Gangliosides GM1 and GM2 Induce Vascular Smooth Muscle Cell Proliferation via Extracellular Signal–Regulated Kinase 1/2 Pathway

Ioanna Gouni-Berthold, Claudia Seul, Yon Ko, Jürgen Hescheler, Agapios Sachinidis

Abstract—Gangliosides, sialic acid–containing glycolipids, accumulate in atherosclerotic vessels and appear to regulate the proliferation of various cell types. Furthermore, vascular smooth muscle cell (VSMC) proliferation is associated with the development and progression of cardiovascular diseases. To demonstrate whether gangliosides are able to modulate the VSMC growth, the effect of gangliosides GM1, GM2, and GM3 on cell DNA synthesis and cell number has been examined. Moreover, we investigated possible intracellular mechanisms by which GM1 and GM2 elicit their mitogenic effects. Stimulation of VSMCs with GM1 and GM2 resulted in a dose-dependent increase in DNA synthesis and cell number, whereas GM3 caused a decrease in DNA synthesis. GM1 and GM2 (50 μmol/L) stimulate phosphorylation of extracellular signal–regulated kinases (ERKs) 1 and 2 and phosphorylation of the c-Jun N-terminal kinase (JNK), with a maximum at 15 minutes, but they do not have an effect on the phosphorylation of p38 mitogen-activated protein kinase (MAPK). GM3 (50 μmol/L), on the other hand, does not stimulate any of the 3 aforementioned MAPKs. Pretreatment of the cells with 20 μmol/L PD 098,059 caused a complete inhibition of ERK1/2 and JNK MAPK, whereas pretreatment with a Ras (farnesyl transferase) inhibitor did not abrogate the GM1- and GM2-induced ERK1/2 phosphorylation. Furthermore, GM1 and GM2 did not activate Raf-1 kinase. Interestingly, pretreatment of VSMCs with 100 nmol/L pertussis toxin resulted in a complete inhibition of the ERK1/2 phosphorylation. Finally, the GM1- and GM2-induced increase in cell number was significantly inhibited by PD 098,059. We may conclude that GM1 and GM2 stimulate ERK1/2 via a pertussis toxin–sensitive Gi-coupled receptor through a Raf-1 kinase–independent pathway. Moreover, the GM1- and GM2-induced VSMC growth is ERK1/2 dependent. (Hypertension. 2001;38:1030-1037.)

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Vascular smooth muscle cell (VSMC) proliferation plays an important role in the development and progression of many cardiovascular diseases, including hypertension1,2 and atherosclerosis.3 Proliferation in most cell types is driven by activation of the mitogen-activated protein kinases (MAPKs), which are serine-threonine kinases performing important functions as mediators of cellular responses to a variety of extracellular stimuli. Three major subfamilies of structurally related MAPKs have been identified in mammalian cells, extracellular signal–regulated kinases (ERKs) 1 and 2, c-Jun N-terminal kinase (JNK), and p38 MAPK.4–7 p38 MAPK and JNK are both activated moderately by classic growth factors and strongly in response to stress stimuli.5–7 Activation of the ERK pathway results in an expression of early growth-response genes, such as c-fos, that are involved in controlling cell proliferation.8,9 The ERK-induced cell proliferation is initiated through ligand binding to cell-surface receptors and mediated largely through either receptor tyrosine kinases or G protein–coupled receptors (GPCRs).10 One pathway involves the activation of receptor tyrosine kinases by traditional polypeptide growth factors, such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and basic fibroblast growth factor (FGF). A second signaling pathway acts through 7 membrane–spanning receptors coupled to heterotrimeric GTP-binding proteins (G proteins). Several ligands that signal via distinct G-protein subtypes have been shown to elicit mitogenic responses.10 Thrombin and lysophosphatidic acid have been shown to stimulate growth via pertussis toxin (PTX)-sensitive GPCRs.11,12 In some but not all cells, vasoactive agents such as angiotensin II and endothelin, which act through GPCRs, have also been shown to initiate proliferation.13 Complex sphingolipids, such as gangliosides, are highly expressed in the outer leaflet of plasma membranes and have been implicated in the regulation of cell growth, differentiation, and programmed cell death.14–16 Furthermore, they have been shown to enhance platelet aggre-
gation and activation\(^1\) as well as to promote platelet adhesion to extracellular matrix collagen.\(^18\) Circulating gangliosides are transported in association with lipoproteins, primarily LDL.\(^19\) Although the physiological importance of gangliosides in the regulation of cell growth is not established, several studies in the past decade have demonstrated that exogenous addition of glycosphingolipids, such as the gangliosides GM1 and GM2, to Swiss 3T3 cells inhibits the PDGF- and EGF-dependent cell growth.\(^14-16\) In this context, it has been suggested that the exogenous ganglioside-dependent suppression of cell growth occurs because of interactions of incorporated gangliosides with the tyrosine kinase receptors, such as the PDGF and EGF receptor,\(^14,15\) or even because of direct chemical-physical interactions between gangliosides and PDGF, resulting in modification of the growth factor-induced signal transduction pathway.\(^20\) GM1, GM2, and GM3 are amphiphatic molecules that belong to a class of anionic glycosphingolipids, which contain 1 molecule of sialic acid (N-acetylneuraminic acid), linked to the sugar residues of a ceramide oligosaccharide.\(^21\) It has been established that several growth factors promote proliferation of VSMCs, thereby contributing to the development and progression of cardiovascular diseases, such as hypertension\(^2,2\) and atherosclerosis.\(^3,21\) In the last years, an accumulation of gangliosides in atherosclerotic vessels has been described by several groups, suggesting a possible role of gangliosides in the pathogenesis of cardiovascular disease.\(^22,23\) Because the cellular mechanisms of gangliosides thereby contributing to the development of cardiovascular disease are still unknown, the purpose of the present study was to examine whether the gangliosides GM1, GM2, and GM3 promote VSMC proliferation and to elucidate the early intracellular transduction pathways mediating their effects on VSMC growth.

