified Eagle's medium (DMEM) containing 20% delipidated fetal calf serum and [methyl-^{3}H]choline (0.5 μCi/dish). The cells were then rinsed twice with medium-199/Earle's (M199; Gibco, Grand Island, NY, U.S.A.). Sphingomyelinase was added to the cells in 0.5 ml of M199. Following the incubation with sphingomyelinase, the cell lipids were extracted with chloroform/methanol (2:1, v/v) and the phospholipids were separated on silica gel G plates developed in chloroform/methanol/glacial acetic acid/water (75:45:12:6, by vol.). The radioactivities corresponding to authentic phosphatidylcholine and sphingomyelin were collected and counted. The amount of radioactivity present in phospholipids from control dishes which were not incubated with sphingomyelinase represented 100%.

Measurement of lipid synthesis

The rates of cholesteryl ester, triacylglycerol and phospholipid synthesis were estimated by labelled oleic acid incorporation into the respective lipid class as previously described [12]. Cholesterol synthesis was determined by [^{14}C]acetate incorporation into cholesterol as described [13].

Cholesterol and oleic acid uptake

CaCo-2 cells were placed on fibronectin-treated micropore polycarbonate filters (Transwells-3412; 0.4 μM pore size; Costar, Cambridge, MA, U.S.A.) separating an upper and lower well as previously described [11]. Medium containing fetal bovine serum was removed from the bottom well and replaced with M199 containing 10 mM-Hepes, without fetal bovine serum, pH 7.4. The medium from the top well was also removed and the cells were washed three times with 1 ml of M199 buffer. Sphingomyelinase, at the concentration indicated for each experiment, was added in aqueous buffer. Control incubations included the buffer vehicle without sphingomyelinase. After the preincubation with the enzyme, the apical medium was replaced with M199 containing 1 mM-taurocholate, 30 μM-mono-olein and 50 μM-[^{14}C]cholesterol (sp. radioactivity 5000 d.p.m./nmol) with or without sphingomyelinase. Micellar solutions were prepared as previously described [14]. After 1, 2 and 4 h, the medium in the basolateral well was removed and lipids were extracted with chloroform/methanol (2:1, v/v). The cells on the micropore filter were washed three times with 1 ml of ice-cold phosphate-buffered saline (137 mM-NaCl, 2.7 mM-KCl, 0.5 mM-MgCl$_2$,6H$_2$O, 1.5 mM-KH$_2$PO$_4$, 8 mM-Na$_2$HPO$_4$, pH 7.4). The cells were scraped carefully from the filters in 1 ml of 0.2 M-NaOH. After neutralizing the alkali with HCl, lipids were extracted from the cell digests with chloroform/methanol (2:1, v/v). The chloroform phase of the lipid extracts was taken to dryness under a stream of nitrogen. The residue was taken up in 0.125 ml of chloroform and applied to silica gel G t.l.c. plates. The plates were developed with hexane/diethyl ether/methanol/acetic acid (70:30:1:1, by vol.), and the cholesterol and cholesteryl esters were visualized by iodine vapours. The bands corresponding to authentic standards of cholesterol and cholesteryl esters were scraped from the plates into 4 ml of liquid scintillation fluid and counted as previously described [11].

To estimate oleic acid uptake, the experimental design was exactly the same as that described for cholesterol uptake. [^{3}H]Oleic acid (25 μM; sp. radioactivity 955 d.p.m./pmol) was solubilized in 1 mM-taurocholate and 30 μM-mono-olein and added to the apical medium. After 1, 2 and 4 h, the cells were washed three times with 1 ml of ice-cold phosphate-buffered saline. The cells were gently scraped from the filters in 1 ml of 0.2 M-NaOH, neutralized with HCl, and the lipids were extracted from the cells in chloroform/methanol (2:1, v/v). The chloroform phase was taken to dryness under a stream of nitrogen and the amount of label in the total lipid fraction was determined by liquid scintillation counting.

