Rhinoviruses Infect Human Epithelial Cells via Ceramide-enriched Membrane Platforms*

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The cell membrane contains very small distinct membrane domains enriched of sphingomyelin and cholesterol that are named rafts. We have shown that the formation of ceramide via activation of the acid sphingomyelinase transforms rafts into ceramide-enriched membrane platforms. These platforms are required for infection of mammalian cells with Pseudomonas aeruginosa, Staphylococcus aureus, or Neisseria gonorrhoeae. In the present study we determined whether the acid sphingomyelinase, ceramide, and ceramide-enriched membrane platforms are also involved in the infection of human cells with pathogenic rhinoviruses. We demonstrate that infection of human epithelial cells with several rhinovirus strains triggers a rapid activation of the acid sphingomyelinase correlating with microtubules- and microfilament-mediated translocation of the enzyme from an intracellular compartment onto the extracellular leaflet of the cell membrane. The activity of the acid sphingomyelinase results in the formation of ceramide in the cell membrane and, finally, large ceramide-enriched membrane platforms. Rhinoviruses colocalize with ceramide-enriched membrane platforms during the infection. The significance of ceramide-enriched membrane platforms for rhinoviral uptake is demonstrated by the finding that genetic deficiency or pharmacological inhibition of the acid sphingomyelinase prevented infection of human epithelial cells by rhinoviruses. The data identify the acid sphingomyelinase and ceramide as key molecules for the infection of human cells with rhinoviruses.

The classic fluid mosaic model of the cell membrane suggests a random distribution of lipids and proteins in the cell membrane (1). This view was challenged by many recent studies indicating that sphingolipids and cholesterol spontaneously separate from other glycosphospholipids in the cell membrane to form distinct microdomains (2, 3). (Glyco)sphingolipids bind to each other via hydrophilic and hydrophobic interactions between their headgroups and the saturated fatty acid side chains, respectively. Void spaces between the bulky glycosphin-golipids seem to be filled with cholesterol via tight interactions of the sterol-ring system with the sphingo sine moiety of the sphingolipids and hydrogen bonds between the C3-hydroxy groups with the hydrophilic headgroups of sphingomyelin. These interactions result in a lateral stabilization and facilitate the formation of distinct membrane domains that were named “rafts” (3), because they float in the “ocean” of the other phospholipids. We have identified a novel membrane domain, i.e. ceramide-enriched membrane platforms (4, 5). Ceramide-enriched membrane platforms are initiated by activation of the acid sphingomyelinase, which has been shown to be stimulated by several receptors, including CD95 (4–9), tumor necrosis factor receptor (10), CD40 (11), or FcγRII (12), pathogenic bacteria, including Neisseria gonorrhoeae, Pseudomonas aeruginosa, and Staphylococcus aureus (13–16), Sindbis virus (17), and many stress stimuli, e.g. radiation, UV light, or cytostatic drugs (18–22). These stimuli do not only activate the enzyme but also induce the translocation of the acid sphingomyelinase from an intracellular compartment onto the extracellular leaflet of the cell membrane (4, 23). The hydrolysis of sphingomyelin that is predominantly located in the outer leaflet of the cell membrane by the acid sphingomyelinase results in the generation of ceramide. The biophysical properties of ceramide predict that ceramide molecules self-associate to small ceramide-enriched domains (4), which spontaneously fuse to one or a few large ceramide-enriched membrane platforms (24). Ceramide-enriched membrane platforms serve to re-organize signaling molecules in the cell, in particular to trap and cluster receptor molecules. Clustering of receptor molecules seems to be necessary for the stimulation of cells via many receptors (for review see Ref. 25). In addition, we have previously shown that ceramide-enriched membrane platforms mediate the infection of mammalian cells with several pathogenic bacteria (13–16). P. aeruginosa, N. gonorrhoeae, and S. aureus trigger the formation of ceramide-enriched membrane platforms and employ these platforms for invasion, induction of apoptosis, and regulation of the cellular cytokine response (15). These studies indicated the central role of ceramide-enriched membrane domains for the infection of mammalian cells with at least some pathogenic bacteria. However, the function of ceramide-enriched membrane platforms for cellular infections with viral pathogens is unknown. Here, we investigated one of the most common viral infections, i.e. infections with human rhinoviruses. Rhinoviruses are small, positive-stranded RNA viruses belonging to the picornavirus family, which infect human respiratory epithelial cells in the upper respiratory tract. In vitro experiments demonstrated that one group of rhinoviruses, named the major group, binds to ICAM-1 (intercellular adhesion molecule 1) to infect cells (26), whereas a second group, the minor group, seems to infect epithelial cells via LDL1 receptors (27). It is unknown whether these receptors