Methods

Culture of VSMCs

Rat aortic VSMCs were isolated from thoracic aortas of Wistar-Kyoto rats (aged 6 to 8 weeks, Charles River Wiga GmbH, Sulzfeld, Germany) by enzymatic dispersion with the use of a slight modification of the method of Chamley-Campbell et al.\(^24\) as described previously.\(^25\)

Determination of DNA Synthesis and Cell Counts

The effect of gangliosides on \(^3\)H\(\text{thymidine}\) incorporation into DNA was assessed as previously described.\(^25\) For cell counting, VSMCs were seeded in 24-well culture plates and cultivated in culture medium until 70\% confluence. Then the medium was replaced by serum-free medium consisting of a mixture of DMEM and Ham’s F-10 medium (1:1 [vol/vol]). After 24 hours, the cells were trypsinized, and cell counting and determination of cell diameter were performed by using the CASY-1 system, which is based on the Coulter counter principle (Scha{"u}re System) as described previously.\(^26\)

Gel Electrophoresis and Immunostaining

VSMCs were seeded in 3-cm Petri dishes and cultivated in culture medium until 70\% confluence. The medium was then replaced by serum-free medium as described above. After another 24 hours of cultivation in serum-free medium, the cells were treated with the gangliosides for different time periods. After removal of the medium, the cells were lysed with RIPA buffer as described previously.\(^27\) After 10 minutes at 0°C, cell lysates were centrifuged at 14 000g for 5 minutes. Protein (10 \(\mu\)g) was analyzed by SDS-PAGE in a 10% acrylamide gel with a thickness of 0.75 mm by using the Mini Gel Protein system (Bio-Rad). After the transfer of proteins to a polyvinylidene difluoride (PVDF) membrane, phosphorylated (activated) MAPKs and phosphorylated Raf-1 kinase were detected by the chemiluminescence Western blotting system (NEN Life Science Products, Inc) as described in the instructions. A phospho-specific ERK1/ERK2 rabbit polyclonal IgG primary antibody (1:1000), a phospho-specific JNK rabbit polyclonal IgG primary antibody (1:1000), a phospho-specific p38 MAPK rabbit polyclonal IgG primary antibody (1:1000), a phospho-specific Raf-1 kinase rabbit polyclonal IgG primary antibody (1:1000), and an alkaline phosphatase–conjugated anti-rabbit secondary antibody (1:5000) were used. The primary antibodies recognize phosphorylated (Thr202/Tyr204) ERK1/2 (also known as p44\(^{\text{mapk}}\)/p42\(^{\text{mapk}}\)), phosphorylated (Thr183/Tyr185) p54 and p46 JNK isoforms, phosphorylated (Thr180/Tyr182) p38 MAPK, and the phosphorylated (Ser259) Raf-1 kinase, respectively. Phosphorylation of the MAPKs on the appropriate amino acid residues is essential for the activation of these MAPKs.\(^5,27\) Laser scanning densitometric analysis of the bands was performed with the 2D scanning densitometer (Biometra) with the use of ScanPack software, version 14.1 A27.

