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Rapid turn-over of plasma membrane sphingomyelin and cholesterol in baby hamster kidney cells after exposure to sphingomyelinase

J. Peter Slotte, Ann-Sofi Härmälä, Christian Jansson and M. Isabella Pörn

Department of Biochemistry and Pharmacy, Åbo Akademi University, Turku (Finland)

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Plasma membrane sphingomyelin in baby hamster kidney (BHK-21) cells was hydrolyzed with sphingomyelinase (*Staphylococcus aureus*) and the effects on membrane cholesterol translocation and the properties of membrane bound adenylate cyclase and Na^+/K^+-ATPase were determined. Exposure of confluent BHK-21 cells to 0.1 U/ml of sphingomyelinase led to the degradation (at 37°C) of about 60% of cell sphingomyelin. No simultaneous hydrolysis of phosphatidylcholine occurred. The hydrolysis of sphingomyelin subsequently led to the translocation (within 40 min) of about 50–60% of cell [^{3}H]cholesterol from a cholesterol oxidase susceptible pool to an oxidase resistant compartment. The translocation of [^{3}H]cholesterol from the cell surface to intracellular membranes was accompanied by a paralleled increase in [^{3}H]cholesterol ester formation. When cells were first exposed to sphingomyelinase (to degrade sphingomyelin) and then incubated without the enzyme in serum-free media, the mass of cell sphingomyelin decreased initially (by 60%), but then began to increase and reached control levels within 3–4 h. The rapid re-synthesis of sphingomyelin was accompanied by an equally rapid normalization of cell [^{3}H]cholesterol distribution. The re-formation of cell sphingomyelin also led to a decreased content of cellular [^{3}H]cholesterol esters, indicating that unesterified [^{3}H]cholesterol was pulled out of the cholesterol ester cycle and transported to the cell surface. Exposure of BHK-21 cells to sphingomyelinase further led to a dramatically decreased activity of ouabain-sensitive Na^+/K^+-ATPase, whereas forskolin-stimulated adenylate cyclase activity was not affected. The activity of Na^+/K^+-ATPase returned to normal in parallel with the normalization of cell sphingomyelin mass and cholesterol distribution. We conclude that sphingomyelin has profound effects on the steady-state distribution of cell cholesterol, and that manipulations of cell sphingomyelin levels directly and reversibly affects the apparent distribution of cholesterol. Changes in the lipid composition of the plasma membrane also appears to selectively affect important metabolic reactions in that compartment.

Introduction

The specific localization of sphingomyelin in plasma membranes of many cell types appears to be a major determinant of the likewise asymmetric distribution of unesterified cholesterol to the same compartment [1,2]. Many types of experiment suggest that sphingomyelin itself may attract cholesterol to yield co-localization in membraneous structures. First, studies with monolayer films at the air/water interphase have revealed that the molecular packing resulting from the interaction of sphingomyelin with cholesterol is significantly tighter than the comparable packing of cholesterol with other phospholipid classes [3–5].

Secondly, we have observed that a selective degradation of membrane sphingomyelin in cultured cells leads to a net flow of cholesterol mass from the cell surface to intracellular membranes [6–8]. The opposite appears also to be true, since addition of sphingomyelin mass to cells from vesicles results in an increased flow of cholesterol from the intracellular regulatory pool to the cell surface [9]). Finally, it also appears that translocation of cholesterol from the cell surface to internal membranes after sphingomyelin degradation is at least in part reversed in parallel with the restoration of cell sphingomyelin content [10].

Abbreviation: BHK-21, baby hamster kidney (21) cells.

Correspondence: J.P. Slotte, Department of Biochemistry and Pharmacy, Åbo Akademi University, SF-20500 Turku, Finland.

Baby hamster kidney (BHK) cells appear to provide a good model to study the effects of sphingomyelin degradation on various metabolic responses, since the rate of sphingomyelin turn-over and re-synthesis after degradation proceeds at a rate of 25% per hour even in serum-free media [11]. This rapid re-synthesis of sphingomyelin mass should make it possible to examine the effects of sphingomyelin degradation and re-synthesis on cholesterol distribution and metabolic reactions within relatively short time periods (0–4 h).