Enzyme assays

HMG-CoA reductase was measured as previously described [11]. Sphingomyelinase activity was measured according to the method of Chatterjee & Ghosh [15] using [^{14}C]sphingomyelin (sp. radioactivity 400 c.p.m./nmol) as the substrate. Briefly, in a final volume of 0.2 ml, the assay mixture contained 25 μmol of Tris/glycine buffer (pH 7.4), 2.5 pmol of MgCl$_2$, 50 nmol of sphingomyelin, 0.5 mg of human serum albumin, 0.1 mg of cutscum and 10–150 μg of protein as the enzyme source. The usual incubation was for 1 h at 37 °C. The reaction was terminated by adding 1 ml of ice-cold 10% trichloroacetic acid. BSA (0.1 mg) was added and the contents were mixed and allowed to settle for 5 min at room temperature before centrifuging for 5 min at 11000 g at 4 °C. A 1 ml portion of the supernatant was extracted with 1 ml of anhydrous ether at 4 °C. The aqueous phase containing the labelled phosphocholine was taken for scintillation counting in 10 ml of scintillation fluid. Controls for the assay contained no protein or protein which had been heated to 60 °C for 10 min, or similar amounts of taurocholate but with heated enzyme. All blank controls contained only background radioactivity.

Chemical and statistical analysis

Cellular and basolateral medium cholesterol mass were estimated by g.l.c. using cholestane as an internal standard [16]. Protein was determined according to the method of Lowry et al. [17]. The unpaired Student's t test was used to determine significance.

RESULTS

Sphingomyelin hydrolysis

To estimate the amount of membrane sphingomyelin which was hydrolysed by sphingomyelinase, CaCo-2 cells were incubated for 48 h with [^{3}H]choline to label the choline-containing phosphilipids. The percentage of label which remained in sphingomyelin and phosphatidylcholine after adding sphingomyelinase to the culture medium was then determined (Fig. 1). The hydrolysis of sphingomyelin by sphingomyelinase was directly related to the concentration of added sphingomyelinase and the duration of the incubation. Maximal hydrolysis of sphingomyelin occurred at a sphingomyelinase concentration of 100 munits/ml. At this concentration, sphingomyelinase had no effect on the percentage of label which remained in phosphatidylcholine. At the highest concentration of sphingomyelinase used (1 unit/ml), the hydrolysis of choline-containing phospholipids was no longer specific for sphingomyelin. Maximal hydrolysis had occurred by 4 h; at this time, 60% of labelled sphingomyelin had been hydrolysed, and the effect plateaued thereafter.
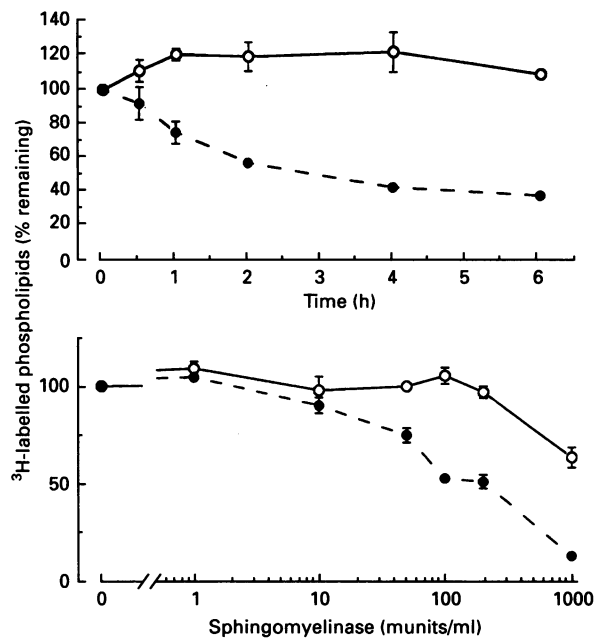
Fig. 1. Effect of sphingomyelinase on the hydrolysis of [³H]choline-labelled phospholipids

On day 10 after plating, the medium was changed to DMEM containing 20 % delipidated fetal calf serum and [methyl-³H]choline (0.5 μCi/well). After 48 h, the medium was removed and the cells were rinsed twice in M199. Sphingomyelinase was added (100 munits/ml for the time course and 4 h for the concentration course) and the radioactivity remaining in sphingomyelin (●) and phosphatidylcholine (○) was determined as described in the Materials and methods section. Each point represents the mean±S.E.M. of three to six individual dishes; '100%' equals 4100 c.p.m. and 38000 c.p.m. for sphingomyelin and phosphatidylcholine respectively. ○, phosphatidylcholine; ●, sphingomyelin.