Materials

PDGF-BB was a gift from Prof Dr Jürgen Hoppe, Physiological Chemistry, University of Würzburg, Würzburg, Germany, and was prepared as described previously.\(^29\) All cell culture reagents were obtained from Gibco-BRL. GM1, GM2, and GM3 were obtained from Sigma Chemical Co, and [methyl-\(^3\)H]thymidine was obtained from Amersham. All phospho-specific antibodies were obtained from New England Biolabs Inc. The farnesyl transferase inhibitor (FTI) III was obtained from Calbiochem.

Statistical Methods

Values are expressed as mean\(\pm\)SD. Statistical analyses of the data were performed with the Mann-Whitney \(U\) test (StatView 5.0, Apple Computer Inc). A value of \(P<0.05\) was considered statistically significant. An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results

Gangliosides GM1 and GM2 Induce DNA Synthesis in VSMCs

Stimulation of VSMCs with 20, 50, and 100 \(\mu\)mol/L GM1 resulted in a concentration-dependent increase in [\(^3\)H]thymidine incorporation from 41\(\pm\)3.5 (basal value) to 69.6\(\pm\)7.1, 180\(\pm\)11, and 190\(\pm\)10 cpm/\(\mu\)g protein, respectively (mean\(\pm\)SD, triplicate determination) (Figure 1A). Because a maximal effect was observed at 50 \(\mu\)mol/L GM1, in the next series of experiments, VSMCs were stimulated with 50 \(\mu\)mol/L GM1, GM2, and GM3. As shown in Figure 1B, stimulation of VSMCs with 50 \(\mu\)mol/L GM1 and GM2 resulted in a 4.3-fold and 6-fold increase in DNA synthesis, respectively, whereas treatment of the cells with 50 \(\mu\)mol/L GM3 caused a 42\% reduction of the DNA synthesis.

GM1 and GM2 Stimulate ERK1/2 in VSMCs

When VSMCs were treated with 50 \(\mu\)mol/L GM1 (Figure 2A) and GM2 (Figure 2B), a marked time-dependent phosphorylation of ERK1/2 was observed, with maximal stimulation at 15 minutes. Phosphorylation was decreased to basal values after 30 minutes of stimulation. To elucidate whether
activation of ERK1/2 by gangliosides occurs via activation of the MAPK kinase (MEK1), we investigated the effect of gangliosides on the phosphorylation of ERK1/2 in VSMCs treated with the selective MEK1 inhibitor 2-(2′/H11032-amino-3′/H11032-methoxyphenyl)-oxanaphthalen-4-one (PD 098,059). As shown in Figure 2A and 2B, the ganglioside-induced phosphorylation of ERK1/2 was abolished, whereas the effect of PDGF-BB was remarkably attenuated.

To demonstrate whether gangliosides GM1 and GM2 are able to stimulate the phosphorylation of p38 MAPK, untreated and PD 098,059-treated cells were stimulated with 50 μmol/L GM1 or GM2 for different time periods (Figure 2C and 2D). As demonstrated, GM1 and GM2 had no effect on p38 MAPK phosphorylation. Interestingly, the PDGF-BB-induced phosphorylation of p38 MAPK was not influenced by PD 098,059.

GM3 Does Not Influence the Effects of GM1- and GM2-Induced ERK1/2 Phosphorylation

Stimulation of VSMCs with 50 μmol/L GM3 had no effect on ERK1/2 stimulation (Figure 3A). To investigate whether GM3 exerts an antagonistic effect on the GM1- and GM2-induced ERK1/2 stimulation, the effect of GM1 and GM2 on ERK1/2 stimulation was examined in VSMCs pretreated with GM3. As demonstrated in Figure 3B, treatment of VSMCs with 50 μmol/L GM3 had no effects on the ERK1/2 stimulation induced by GM1 or GM2.