In this study we have examined kinetics of sphingomyelinase-induced cholesterol translocation between the cell surface and the cell interior, together with the normalization of cholesterol distribution as paralleled by the normalization of cell sphingomyelin mass. To gain further insight into the effects of sphingomyelin degradation on the metabolic functions of the plasma membrane, we measured activities of forskolin-stimulated adenylate cyclase and ouabain-sensitive ^{86}Rb-uptake in untreated and sphingomyelin-depleted BHK-21 cells.

Experimental

Cell culture. Baby hamster kidney cells (BHK-21) were cultured in Glasgow medium (GIBCO) supplemented with 10% tryptose phosphate broth and 5% fetal calf serum. Cells for experiments were seeded in 35 mm diameter cell culture dishes (1 : 20 split) and were used 2–3 days after plating.

Degradation of [^{3}H]sphingomyelin. To determine the amount of cell sphingomyelin that was degradable by exogenously added sphingomyelinase, cellular sphingomyelins and phosphatidylcholines were labeled with [*methyl*-^{3}H]choline chloride (Amersham International, 75 Ci/mmol) as follows: Cells were cultured in growth medium supplemented with 2 μCi/ml of [*methyl*-^{3}H]choline chloride (28 μM final choline concentration) for 48 h. Cells were then rinsed (3 × 2 ml) with phosphate-buffered saline and fixed for 10 min at 4°C with 1% glutaraldehyde in phosphate-buffered saline. After the fixation, cells were rinsed (3 × 2 ml) and exposed to 100 mU/ml of sphingomyelinase (*Staphylococcus aureus*, Sigma Chemicals) in phosphate-buffered saline for up to 40 min. The cellular content of [^{3}H]sphingomyelin in control and treated cells was determined by thin-layer chromatography of the total lipid extract.

Labeling of cells with [^{3}H]cholesterol. After plating in dishes, the cells were grown for 48 h in growth medium containing 5% fetal calf serum with [^{3}H]cholesterol (5–10 μCi/ml serum; Du Pont New England Nuclear, 60 Ci/mmol). The cells were incubated for 3–5 h in serum-free medium prior to the experiments.

Incubation procedures. The incubation protocols used were as follows: native cells were either exposed to sphingomyelinase (100 mU/ml) continuously for up to 90 min and then taken for oxidation studies and for [^{3}H]cholesterol ester analysis, or were exposed to sphingomyelinase during a 30 min pulse. After the 30 min exposure, cells were rinsed with phosphate-buffered saline (3 × 2 ml). Then 1 ml of serum-free HAM F12 medium was added, and the cells incubated at 37°C for time periods up to 5 h in order to allow for re-synthesis of sphingomyelin mass.

Oxidation of cell [^{3}H]cholesterol. Confluent native [^{3}H]cholesterol-labeled cells, pre-treated with sphingomyelinase as indicated separately (but without prior fixation), were first rinsed once with ice-cold phosphate-buffered saline and then fixed for 10 min (0°C) with 1% glutaraldehyde in phosphate-buffered saline. The fixative was then removed and the cells rinsed (3 × 2 ml) with ice-cold phosphate-buffered saline. 1.0 ml HAM F-12 medium (serum-free) containing 1 U/ml of cholesterol oxidase (*Brevibacterium*; Beckman, Carlsbad, CA) and 0.1 U/ml of sphingomyelinase was added, and the cells were incubated at 37°C for 30 min. The addition of sphingomyelinase to the oxidation buffer is explained by the fact that cell cholesterol is not susceptible for oxidation without manipulations of lipid packing in the plasma membrane. We have characterized the effects of sphingomyelin degradation on cholesterol oxidizability and developed this cholesterol oxidase assay [7]. At the end of the incubation, the dishes were chilled, rinsed with phosphate-buffered saline and stored frozen (−20°C) until lipid analysis was performed.