Fig. 2. Effect of sphingomyelinase on the incorporation of [¹⁴C]acetate into cellular lipids

CaCo-2 cells were incubated overnight in M199. The next morning, the cells were incubated for 4 h with sphingomyelinase at the concentrations given. During the last 2 h of incubation, [¹⁴C]acetate (5 mM; specific radioactivity 150 d.p.m./nmol) was added and the incorporation of labelled acetate into cell total lipids, fatty acids and unesterified cholesterol was determined as described in the Materials and methods section. Values represent the means±S.E.M. of three individual dishes.

Effect of sphingomyelinase on cellular cholesterol metabolism

To investigate the effect of membrane sphingomyelin hydrolysis on cholesterol synthesis, CaCo-2 cells were incubated with sphingomyelinase, and the incorporation of labelled acetate into total lipids, fatty acids and cholesterol was determined. In cells incubated with sphingomyelinase, there was a dose-dependent decrease in the incorporation of acetate into unesterified cholesterol (Fig. 2). The effect of sphingomyelinase on cholesterol synthesis was specific, as sphingomyelinase had no significant effect on the incorporation of acetate into total lipids or fatty acids.

Data in Fig. 3 show that, in whole homogenates prepared from CaCo-2 cells incubated with increasing concentrations of sphingomyelinase, HMG-CoA reductase activity was inhibited by 54%. Sphingomyelinase had no effect on HMG-CoA reductase activity when added to the in vitro assay for reductase, suggesting that intact cells were required for this inhibitory effect (results not shown).

To investigate the effect of sphingomyelin hydrolysis on cholesteryl ester synthesis, CaCo-2 cells were incubated with sphingomyelinase and the incorporation of labelled oleate into cholesteryl esters, triacylglycerols and phospholipids was determined (Fig. 4). In a dose-dependent manner, the hydrolysis of membrane sphingomyelin resulted in up to 4-fold increase in the synthesis of cholesteryl esters. In contrast, the rate of incorporation of labelled oleate into triacylglycerols was not altered. There was a modest decrease in the incorporation of oleate into phospholipids which occurred at the higher concentrations of sphingomyelinase.
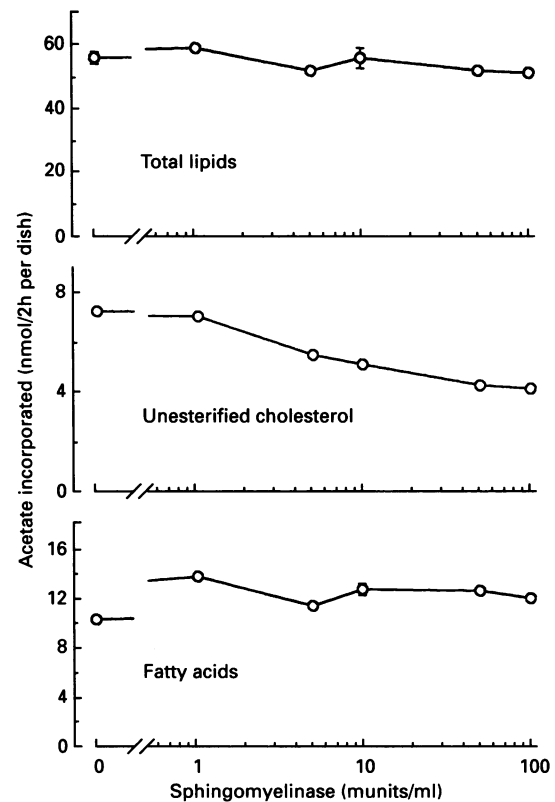
Effect of sphingomyelinase on cellular cholesterol mass

To determine whether the hydrolysis of membrane sphingomyelin altered the cholesterol content of the cells, unesterified and esterified cholesterol were measured in CaCo-2 cells incubated for 4 h with sphingomyelinase. Table 1 shows data from three separate experiments. Total cellular cholesterol was not altered by the addition of sphingomyelinase. The percentage of total cholesterol which was present in the esterified form, however, was significantly increased in the three individual experiments. The amount of unesterified cholesterol was decreased by approx. 1.5 μg/mg of protein. It appears that, following sphingomyelin hydrolysis, there occurs an influx of membrane cholesterol which leads in turn to an increase in cholesteryl ester synthesis, thereby causing an increase in cholesteryl ester mass within these cells.