GM1 and GM2 Stimulate JNK Phosphorylation in VSMCs

Next, VSMCs were stimulated for different time of periods with 50 μmol/L of GM1 or GM2, and their effect on JNK

Figure 1. A, Effect of ganglioside GM1 on DNA synthesis. Confluent cells (24-well plates) were precultured for 24 hours in serum-free medium. Then cells were treated with different concentrations of GM1. After 20 hours of incubation, cells were exposed to 3 μCi/mL [3H]thymidine. Four hours later, the reaction was terminated, and cell protein and [3H]thymidine incorporation into cell DNA was quantified (1 representative experiment performed in triplicate, mean±SD). *P<0.05 vs control. B, Effect of 50 μmol/L of GM1, GM2, and GM3 on DNA synthesis. Data are expressed as the percent increase above basal value (mean±SD of 3 separate independent experiments, each performed in triplicate). **P<0.05 for GM1 or GM2 effect vs control.

Figure 2. Effects of the gangliosides GM1 and GM2 on ERK1/2 and p38 MAPK phosphorylation. VSMCs were precultured in serum-free medium for 24 hours. Cells were then treated with 20 μmol/L PD 098,059 for 3 hours before stimulation with 50 μmol/L GM1 and GM2 for different time periods or with 50 ng/mL PDGF-BB for 5 minutes. Equal amounts of protein (10 μg per lane) were analyzed by the chemiluminescence Western blotting method using specific antibodies that recognize the phosphorylated ERK1/2 (P-ERK1/P-ERK2) (A and B) and phosphorylated p38 MAPK (P-p38) (C and D). Equal amounts of protein (10 μg per lane) were analyzed by using antibodies that recognize total amount of ERK1/2 and p38 MAPK (bottom of panels A through D).
phosphorylation was examined. As demonstrated in Figure 4A, GM2 stimulated the phosphorylation of JNK, with a maximum at 15 minutes. Pretreatment of VSMCs with PD 098,059 resulted in a complete inhibition of the effect of GM2 and PDGF-BB on JNK activation. GM1 (50 μmol/L) also stimulated the phosphorylation of JNK after 15 minutes. Quantification of the band densities by laser scanning densitometry obtained by separate experiments showed that GM1 and GM2 caused, at 15 minutes, 127±35% and 133±41% (mean±SD, n=6) increases of ERK1/2 phosphorylation and 124±16% and 198±25% (mean±SD, n=4) increases of JNK phosphorylation, respectively (Figure 4B). GM3 had no effect on p38 and JNK phosphorylation (data not shown).

Stimulation of ERK1/2 by GM1 and GM2 Is PTX Dependent
To address whether Gα proteins are involved in the signal transduction pathway of GM1 and GM2, we examined the effect of both gangliosides on ERK1/2 phosphorylation in PTX-treated cells. Interestingly, treatment of the VSMCs with 100 nmol/L PTX resulted in an almost complete inhibition of the GM1- and GM2-induced phosphorylation of ERK1/2 (Figure 5).

GM1 and GM2 Do Not Stimulate Raf-1 Kinase and Ras
To investigate whether GM1 and GM2 activate MEK1 through Raf-1 kinase stimulation, we investigated the effects of GM1 and GM2 on Raf-1 kinase phosphorylation. As seen in Figure 6, there was no effect of GM1 or GM2 on Raf-1 kinase phosphorylation. Furthermore, we examined whether pharmacological blockade of the Ras pathway would reverse the GM1- and GM2-induced ERK1/2 phosphorylation. For this purpose, we used an FTI (25 μmol/L).31 Cells were preincubated with FTI for 4 hours, and then GM1 (50 μmol/L) or GM2 (50 μmol/L) was added. As seen in Figure 7, the Ras inhibitor FTI did not affect the GM1- or GM2-induced ERK1/2 phosphorylation.

PD 098,059 Inhibits GM1- and GM2-Induced VSMC Proliferation
Finally, to investigate whether the growth-promoting effects of GM1 and GM2 are mediated by ERK1/2, cells were treated with 20 μmol/L PD 098,059 and then stimulated with the aforementioned gangliosides. Control experiments were performed after stimulation of the PD 098,059–treated cells with 50 ng/mL PDGF-BB. GM1, GM2 (each 50 μmol/L), and PDGF-BB caused a 66±12%, 44±6%, and 99±11% increase in cell number, respectively (mean±SD, 3 separate independent experiments). The cell diameter of unstimulated cells was 15.4±0.5 μm. After stimulation with GM1, GM2, and PDGF-BB, there was an increase of 10±2% (for each ganglioside) and 15±2% for PDGF-BB. Treatment of cells with 20 μmol/L PD 098,059 resulted in a complete inhibition of the GM2-induced increase in cell number. Furthermore, the GM1-induced and the PDGF-BB–induced increase in cell
number was markedly reduced from 66±12% and 99±11% to 23±5% and 28±8%, respectively (Figure 8).