^{86}Rb influx. To determine the effects of sphingomyelin degradation on the activity of plasma membrane Na^+/K^+-ATPase in intact native cells (un-fixed), the ouabain-sensitive uptake of ^{86}Rb (1–8 mCi/mg, Amersham) was determined exactly as described [12]. Cells were treated either with sphingomyelinase for 30 min at 37°C before exposure to ouabain and ^{86}Rb, or were exposed to sphingomyelinase and then incubated for 4 h in serum-free media without the enzyme (to allow for sphingomyelin re-synthesis) before ouabain and ^{86}Rb were added. Control cells were not exposed to sphingomyelinase. Dishes were then treated for 10 min at 37°C with 1 mM ouabain before exposure to 0.5 μCi/ml of ^{86}Rb (2.5 min at 37°C). Some sets of dishes were exposed to ^{86}Rb without prior treatment of ouabain. The dishes were rapidly (less than 10 s) rinsed in beakers (500 ml) containing ice-cold 0.1 M $MgCl_2$. The cellular content of ^{86}Rb was determined from an aliquot of the cells. To correct for variable protein content per dish, cells were pre-labeled for 10 h with 1 μCi/ml of [^{3}H]thymidine (27 Ci/mmol, Amersham), and the ^{86}Rb counts were normalized to the [^{3}H]-thymidine counts. The protein content of some sets of dishes was also determined.

Conversion of [^{3}H]adenine to cyclic [^{3}H]AMP. To determine the effects of sphingomyelin degradation on

the activity of plasma membrane adenylate cyclase, the uptake and conversion of [^{3}H]adenine (23.5 Ci/mmol, Amersham) to cyclic [^{3}H]AMP was determined in intact cells. Cells were either exposed to sphingomyelinase (0.1 U/ml) for 30 min or exposed to sphingomyelinase (30 min) and allowed to recover without sphingomyelinase for 4 h. Control cells were not exposed to sphingomyelinase. Cells were then exposed for 60 min to [^{3}H] adenine (7.5 μCi/ml) in serum-free HAM F12 medium containing 1 mM 3-isobutyl-1-methylxanthine. At the end of the incubation, 15 μM forskolin was added and the cells incubated for an additional 15 min. The conversion of [^{3}H]adenine to cyclic [^{3}H]AMP was determined by ion exchange chromatography [13].

Lipid chemistry. Total cell lipids from control or sphingomyelinase-treated cells were extracted with hexane/2-propanol (3:2, v/v; 2 × 2 ml/dish for 20 min each). The organic solvent was evaporated, and the total lipids dissolved in chloroform and spotted on heat-activated Kieselgel 60 thin-layer chromatography plates (Merck, F.R.G.). The plates were developed with chloroform/methanol/acetic acid/water (25:15:4:2, v/v; [14]), air dried, and stained with iodine. The [^{3}H]sphingomyelin spots were scraped into scintillation vials and counted for radioactivity.

[^{3}H]Sterols in the total lipid extracts were separated on thin-layer chromatography sheets (Kodak Chromagram sheets) with hexane/diethyl ether/acetic acid (130:30:2, v/v) as developing solvent. Lipid spots were detected with I_2 staining. Spots for [^{3}H]cholesterol, [^{3}H]cholestenone, and [^{3}H]cholesterol esters were identified from standards run in parallel. The appropriate spots were marked, the I_2 stain was removed and the spots cut into scintillation vials. The radioactivity was counted in an LKB RackBeta liquid scintillation counter.

Results

Degradation of cell [^{3}H]sphingomyelin

To determine the extent of cell sphingomyelin that was susceptible to hydrolysis by extracellularly added sphingomyelinase, cellular phosphatidylcholines and sphingomyelins were labeled to constant specific activity with [*methyl*-^{3}H]choline chloride [11]. Treatment of such labeled cells with sphingomyelinase led within 40 min to the degradation of about 60% of cell [^{3}H] sphingomyelin (Fig. 1). No degradation of ^{3}H-labeled phosphatidylcholine was evident during the sphingomyelinase treatment (data not shown).