Effect of sphingomyelinase on cholesterol uptake

The effect of sphingomyelin hydrolysis on the uptake and secretion of micellar cholesterol was then studied. CaCo-2 cells were grown on semi-permeable micropore filters separating upper and lower wells. Following a 4 h preincubation with sphingomyelinase, ¹⁴C-labelled cholesterol solubilized in a bile salt micelle was added to the apical medium for 1, 2 and 4 h. The amount of label associated with the cell and that found in the basolateral medium was then determined (Fig. 5). In CaCo-2 cells preincu-
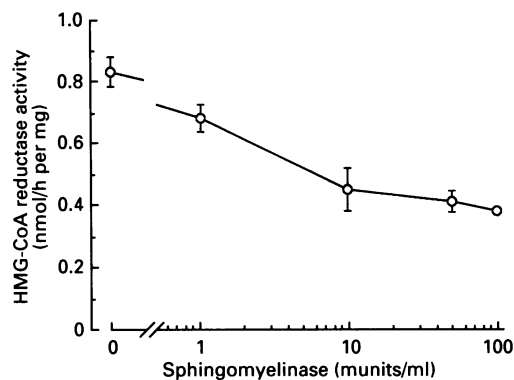
Fig. 3. Effect of sphingomyelinase on HMG-CoA reductase activity in CaCo-2 cells

CaCo-2 cells were incubated overnight in M199. The next morning, the cells were incubated for 4 h with increasing concentrations of sphingomyelinase. At the end of the incubation, the cells were rinsed twice with ice-cold phosphate-buffered saline. The cells were then homogenized in buffer containing 0.04 M-KH_2PO_4, 0.05 M-KCl, 0.25 M-sucrose and 0.03 M-EDTA, pH 7.2. The whole homogenate was used to measure HMG-CoA reductase activity as described in the Materials and methods section. The values represent the means $\pm$ S.E.M. of three individual dishes.
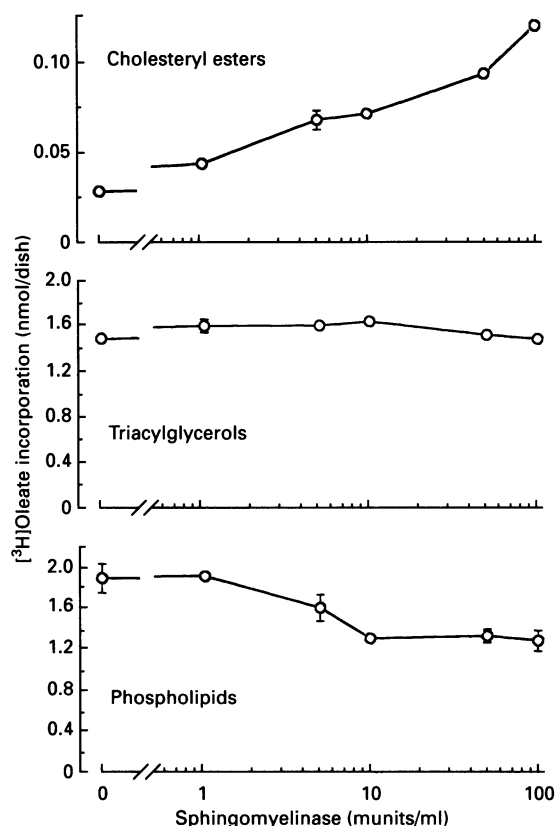

Fig. 4. Effect of sphingomyelinase on [³H]oleate incorporation into cellular lipids

CaCo-2 cells were incubated overnight in M199. The next morning, the cells were incubated for 4 h with increasing concentrations of sphingomyelinase. During the last 2 h of the incubation, [³H]oleate attached to albumin (50 μM; specific radioactivity 725 d.p.m./pmol) was added and the incorporation of labelled oleate into cholesteryl esters, triacylglycerols, and phospholipids was determined as described in the Materials and methods section. Data are expressed as the means $\pm$ S.E.M. of three individual dishes.

bated with sphingomyelinase there was significantly less micellar cholesterol associated with the cell at each time point compared with the amount observed in control cells. The percentages of labelled cellular cholesterol which were found in the esterified form was similar in control cells and cells preincubated with sphingomyelinase.