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† The abbreviations used are: LDL, low density lipoprotein; MEM, minimal essential medium; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; Z-VAD-fmk, benzyloxycarbonyl-VAD-fluoromethyl ketone; PBS, phosphate-buffered saline; DAG, diacylglycerol.
participate in vivo, i.e. in the nasal tissue, in the infection with rhinoviruses. Recent studies showed that cholesterol-enriched membrane domains are involved in cellular infections with rhinoviruses, because the infection of human cells was inhibited by pre-treatment with methyl-β-cyclodextrin (28), which interferes with the cholesterol metabolism and destroys small sphingolipid- and cholesterol-enriched membrane rafts (29). These data suggest an important function of distinct membrane domains for the infection of human cells with rhinovirus. Here, we investigated the role of ceramide-enriched membrane platforms for rhinoviral infections. We demonstrate that major and minor group rhinoviruses induce an activation and translocation of the acid sphingomyelinase onto the extracellular leaflet of the cell membrane. The translocation of the acid sphingomyelinase is mediated by microtubules and microfilaments. The activity of the acid sphingomyelinase results in a reorganization of small membrane rafts and the formation of ceramide-enriched membrane platforms. Inhibition of the acid sphingomyelinase by the drugs amitriptyline and imipramine or genetic deficiency of the enzyme prevented the generation of ceramide-enriched membrane platforms upon infection and, most important, blocked cellular uptake of rhinoviruses. These studies indicate a central role of acid sphingomyelinase-generated ceramide in the infection of human epithelial cells with rhinoviruses.

MATERIALS AND METHODS

Cells and Viruses—Chang, HeLa epithelial cells, WI-38 fibroblasts, and acid sphingomyelinase-deficient or normal control fibroblasts were cultured in RPMI 1640 (Chang cells) or MEM (all other cells) supplemented with 10% FCS, 10 mM HEPES, 2 mM t-glutamine, 1 mM sodium pyruvate, 100 μM non-essential amino acids, 100 units/ml penicillin, 100 μg/ml streptomycin (all purchased from Invitrogen).

Human rhinovirus types 2, 14, and 16 (RV2, RV14, and RV16) were from ATCC and propagated by infection of HeLa cells with a multiplicity of infection of 0.01 (1 virus/100 cells) in MEM supplemented with 2% FCS and 20 mM MgCl2. Cells were infected for 1 h at 37 °C and further cultured in standard medium for 3–4 days at 33 °C. Samples were then centrifuged for 10 min at 600 g to pellet cellular debris, virus-containing supernatants were collected, stored at −80 °C, and served as viral stocks. Supernatants from non-infected cells served as controls in the infection experiments described below.

Infections and Inhibitors—Cells were infected by addition of RV2, RV14, or RV16 at a multiplicity of infection of 10 viruses/cell (10:1) in a medium consisting of MEM, 2% FCS, and 20 mM MgCl2. If the experiments involved incubations longer than 120 min, we replaced the infection medium with fresh MEM/10% FCS as above at that time. Infection of epithelial cells with rhinoviruses was quantified employing classic plaque-forming assays or determining the ratio of dead and live cells 48 h after infection with rhinoviruses. The latter was performed by flow cytometry analysis after trypan staining of the cells and staining with FITC-Annexin V to detect dead cells. Inhibition of the acid sphingomyelinase in infected cells was achieved by a 20-min preincubation of the cells with 25 μM amitriptyline or 30 μM imipramine, respectively, in MEM supplemented with 2 mM t-glutamine prior to viral infection.

To identify mechanisms of acid sphingomyelinase-translocation we inhibited microtubules by pretreatment with nocodazole (20 μM), microfilaments by cytochalasin B (5 μM), and caspases by Z-VAD-fmk (1 μM) for 20 min prior to infection.