Acute effects of sphingomyelin degradation on [^{3}H] cholesterol mobilization

In untreated BHK-21 cells, about 70% of the cell unesterified [^{3}H]cholesterol was susceptible to oxidation by cholesterol oxidase (Fig. 2A). However, when cell

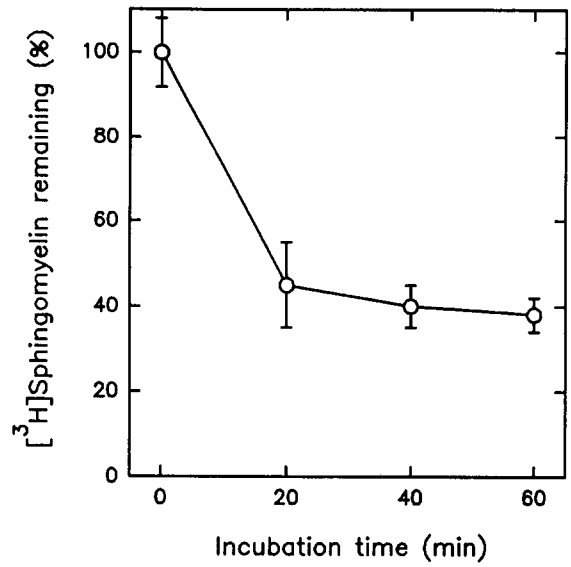

Fig. 1. Sphingomyelinase-induced degradation of sphingomyelin in BHK-21 cells. The choline-containing phospholipids in BHK-21 cells were labeled with [^{3}H]choline for 48 h. After a post-incubation in serum-free media (3–5 h), cells were fixed (1% glutaraldehyde, 10 min at 0°C), and exposed to 100 mU/ml of sphingomyelinase for the indicated periods of time. The content of [^{3}H]sphingomyelin in the cellular total lipid extract was determined by thin-layer chromatography, and values for treated cells are expressed relative to untreated cells. The amount of [^{3}H]sphingomyelin in untreated cells corresponded to about 10500 cpm/dish. Values are averages for duplicate dishes from two independent experiments (± range, $n = 4$).

sphingomyelin mass was reduced by treatment of cells with sphingomyelinase, the fraction of cell [^{3}H]cholesterol that was oxidizable by cholesterol oxidase decreased from 70% to as low as 10%. This rapid and extensive translocation of cell surface [^{3}H]cholesterol to

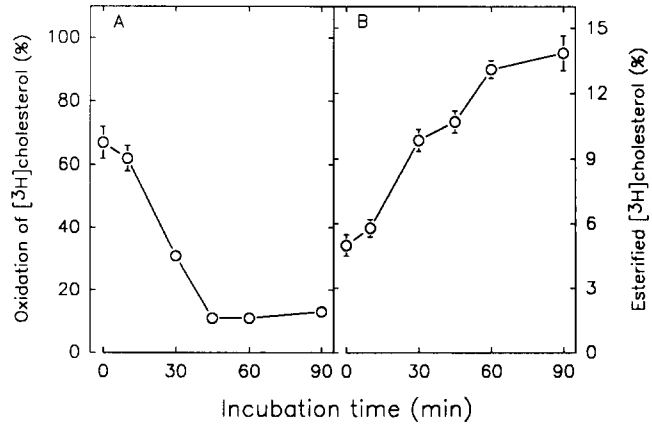

Fig. 2. Effects of sphingomyelin depletion on [^{3}H]cholesterol translocation and esterification in BHK-21 cells. Cells were labeled with [^{3}H]cholesterol for 48 h and then incubated in serum-free media for 3 h prior to exposure to sphingomyelinase (0.1 U/ml). Cell [^{3}H]cholesterol was oxidized by cholesterol oxidase and the formation of [^{3}H]cholestenone (panel A) and [^{3}H]cholesterol esters (panel B) was determined from the total lipid extract by thin-layer chromatography. Values are averages ± range from two separate experiments ($n = 5$ or 6).

intracellular compartments was completed within 45 min, and the fraction of cell [^{3}H]cholesterol that was susceptible to oxidation remained extremely low as long as the sphingomyelinase was present in the incubation medium (to prevent re-synthesis of sphingomyelin; Fig. 2A). The hydrolysis of cell sphingomyelin also led to an equally dramatic and rapid increase in the formation of endogenously synthesized [^{3}H]cholesterol esters (Fig. 2B).