Labelled cholesteryl esters were not detectable in the baso-lateral medium over the 4 h incubation period. The amount of labelled unesterified cholesterol which was secreted into the basolateral medium by cells incubated with sphingomyelinase was very similar to the amount secreted from control cells. Because sphingomyelin hydrolysis causes significant alterations in cellular esterified and unesterified cholesterol mass, the specific radioactivity of the intracellular labelled cholesterol will be different between control cells and cells incubated with sphingo-myelinase. To circumvent this problem in estimating the amount of cholesterol being secreted by the cells, cholesterol mass was

measured in the basolateral medium of control cells and of cells preincubated with sphingomyelinase. The results are shown in the last column of Table 1. In all three experiments, less unesterified cholesterol was found in the basolateral medium of

Table 1. Effect of sphingomyelinase on cellular cholesterol and cholesterol secreted into the basolateral medium

CaCo-2 cells were cultured on semi-permeable micropore filters. On the day of the experiment, the cells were washed and incubated apically for 4 h with M199 containing sphingomyelinase (100 munits/ml). At the end of the incubation, the cells were washed with ice-cold phosphate-buffered saline, scraped from the filters, and lipids were extracted with chloroform/methanol (2:1, v/v). Lipids were extracted from the medium of six combined lower wells. Cholesterol was determined by g.l.c. as described in the Materials and methods section. The data are expressed as means $\pm$ S.E.M. from 6–12 filters (actual numbers given in parentheses). Data for medium cholesterol are expressed per six wells, assayed in duplicate. *$P < 0.05$; **$P < 0.001$ versus controls (Student's t test).

Experiment	Cellular cholesterol (μg/mg of protein)		Medium cholesterol (μg/six wells)	
	Unesterified	Ester	Unesterified	Ester
1				
Control (5)	16.5 ± 0.24	0.80 ± 0.39	8.5	–
Sphingomyelinase (6)	15.1 ± 0.19*	1.66 ± 0.31*	4.4	–
2				
Control (12)	18.8 ± 0.40	1.91 ± 0.33	4.7	–
Sphingomyelinase (11)	16.9 ± 0.19ß	2.88 ± 0.34*	3.6	–
3				
Control (6)	16.1 ± 0.65	1.00 ± 0.14	9.7	–
Sphingomyelinase (6)	14.9 ± 0.72	1.50 ± 0.16*	8.2	–

Fig. 6. Effect of sphingomyelinase on the uptake of micellar [^{3}H]oleic acid into cellular total lipids

CaCo-2 cells were cultured and incubated as described in the legend to Fig. 5. After a 4 h incubation with sphingomyelinase, 25 μM-[^{3}H]oleic acid (specific radioactivity 995 d.p.m./pmol), solubilized in 1 mM-taurocholate and 30 μM-mono-olein, was added to the apical medium. Sphingomyelinase was added back to those incubations which contained it originally. After 1, 2 and 4 h the amount of labelled oleate found in cellular total lipids was determined. The data are expressed as the means $\pm$ S.E.M. of four separate experiments done in triplicate dishes. O, Control; ●, sphingomyelinase-treated.

Table 2. Sphingomyelinase activity in human pancreatic juice, human duodenal biopsies and CaCo-2 cells

Pancreatic juice was obtained from three different patients who were undergoing diagnostic and/or therapeutic endoscopic pancreatography. Biopsies were obtained from two patients who were undergoing endoscopic small-intestinal biopsy as part of their evaluation. The biopsies were homogenized in phosphate-buffered saline and used for the assay. Sphingomyelinase activity was estimated as described in the Materials and methods section. The values represent the means of triplicate assays. CaCo-2 cells (10 mg of protein) were homogenized in 7 ml of phosphate-buffered saline at 4 °C. The whole homogenate was centrifuged at 105000 g for 1 h. The resulting membrane pellet was dispersed in phosphate-buffered saline, and along with the cytosol was assayed for sphingomyelinase activity as described in the Materials and methods section.

Fig. 5. Effect of sphingomyelinase on the uptake and secretion of micellar [^{14}C]cholesterol