Fluorescence Microscopy—Cells were grown on coverslips overnight, washed in MEM, 2% FCS, and 20 mM MgCl2 and infected with RV2, RV14, or RV16 for the indicated time as above. Cells were washed in PBS, 5 mM each MgCl2 and CaCl2 and fixed in 1% paraformaldehyde in PBS (pH 7.4) at room temperature for at least 10 min. Cells were washed twice in PBS and blocked by a 15-min incubation in PBS supplemented with 0.025% Tween 20. Cells were washed again in PBS and stained consecutively each at room temperature with polyclonal goat anti-acid sphingomyelinase antibodies (diluted 1:100 in PBS and 5% FCS; kindly provided by Dr. K. Sandhoff, University of Bonn, Germany) (4) or monoclonal mouse anti- ceramide antibodies 15B4 (diluted 1:50 in PBS and 5% FCS, Alexis Inc.). The primary antibodies were visualized by staining with FITC- and Cy3-coupled secondary antibodies (1:500 dilution in PBS, 5% FCS). The cells were washed three times in PBS supplemented with 0.025% Tween 20 between each staining step. Cells were finally washed three times in PBS, mounted in Mowiol and analyzed by using a Leica DMIRE 2 confocal laser scanning microscope.

Acid Sphingomyelinase Activity—To determine the activity of the acid sphingomyelinase, cells were infected as above or left un-infected. Cells were lysed in 50 mM Tris (pH 7.5), 1 mM bacitracine, 1 mM benzamidine, 1 mM sodium orthovanadate, 10 μg/ml aprotinin/leupeptin, 0.1 mg/ml soybean trypsin inhibitor, and 0.2% Triton X-100. The samples were immediately sonicated three times 10 s each, nuclei were removed by 5 min of centrifugation at 600 × g, and the supernatants were supplemented with the same volume of a buffer consisting of 50 mM Tris (pH 7.5), 5% Nonidet P-40, 1% Triton X-100, 1 mM sodium orthovanadate, and 10 μg/ml aprotinin/leupeptin were added. Acid sphingomyelase was immunoprecipitated for 60 min employing a goat anti-sphingomyelinase antibody. Immunocomplexes were immobilized on protein A/G-agarose, washed three times in 0.2% Triton X-100, 50 mM sodium acetate, 1 mM sodium orthovanadate, and 10 μg/ml aprotinin/leupeptin and resuspended in 20 μl of 1M KCl and extracted, and the organic phase was dried, resuspended in 20 μl of 1M KCl, 2% FCS, and 20 mM MgCl2 and infected with RV2, RV14, and RV16. The kinase reaction was performed for 30 min at room temperature, and the samples were extracted in 1 ml of CHCl3:CH3OH:1 n HCl (100:100:1), the lower phase was collected, dried, and subjected to alkaline hydrolysis of diacylglycerol in 0.1 M methanolic KOH at 37 °C for 60 min. Samples were then lyophilized, the lipids were dissolved in 20 μl of 1M CH3COOH (50:30:8:4, v/v) and dried, and sphingomyelin was identified in iodine vapor by comigration with an authentic standard. The spots were visualized by staining with 0.025% CoCl2 in 1M CH3COOH:1% CH3OH:3% HCl. The spots were finally washed three times in PBS, mounted in Mowiol and analyzed by using a Leica DMIRE 2 confocal laser scanning microscope.

Ceramide Monitoring by the DAG Kinase Assay—Cells were infected as above, extracted in CHCl3:CH3OH:1 n HCl (100:100:1), the lower phase was collected, dried, and subjected to alkaline hydrolysis of diacylglycerol at 37 °C for 60 min. Samples were lyophilized, the lipids were dissolved in 20 μl of 1M CH3COOH (50:30:8:4, v/v) and dried, and sphingomyelin was identified in iodine vapor by comigration with an authentic standard. The spots were visualized by staining with 0.025% CoCl2 in 1M CH3COOH:1% CH3OH:3% HCl. The spots were finally washed three times in PBS, mounted in Mowiol and analyzed by using a Leica DMIRE 2 confocal laser scanning microscope.