Effects of sphingomyelin re-synthesis on [^{3}H]cholesterol mobilization

To test for the effects of sphingomyelin re-synthesis on the normalization of cellular [^{3}H]cholesterol translocation, un-fixed native cells were first exposed to sphingomyelinase, but then post-incubated in serum-free medium to allow for the re-synthesis of sphingomyelin mass. As illustrated in Fig. 3, BHK-21 cells were able to restore the sphingomyelin content within about 3–4 h when the cells were allowed to recover from the sphingomyelinase treatment. This rapid re-synthesis of cell sphingomyelin in un-fixed BHK-21 cells was very similar to that observed by Allan and Quinn [11].

It was observed that the restoration of the cell sphingomyelin content also restored the apparently normal distribution of [^{3}H]cholesterol within the cells, as probed with cholesterol oxidase. Within 2–3 h after the removal of sphingomyelinase from the incubation medium, the fraction of cell [^{3}H]cholesterol that was susceptible to oxidation by cholesterol oxidase had increased from 20–25% to about 60% (Fig. 4A). The kinetics of the normalization of cell [^{3}H]cholesterol distribution followed fairly well the kinetics of the recovery of cell sphingomyelin mass (Fig. 4A vs. Fig. 3).

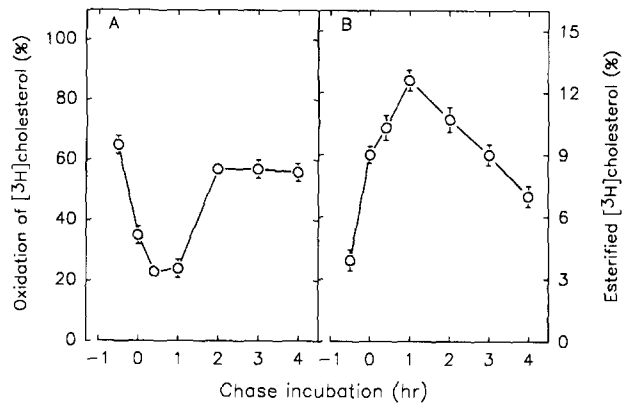

Fig. 4. Effects of sphingomyelin mass recovery on [^{3}H]cholesterol distribution and [^{3}H]cholesterol ester content. BHK-21 cells, labeled for 48 h with [^{3}H]cholesterol, were exposed for 30 min to sphingomyelinase, and then incubated in serum-free media for indicated time periods. The oxidizability of [^{3}H]cholesterol (panel A), and the content of [^{3}H]cholesterol esters (panel B) was determined. Values are averages (± range) of duplicate dishes from three separate and representative experiments ($n = 6$).

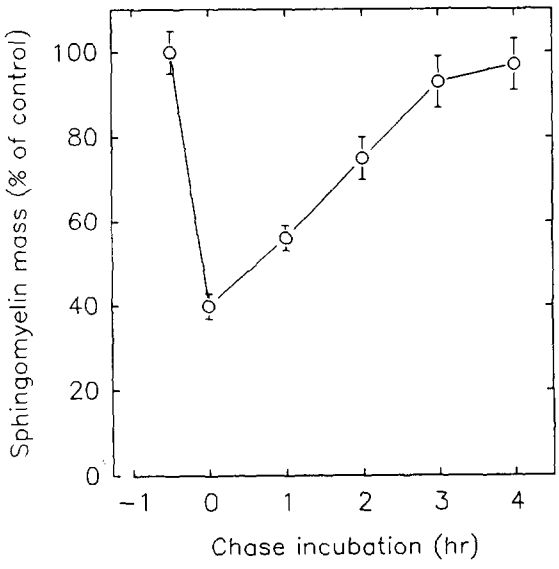

Fig. 3. Recovery of sphingomyelin mass after initial degradation by sphingomyelinase. Cells were first exposed for 30 min to sphingomyelinase (except control cells), and were then allowed to recover in serum-free media for indicated time periods. The content of sphingomyelin mass in control and sphingomyelinase-treated (and recovered) BHK-21 cells was determined from the total lipid extract by thin-layer chromatography and densitometric scanning. Values (± range) are averages of duplicates from one representative experiment, and are given relative to the mass of sphingomyelin in untreated cells (100%).