CaCo-2 cells, cultured on semi-permeable micropore filters, were incubated overnight in M199. The next morning, the cells were rinsed twice with M199, and sphingomyelinase (100 munits/ml) was added to the apical medium. After 4 h, 50 μM-[^{14}C]cholesterol (specific radioactivity 5000 d.p.m./nmol), solubilized in 1 mM-taurocholate and 30 μM-mono-olein, was added to the apical medium. Sphingomyelinase was added back to those incubations which contained it originally. After 1, 2 and 4 h the amount of labelled cholesterol associated with the cell (a), that esterified within the cell (b), and that secreted into basolateral medium (c) were determined as described in the Materials and methods section. The data are expressed as the means of two transwells at each time point. This experiment is representative of four separate experiments, all showing similar results. O, Control; ●, sphingomyelinase-treated.
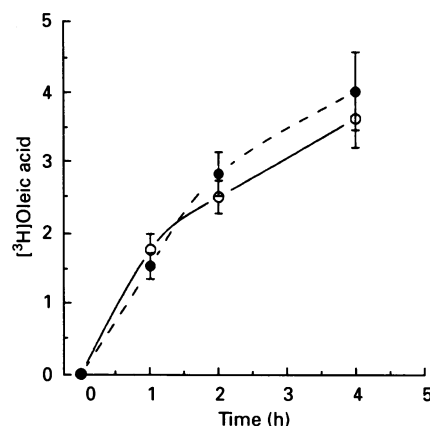
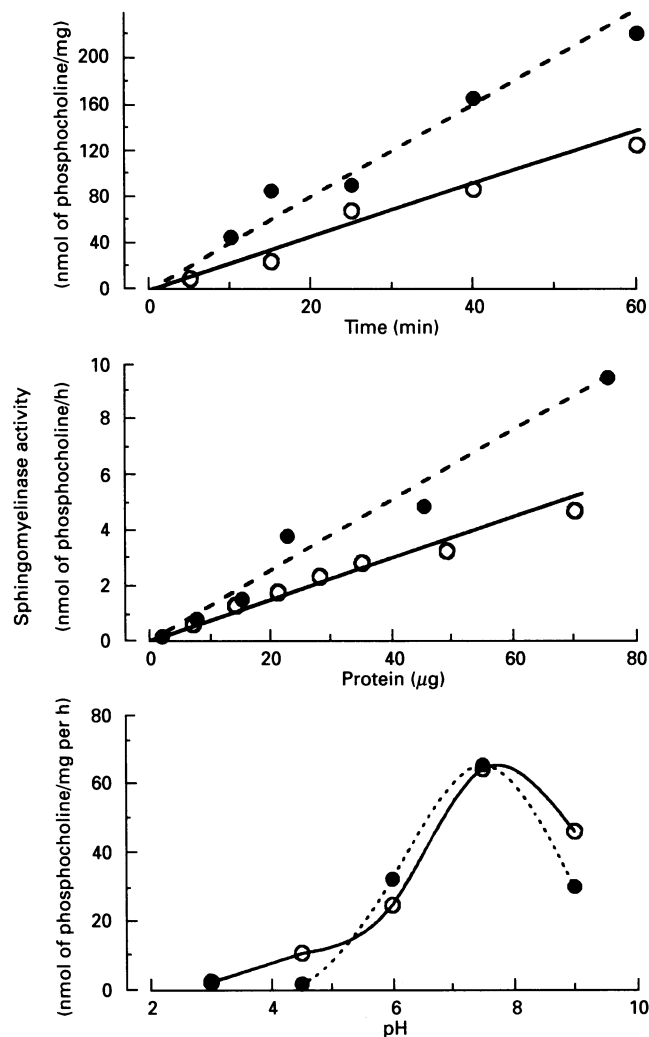|	Sphingomyelinase activity (nmol of phosphocholine/ h per mg of protein)
Pancreatic juice	
Sample 1 (bile-contaminated)	
— Taurocholate	120
+ Taurocholate	180
Sample 2	
— Taurocholate	10.1
+ Taurocholate	69.4
Sample 3	
— Taurocholate	4.74
+ Taurocholate	46.7
Duodenal biopsies	
Sample 1	526
Sample 2	229
CaCo-2 cells	
Membrane pellet	78.3
Cytosol	0–1.5

Fig. 7. Sphingomyelinase activity in CaCo-2 cells and in human pancreatic juice

Total membranes of CaCo-2 cells were prepared by centrifuging a whole-homogenate preparation at 105000 g for 1 h. The membrane preparation was dispersed in phosphate-buffered saline and sphingomyelinase activity was determined using 0.125 mg of protein. Sphingomyelinase activity was estimated in pancreatic juice contaminated with bile. The amount of protein used for the assay was 0.050 mg. The pH of the assay was adjusted with either 125 mM-sodium acetate/acetic acid buffer (pH 3.0–6.0) or 125 mM-Tris/glycine buffer (pH 7.0–9.0). The data are expressed as the means of duplicate assays at each point or triplicate assays at each pH. O, CaCo-2 cell membranes; ●, pancreatic juice.