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RESULTS

Rhinoviruses Activate the Acid Sphingomyelinase and Induce the Formation of Ceramide-enriched Membrane Platforms—To gain insight into the mechanisms of the infection of mammalian cells with rhinoviruses, we tested whether alterations of the plasma membrane are associated with the infection. To this end we measured the activity of the acid sphingomyelinase upon infection, which has been previously shown by us to be a key enzyme in the infection of mammalian cells with at least some pathogenic bacteria (13–16). Infection with different rhinovirus strains, i.e. RV2, RV14, and RV16 induced a rapid activation of the acid sphingomyelinase in human epi-
Rhinoviral infections activate the acid sphingomyelinase (ASM) and trigger the release of ceramide. A, human Chang epithelial cells were infected with RV2, RV14, or RV16, respectively, for the indicated time, and the activity of the acid sphingomyelinase was determined by consumption of $[^{14}C]$ sphingomyelin. Shown are the mean ± S.D. of three independent experiments determining the activity of the acid sphingomyelinase. B and C, activation of the acid sphingomyelinase upon infection of human Chang epithelial cells with RV2, RV14, or RV16 correlates with a rapid release of ceramide (B) and a concomitant consumption of sphingomyelin (C). Cellular ceramide was determined by the DAG-kinase assay. Consumption of sphingomyelin was quantified by incubation of the cells with $[^{3}H]$ choline chloride to label endogenous sphingomyelin. Displayed is the mean ± S.D. of each three independent studies.

The results demonstrate that RV2, RV14, and RV16 very rapidly triggered the formation of large ceramide-enriched membrane platforms in human epithelial cells (Fig. 2A). Ceramide-enriched membrane platforms localized in the outer leaflet of the cell membrane as indicated by confocal microscopy studies in intact cells (Fig. 2A). A quantitative analysis reveals that formation of ceramide-enriched membrane platforms rapidly occurs within 5 min after infection, peaks at 15–20 min, and declines thereafter (Fig. 2B). Simultaneous staining of the cells with anti-ceramide and anti-acid sphingomyelinase antibodies demonstrated that the acid sphingomyelinase very rapidly translocated from an intracellular compartment onto the cell surface and clustered in ceramide-enriched membrane platforms after infection with RV2, RV14, or RV16 (Fig. 2C). Control studies indicated that supernatants from non-infected producer cells did not induce the formation of ceramide-enriched membrane platforms (not shown).

Previous studies (4, 11) revealed that the acid sphingomyelinase translocates from an intracellular compartment onto the outer leaflet of the cell membrane upon stimulation via CD95 or CD40. Furthermore, electron microscopy images indicated that the acid sphingomyelinase localizes to intracellular vesicles prior to stimulation (23) suggesting that a fusion of those vesicles with the cell membrane mediates the surface exposure of the enzyme. We, therefore, investigated whether cytoskeleton elements that may mediate the fusion of vesicles with the cell membrane are involved in the translocation of the acid sphingomyelinase. The results (Fig. 2D) reveal that inhibition of microtubules by nocodazole and microfilaments by cytochalasin B prevented translocation of the acid sphingomyelinase onto the cell surface indicating that a rearrangement of the cytoskeleton is required for acid sphingomyelinase surface exposure. Furthermore, previous studies from us (30) suggested that caspases are also involved in the translocation and activation of the acid sphingomyelinase upon stimulation of death receptors. We, therefore, tested whether a broad-spectrum caspase inhibitor, Z-VAD-fmk, altered the translocation of the acid sphingomyelinase onto the cell membrane upon rhinoviral infection. The data, however, indicate that Z-VAD-fmk did not affect the translocation of the acid sphingomyelinase onto the extracellular leaflet of the cell membrane (Fig. 2D).