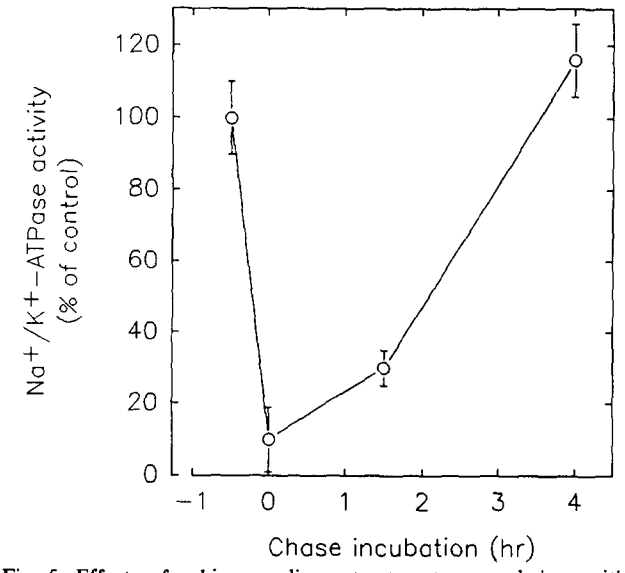

Fig. 5. Effects of sphingomyelinase treatment on ouabain-sensitive ^{86}Rb uptake. Confluent BHK-21 cells were exposed to sphingomyelinase (0.1 U/ml) for 30 min, and some dishes were allowed to recover for additional time periods in serum-free media. Control cells were not exposed to sphingomyelinase. Uptake or ^{86}Rb in these pre-treated cells (with or without ouabain) was determined during a 2.5 min pulse. Values (± S.D.) are given for quadruplicate dishes from two independent experiments ($n = 7$ or 8), and are given relative to ouabain-sensitive ^{86}Rb uptake in untreated cells. The ouabain-sensitive ^{86}Rb uptake was about 40% of total ^{86}Rb uptake, total uptake being about $2 \cdot 10^4$ cpm/mg cell protein.

The recovery of cell sphingomyelin mass and the normalization of cellular [^{3}H]cholesterol distribution was further accompanied by a decreased cellular content of [^{3}H]cholesterol esters (Fig. 4B).

Effects of sphingomyelin degradation of the activity of membrane-bound enzymes

The catalytic activity of many membrane-bound enzymes (e.g., in the plasma membrane) are known to be markedly affected by changes in the lipid composition of their microenvironment [15]. Since treatment of cells with sphingomyelinase results in the loss of both sphingomyelin and cholesterol from plasma membranes, this treatment can also be expected to result in changes of the catalytic activities of several membrane-bound enzymes. To test for this possibility, we measured the activities of two membrane-bound enzymes, the Na^+/K^+-ATPase and the adenylate cyclase.

We observed that the ouabain-sensitive uptake of ^{86}Rb by BHK-21 cells was dramatically reduced (80–90% inhibition) in sphingomyelin depleted cells (Fig. 5). However, cells which were allowed to recover from the sphingomyelinase treatment normalized their ouabain-sensitive Na^+/K^+-ATPase activity within 4 h. In contrast to these results, we did not observe a reduction in the forskolin-stimulated activity of adenylate cyclase in cells whose sphingomyelin mass was degraded by the action of sphingomyelinase (Fig. 6).