CaCo-2 cells preincubated with sphingomyelinase than in that of controls. Cholesteryl ester mass was not measurable in any of the experiments.

In contrast to the effect of sphingomyelin hydrolysis on cholesterol uptake, the uptake of labelled oleic acid into total cell lipids was not altered by sphingomyelinase (Fig. 6). In an experiment performed exactly as described for cholesterol uptake, the amounts of labelled oleate found in the total lipids at 1, 2 and 4 h was similar in control cells and in cells incubated with sphingomyelinase.

Sphingomyelinase activity in human pancreatic juice, human duodenal biopsies and CaCo-2 cells

Since exogenously added sphingomyelinase led to an alteration in cholesterol metabolism and a decrease in cholesterol absorption in CaCo-2 cells, it was questioned whether the pancreas

could be playing a role in regulating intestinal cholesterol metabolism by secreting sphingomyelinase into the lumen. To investigate this, human pancreatic juice was obtained by direct cannulation of the pancreatic duct in patients undergoing diagnostic or therapeutic endoscopic retrograde pancreatography. The pancreatic secretions were then analysed for sphingomyelinase activity using labelled sphingomyelin as a substrate (Table 2, Fig. 7). Fortuitously, the first sample collected was visibly contaminated with bile; sphingomyelinase activity was easily detectable in this sample. In contrast, in pancreatic juice without visible bile contamination, sphingomyelin hydrolysis activity was substantially lower. On addition of 5 mM-taurocholate to the specimens containing pure pancreatic juice, sphingomyelinase activity was enhanced 7–10-fold, suggesting that bile salts were required for full expression of pancreatic sphingomyelinase activity. As shown in Fig. 7, pancreatic juice sphingomyelinase activity was maximal at neutral pH.

The results of an earlier study suggested that porcine intestinal mucosa also contains sphingomyelinase activity [18]. To investigate whether human small intestinal mucosa contains sphingomyelinase activity, biopsies were obtained from the distal duodenum in two different patients undergoing endoscopic small-intestinal biopsies as part of their evaluation. Table 2 shows the sphingomyelinase activities of whole homogenates prepared from the two biopsies. Compared with the activities observed in the pancreatic secretions, sphingomyelinase activities were substantially higher in the duodenal mucosa.

The rate of sphingomyelin hydrolysis was also measured in the cytosol and in a total membrane preparation from CaCo-2 cells (Table 2 and Fig. 7). Essentially all of the sphingomyelinase activity was associated with the membrane fraction, as negligible activity was observed in the cytosol. Data in Fig. 7 demonstrate that this membrane-associated activity was also maximal at neutral pH. When taurocholate was added to the sphingomyelinase assay containing CaCo-2 cell membranes, the activity was not enhanced (results not shown). This suggests that, unlike pancreatic sphingomyelinase activity, taurocholate is not required for the full expression of the cellular enzyme.

DISCUSSION

Data from the present study provide strong support for a regulatory role for apical membrane sphingomyelin content in the absorption of micellar cholesterol by the small-intestinal absorptive cell. It has been demonstrated that this membrane phospholipid has a high affinity for cholesterol, suggesting that sphingomyelin is partially responsible for maintaining the solubility of cholesterol within the membrane [19,20]. When the plasma-membrane sphingomyelin content is decreased, cholesterol leaves the plasma membrane and enters the cell [9]. Conversely, if sphingomyelin is incorporated into cellular plasma membranes, there occurs a marked redistribution of intracellular cholesterol pools to the plasma membrane [21]. Since the rate of transfer of cholesterol from micelles to the absorptive cell membrane depends upon the partitioning of cholesterol between the micelle and the cell membrane [2,3,22], it makes sense that, when the brush-border membrane contains less sphingomyelin, less micellar cholesterol is taken up due to a decrease in the capacity of the brush-border membrane to solubilize, and therefore accept, the sterol. These conditions would favour the partitioning of luminal cholesterol to the micelle. In CaCo-2 cells, in which the labelled sphingomyelin content was decreased by 60%, approx. 50% less micellar cholesterol was taken up compared with the amount taken up by control cells.