Inhibition of the Acid Sphingomyelinase Prevents the Formation of Ceramide-enriched Membrane Platforms upon Rhinoviral Infection—To confirm that the acid sphingomyelinase releases ceramide upon infection with RV2, RV14 or RV16, we treated human epithelial cells with pharmacological inhibitors of the acid sphingomyelinase, i.e. imipramine or amitriptyline (31). Control experiments demonstrated a reduction of the basal acid sphingomyelinase activity by 70 ± 11% by imipramine and 75 ± 8% by amitriptyline, respectively (Fig. 3A). Next, we infected Chang epithelial cells that were pre-treated with imipramine or amitriptyline with RV2, RV14, or RV16 and determined the release of ceramide as well as the formation of ceramide-enriched membrane platforms. The results indicate that inhibition of the acid sphingomyelinase prevented the release of ceramide as determined by the DAG-kinase assay (Fig. 3B) and the formation of ceramide-enriched membrane platforms (Fig. 3C). Very similar data were obtained upon infection of human acid sphingomyelinase-deficient or wild type control fibroblasts. Infection of normal fibroblasts resulted in formation of ceramide (Fig. 3D) and ceramide-enriched membrane platforms (not shown), events that were absent in acid sphingomyelinase-deficient cells (Fig. 3D). These data suggest that RV2, RV14, and RV16 induce the formation of ceramide-enriched membrane platforms via an activation of the acid sphingomyelinase.
Rhinoviral infections induce the formation of ceramide-enriched membrane platforms. A, RV2, RV14, and RV16 induce the formation of ceramide-enriched membrane platforms on human epithelial cells. Cells were infected for 5 min, fixed, stained with monoclonal Cy3-coupled anti-ceramide-antibodies, and analyzed by confocal microscopy. The results indicate the formation of large ceramide-enriched membrane platforms upon rhinoviral infection. The studies are representative for each five independent experiments. B, a quantitative analysis of the formation of ceramide-enriched membrane platforms upon infection of Chang epithelial cells with RV2, RV14, or RV16, respectively, indicates a rapid formation of these platforms. Formation of ceramide-enriched membrane platforms peaks 15 min after rhinoviral infection and declines thereafter. To quantify the formation of ceramide-enriched membrane domains, cells were infected as indicated, fixed, and stained with Cy3-anti-ceramide antibodies. The number of cells displaying a ceramide-enriched membrane platform was determined by counting at least 300 cells/sample on a confocal microscope. The results represent the mean ± S.D. of three independent studies. C, confocal microscopy studies indicate that infection of HeLa epithelial cells with RV2, RV14, or RV16 induces a translocation of the acid sphingomyelinase onto the cell surface and the formation of ceramide-enriched membrane platforms. Surface acid sphingomyelinase localizes within ceramide-enriched membrane platforms as indicated by the overlays (Cy3 plus FITC). Non-infected cells display only marginal amounts of ceramide or acid sphingomyelinase on the cell surface. Cells were infected for 10 min with RV2, RV14, or RV16, fixed in paraformaldehyde and stained with Cy3-conjugated anti-ceramide and FITC-coupled anti-acid sphingomyelinase antibodies. The samples were analyzed by confocal microscopy and are representative for at least 300 cells analyzed in three independent infection experiments. Similar results were obtained with Chang epithelial cells. D, translocation of the acid sphingomyelinase onto the cell surface upon rhinoviral infection is prevented by blocking microtubules or microfilaments by preincubation of cells with nocodazole (20 μM) or cytochalasin B (5 μM). Translocation was determined by staining of the cells with FITC-anti-acid sphingomyelinase antibodies and microscopy analysis of the percentage of cells that were positive for surface acid sphingomyelinase 15 min after infection with the indicated strains. Displayed are mean ± S.D. of three independent experiments analyzing at least 300 cells each.
Genetic Deficiency or Pharmacological Inhibition of the Acid Sphingomyelinase Blocks Uptake of Rhinoviruses by Human Epithelial Cells—To determine the significance of the acid sphingomyelinase and ceramide-enriched membrane platforms for the infection of human cells with rhinoviruses, we employed cells genetically deficient for the acid sphingomyelinase or blocked the enzyme in human Chang epithelial cells by incubation with imipramine or amitriptyline. The infection of the cells with RV2, RV14, and RV16 was determined by measuring the cytopathic effect of the viruses. The results reveal that inhibition of the acid sphingomyelinase by pre-treatment of epithelial cells with amitriptyline or imipramine almost completely prevented the infection of human epithelial cells with rhinoviruses (Fig. 4, A and B). The pharmacological studies were confirmed by infection of acid sphingomyelinase-deficient and wild type control fibroblasts with RV2, RV14, and RV16 (Fig. 4B). These experiments demonstrated an almost complete inhibition of rhinoviral infection of cells that were genetically deficient for the acid sphingomyelinase, while wild type fibroblasts were rapidly infected with rhinoviruses. The genetic and pharmacological data demonstrate the significance of the acid sphingomyelinase and ceramide for the uptake of rhinoviruses by human epithelial cells. Controls indicate that the treatment of the virus stocks with imipramine or amitriptyline did not inhibit the infection excluding any effect of the drugs on the virus per se (not shown).

DISCUSSION

The present manuscript indicates a central role of ceramide-enriched membrane platforms for the infection of mammalian cells with rhinoviruses. Ceramide-enriched membrane platforms are generated by the activity of the acid sphingomyelinase that translocates onto the extracellular leaflet of the cell membrane and thereby releases ceramide after infection. Cer-
amide-enriched membrane platforms are required for uptake of epithelial cells with rhinoviruses as indicated in experiments employing pharmacological inhibitors of the acid sphingomyelinase or cells genetically deficient for the acid sphingomyelinase.