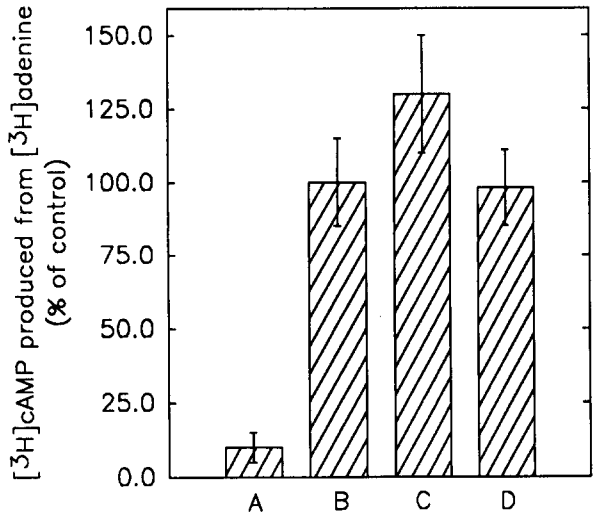

Fig. 6. Effects of sphingomyelinase treatment on forskolin-stimulated adenylate cyclase activity. Confluent BHK-21 cells were exposed to sphingomyelinase (0.1 U/ml) for 30 min, and some dishes were allowed to recover for 4 h in serum-free media. Control cells were not exposed to sphingomyelinase. Cells were then pulsed for 1 h at 37°C with [^{3}H]adenine and exposed for 15 min to 15 μM forskolin. The uptake of [^{3}H]adenine and the conversion to [^{3}H]cAMP was determined from cell extracts as described under Experimental. Values are averages (±S.D.) from four separate experiments ($n = 16$ per group). Bar A: untreated cells (no sphingomyelinase and no forskolin), B: untreated control cells plus forskolin (given as 100% ±S.D.), C: sphingomyelinase-treated cells plus forskolin, D: sphingomyelinase-treated and recovered cells plus forskolin.

Discussion

This study has addressed questions related to the inter-relationship between plasma membrane sphingomyelin on one hand and the distribution of cholesterol on the other. Since the turn-over and re-synthesis of plasma membrane sphingomyelin is very efficient in BHK-21 cells [11], this cell type was chosen as an experimental model. In good agreement with the results of Allan and Quinn [11], we observed that about 60% of BHK-21 sphingomyelin was degradable by sphingomyelinase. In further agreement, we also observed that the rate of sphingomyelin re-synthesis in BHK-21 cells, after initial exposure to sphingomyelinase, was in the range of 25% per h (Fig. 3). It has been suggested that the rapid reformation of sphingomyelin mass in BHK-21 cells is at least in part due to transfer of phosphorylcholine from phosphatidylcholine to ceramide in the plasma membrane compartment [11]. However, recent reports on the rat liver system have indicated that the bulk of sphingomyelin synthesis actually takes place in the *cis* and *medial* cisternae of the Golgi apparatus [16]. A rapid restoration of the cellular sphingomyelin content after initial degradation by sphingomyelinase appears not to be a universal feature of cells, however, since neither human neuroblastoma cells nor skin fibroblasts displayed a comparably fast re-synthesis of sphingomyelin mass [10].

The metabolic effects of sphingomyelin removal from plasma membranes were dramatic in the BHK-21 cell model. Degradation of sphingomyelin led within 40 min to the disappearance of cholesterol from the cell surface, as probed with cholesterol oxidase. The fraction of cell unesterified cholesterol that was susceptible to oxidation by cholesterol oxidase decreased by 50–60% , suggesting that a fairly large amount of the unesterified cholesterol was translocated from the cell surface to intracellular compartments. The mobilized cholesterol was not available for efflux [8], but rather appeared in intracellular compartments, as evidenced by its appearance as [^{3}H]cholesterol esters (cf. [8] and Fig. 2B). The magnitude of cholesterol translocation and the swiftness of the process in BHK-21 cells has not previously been seen in other cell types [7,10], and may be related to the exceptional capacity of BHK-21 cells to turn-over plasma membrane sphingomyelin.

The increased conversion of plasma membrane [^{3}H]cholesterol to [^{3}H]cholesterol esters after degradation of cell sphingomyelin is evidence that at least a fraction of the cholesterol at the cell surface was translocated to the endoplasmic reticulum. Experiments performed with a steroidogenic cell type (i.e., mouse Leydig tumor cells) has revealed that cell surface cholesterol is translocated to the mitochondria as well, subsequent to sphingomyelin degradation (Pörn, Tenhunen and Slotte, manuscript in preparation). It is therefore likely that

sphingomyelin degradation results in the transport of cell surface cholesterol to the interior of the cells, where it partitions (passively?) into various organellar membranes.