Slotte & Bierman [9] and Gupta & Rudney [10] investigated the effect of plasma-membrane sphingomyelin hydrolysis on

cholesterol metabolism in a variety of cell types. These investigators demonstrated that, on hydrolysis of sphingomyelin, there occurred an influx of plasma-membrane cholesterol into intracellular metabolic pools of cholesterol. With the expansion of these pools, the activity of HMG-CoA reductase was decreased and acyl-CoA:cholesterol acyltransferase (ACAT) activity was increased. The human intestinal cell, CaCo-2, responded similarly to the hydrolysis of membrane sphingomyelin. The rate of cholesterol synthesis was decreased by 43%, and this was accompanied by a 54% decrease in HMG-CoA reductase activity. Moreover, with the expansion of the ACAT substrate pool, the rate of cholesterol esterification was increased 4-fold. In IEC-6 cells, Gupta & Rudney [10] demonstrated that the products of sphingomyelin hydrolysis, i.e. ceramide phosphocholine, did not alter cellular cholesterol metabolism; rather, it was postulated that, from the influx of membrane cholesterol, an oxysterol was generated at the putative regulatory site causing the regulation of cholesterol metabolism. Further studies would be required to investigate whether this occurs in CaCo-2 cells.

The addition of sphingomyelinase and the subsequent hydrolysis of sphingomyelin resulted in significant changes in cholesterol mass within CaCo-2 cells. The mass of cholesteryl esters increased by approx. 50%, and unesterified cholesterol levels were decreased by 1.5 μg/mg of protein. Total cholesterol mass was unchanged by sphingomyelin hydrolysis, suggesting that unesterified cholesterol in the brush-border membrane, rather than effluxing from the cell, was being channelled into an ester storage pool within the cell. These major shifts in cellular cholesterol pools, however, make the interpretation of data obtained by using labelled cholesterol to estimate cholesterol esterification and basolateral secretion very difficult. For example, a substantial influx of membrane cholesterol into the substrate pool of ACAT will dilute the labelled cholesterol in this particular pool. Without knowledge of the specific radioactivity of this particular cholesterol pool, the calculated rate of esterification of the labelled cholesterol taken up by the cell will be underestimated. The observation that a similar percentage of absorbed cholesterol was found in the esterified form both in the presence and the absence of sphingomyelinase suggests that the esterification of absorbed cholesterol was probably enhanced by sphingomyelin hydrolysis. Without information on the specific radioactivity of intracellular cholesterol, however, this remains conjecture.

For these reasons, the cholesterol mass was also measured in the basolateral medium to determine the effect of sphingomyelin hydrolysis on the secretion of cholesterol. In three separate experiments, cells preincubated with sphingomyelinase secreted less cholesterol into the basolateral medium than did control cells. Further studies are required to determine the effect of sphingomyelin hydrolysis on lipoprotein assembly and/or secretion. However, the data presented here suggest that the depletion of sphingomyelin from the apical membranes of CaCo-2 cells results in a decrease in the uptake and secretion of cholesterol.

To our knowledge, this is the first study which demonstrates the existence of sphingomyelinase activity in human intestinal mucosa and human pancreatic juice. At this time, it is not known whether this activity represents a specific enzyme which hydrolyses only sphingomyelin, or whether the activity represents a non-specific lipase capable of sphingomyelin hydrolysis. Nilsson [18] previously observed sphingomyelinase activity in human duodenal contents. He postulated, however, that this activity originated from the mucosa of the intestine, as pig mucosal

homogenates hydrolysed sphingomyelin whereas rat pancreatic juice did not. As with another pancreatic lipase (cholesterol esterase), pancreatic sphingomyelinase activity was maximal at neutral pH and required taurocholate for full expression [23,24]. An obvious function of pancreatic sphingomyelinase may be to hydrolyse dietary sphingolipids. It is quite possible, however, that within the intestinal lumen, pancreatic sphingomyelinase could lead to the remodelling of the brush-border membrane of the absorptive cell. The observation that human duodenal mucosa and CaCo-2 cells contain a membrane-bound sphingomyelinase suggests that the endogenous enzyme could also alter the amount of sphingomyelin present in the brush-border membrane. It is tempting to speculate that variability in the sphingomyelin content of the brush-border membrane of the absorptive cell may be one of the factors which could contribute to the large individual variation seen in cholesterol absorption among human subjects. Sphingomyelinase activities present within intestinal cells and in pancreatic juice could regulate intestinal cholesterol absorption by altering the amount of sphingomyelin in the plasma membrane of absorptive cells.

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