At present the exact function of ceramide-enriched membrane platforms during the internalization of viruses is unknown. In analogy to previous data for CD95 (4, 5), CD40 (11), or FcγRII (12), ceramide-enriched membrane platforms may serve to cluster receptors of rhinoviruses, in particular ICAM-1 for RV14 and RV16 and members of the LDL receptor family, i.e. LDLR, VLDLR, and LRP, for RV2, respectively. However, future studies are required to define whether ICAM- or LDL-receptor molecules cluster upon infection with rhinoviruses and whether their clustering is involved in uptake of rhinoviruses. Second, the formation of membrane platforms may recruit intracellular signaling molecules to the entry site of the virus. The assembly of intracellular signaling molecules with a virus-binding receptor within a distinct domain of the cell membrane may permit the receptor to transmit the signal into the cell that finally mediates internalization of the virus. Third, ceramide-enriched membrane platforms may serve the reorganization of the cytoskeleton and may function as anchor for actin filaments. In this context it is interesting to note that

![Diagram of FITC-Annexin V binding in infected and non-infected cells.](http://www.jbc.org/)
ceramide was recently implicated to serve as a binding site for actin filaments that mediate the fusion of phagosomes with lysosomes (32). Whether similar mechanisms apply for the internalization of rhinoviruses is presently unknown.

Our studies are in accordance with previous studies of Jan et al. (17) demonstrating a rapid activation of the acid sphingomyelase and a concomitant release of ceramide upon infection of neuroblastoma cells with Sindbis virus. Activation of the acid sphingomyelase was linked by the authors to internalization of the virus and/or fusion of endosomal membranes. A function of distinct membrane domains in rhinoviral infection is supported by the recent findings that rhinoviral infections are prevented by treatment of cells with β-cyclodextrin (28) leading to the destruction of membrane rafts. Destruction of rafts has been previously shown to prevent the formation of ceramide-enriched membrane platforms upon CD95 stimulation (4) suggesting that the integrity of rafts is required for an efficient release of ceramide by surface acid sphingomyelins.

We confirm previous observations showing a translocation of the acid sphingomyelinas into the extracellular leaflet of the cell membrane upon stimulation (4, 11, 12). The translocation of the acid sphingomyelinas involves microtubules and microfilaments consistent with previous findings in CD95-stimulated lymphocytes that showed a noclodazole and cytochalasin D-sensitive translocation of the acid sphingomyelinase (30). However, rhinoviruses do not employ caspases for cytochalasin D-sensitive translocation of the acid sphingomyelinas. The release of the acid sphingomyelinas by surface acid sphingomyelinas involves microtubules and microfilaments and is re-organized via different pathways upon receptor activation or rhinoviral infection. Mobilization and fusion of acid sphingomyelinas-containing vesicles results in the exposure of intra-vascular acid sphingomyelinas onto the extracellular leaflet of the cell membrane, which also contains most of the ceramide-enriched membrane in the cell membrane.

The molecular mechanisms resulting in stimulation of the enzymatic activity of the acid sphingomyelinas are also poorly characterized. Previously, oxygen radicals were implied in the activation of the enzyme (33, 34), in particular because an oxidation of the cysteine residue 629 of the acid sphingomyelinas activates the acid sphingomyelinase in vitro (34). However, experiments employing Tyrion, a chemical mimetic of superoxide dismutase removing O\textsubscript{2} from the intracellular and extracellular environment, and dithiothreitol did not show an inhibition of acid sphingomyelinas activation upon infection or of the viral uptake into Chang cells. This suggests that rhinoviruses do not activate the acid sphingomyelinas by a redox mechanism.

Imipramine and amitriptyline have been previously shown by several authors to inhibit the acid sphingomyelinas (13, 14, 31, 35). Sandhoff and colleagues demonstrated that these drugs interfere with the binding of the acid sphingomyelinas with the lipid bilayer (31, 35). The cationic moiety of imipramine and amitriptyline competes with the acid sphingomyelinas for the binding site on the membrane and, thus, displaces the enzyme from the membrane (31, 35). The release of the acid sphingomyelinas from the membrane into the vesicular space results in proteolytic degradation of the enzyme in intracellular vesicles and reduction of the cellular acid sphingomyelinase-activity.

The present data identify a novel mechanism of viral infection of human cells. This study reveals the central role of the acid sphingomyelins and ceramide-enriched membrane plat-
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