The sphingomyelinase-induced distribution of a large fraction of cell surface cholesterol to intracellular compartments was not an enduring situation, since the recovery of cell sphingomyelin mass simultaneously resulted in the normalization of cell cholesterol distribution. A similar trend of paralleled recovery of sphingomyelin mass and a normal cholesterol distribution was previously observed in both human neuroblastoma cells and in human fibroblasts [10]. The extent of recovery and the pace of the processes in the other cell types were not, however, comparable to that observed in this study with BHK-21 cells.

The flow of intracellular cholesterol to the cell surface concomitant with the restoration of sphingomyelin mass also led to a decreased cellular content of [^{3}H]cholesterol esters. The formation of cholesterol esters by the acyl-CoA : cholesterol acyl transferase (EC 2.3.1.26) reaction in the endoplasmic reticulum is known to be balanced by the controlled hydrolysis of formed cholesterol esters by a neutral cholesterol ester hydrolase (EC 3.1.1.13; [17]). The apparent purpose of this 'cholesterol ester cycle' is to provide a mean for cells to rapidly mobilize unesterified cholesterol from cholesterol ester stores. Clearly, unesterified [^{3}H]cholesterol was mobilized from this cholesterol ester cycle as a consequence of the formation of sphingomyelin mass in the plasma membranes.

The metabolic reactions in the plasma membranes of sphingomyelin depleted cells obviously cannot be the same as those observed in untreated cells, since the mass of both sphingomyelin and cholesterol in the membrane is markedly reduced. The catalytic activity of many enzymes residing in plasma membranes are known to be sensitive to changes in the lipid environment [15]. We chose to examine the effects of sphingomyelin degradation on the activities of two different types of membrane bound enzymes. The Na^+/K^+-ATPase is a membrane-penetrating protein that pumps 3 moles of Na^+ out of cells for each pair of K^+ pumped into cells, at the expense of ATP [18]. Adenylate cyclase, on the other hand, converts ATP to cyclic AMP, and is thought to be a complex of protein subunits residing in the endoleaflet of the plasma membrane [19]. It is unlikely that the enzyme resides in a sphingomyelin-rich environment, since this phospholipid class is predominantly distributed in favor of the exoleaflet [20].

Degradation of plasma membrane sphingomyelin led in this study to an almost complete inhibition of the ouabain-sensitive Na^+/K^+-ATPase activity, whereas the rate of non-specific ^{86}Rb uptake into cells was not affected. Our present data do not allow us to explain whether the reduced content of sphingomyelin or cholesterol affected the Na^+/K^+-ATPase activity in the membrane, since the drop in plasma membrane content of these two lipids was almost completely paralleled. The inactivation of Na^+/K^+-ATPase could also have been caused by the likely formation of sphingosine from the ceramides produced in the sphingomyelinase reaction [21], since sphingosine is a potent inhibitor of Na^+/K^+-ATPase [22]. However, full Na^+/K^+-ATPase activity was observed in sphingomyelinase-treated cells that were allowed to recover, indicating that the sphingomyelinase-induced effects on the ion channel were reversible.

Surprisingly, the effects of sphingomyelin degradation and cholesterol translocation on the activity of forskolin-stimulated adenylate cyclase were small or non-existent. This finding contrasts with reports from Houslay and co-workers [23,24], showing that manipulations of the cholesterol content in isolated plasma membranes from rat liver markedly affects both glucagon- and NaF-stimulated adenylate cyclase activities. The lack of effects of sphingomyelin degradation and cholesterol translocation on forskolin-stimulated adenylate cyclase activity is fairly difficult to interpret. It may indicate, however that the sphingomyelinase treatment led to only minimal perturbations in the lipid composition of the microdomains in the endoleaflet where the enzyme complex resides. If this is true, it follows that sphingomyelin degradation and cholesterol translocation only selectively affects metabolic reactions taking place in the plasma membranes.

In conclusion it can be said that this study has demonstrated an unequivocal functional and dynamic linkage between the co-localization of sphingomyelin and cholesterol at the cell surface. Since cholesterol interacts preferentially with sphingomyelin, both in model membranes as well as in intact cell membranes, it is likely that sphingomyelin has molecular properties that specifically attracts cholesterol to itself or captures it to yield co-localization. Future studies in this field must address questions related to the unique properties of sphingomyelin in relation to its interactions with cholesterol.

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