Discussion

In the last years, there has been increasing evidence that gangliosides may be involved in the development of cardiovascular diseases. In this context, several groups have described an accumulation of gangliosides in atherosclerotic vessels.22,23 Furthermore, it has been reported that gangliosides modulate cell growth and/or differentiation of various mammalian cells. For example, it has been shown that exogenous addition of gangliosides into culture medium inhibits cellular growth via suppression of the tyrosine phosphorylation of several growth factor receptors.14,15 Recently, it has been demonstrated that gangliosides are also able to interact with PDGF-BB.20 In particular, gangliosides cause an inhibition of the tyrosine phosphorylation of the PDGF β-receptor as well as of other early intracellular events and, therefore, lead to a reduction of the proliferative effects of PDGF-BB in VSMCs.20 Moreover, it has been shown that interactions of FGF-2 with GM1 and GM2 result in an attenuation of the FGF-2–induced proliferative effects in endothelial cells.32 In the present study, we demonstrate that GM1 and GM2, per se, stimulate cell DNA synthesis, an increase in cell number, and an increase in cell size (which is due to the increase in cell diameter). Because an increase in cell size occurs during the S and M phases of the cell cycle, it is most likely that the increase in the cell size induced by the gangliosides and by PDGF-BB reflects cells found in the S or M phase. However, hypertrophy or even hyperploidy of cells cannot be excluded. Furthermore, we demonstrated that GM1 and GM2 stimulate ERK1/2 and JNK but not p38 MAPK, whereas GM3 suppresses VSMC growth and has no effect on ERK1/2, JNK, and p38 MAPK stimulation. We also demonstrated that GM3 had no antagonistic effect on the GM1/2-induced ERK1/2 phosphorylation. However, the maximal effect of ERK1/2 varied between 5 minutes (Figure

Figure 5. Effects of PTX on GM1- and GM2-induced phosphorylation of ERK1/2. VSMCs were precultured in serum-free medium for 24 hours. Cells were then treated with 100 ng/mL PTX for 4 hours and were then stimulated with 50 μmol/L GM1 (top panel) or GM2 (bottom panel) for 15 minutes. Equal amounts of protein (10 μg per lane) were analyzed by chemiluminescence Western blotting using specific antibodies that recognize P-ERK1/P-ERK2 (representative blot from 2 separate independent experiments). At the bottom of each panel, 10 μg protein was analyzed by chemiluminescence Western blotting using specific antibodies that recognize total ERK1/2.

Figure 6. Effect of GM1 and GM2 on Raf-1 kinase phosphorylation. VSMCs were precultured in serum-free medium for 24 hours and then stimulated with 50 μmol/L GM1 and GM2 for different time periods. Equal amounts of protein (10 μg per lane) were analyzed by the chemiluminescence Western blotting method using phospho-specific Raf-1 kinase antibodies (representative blot from 2 separate independent experiments). Arrow shows the phosphorylated 74-kDa Raf-1 kinase protein (P-Raf-1).

Figure 7. Effect of FTI on GM1- and GM2-induced ERK1/2 stimulation. VSMCs were precultured in serum-free medium for 24 hours. Cells were then treated with 25 μmol/L FTI for 4 hours and then stimulated with 50 μmol/L GM1 and GM2 for different time periods. Equal amounts of protein (10 μg per lane) were analyzed by the chemiluminescence Western blotting method (representative blot from 2 separate independent experiments). At the bottom, PDVF membrane was stripped and rebotted with total ERK1/2 antibodies.

Figure 8. Effect of PD 098,059 on the GM1- and GM2-induced increase in cell number. VSMCs were precultured in 24-well plates for 24 hours in serum-free medium. Then cells were treated with 20 μmol/L PD 098,059 for 3 hours. Cells were then stimulated with 50 μmol/L GM1 and GM2 or with 50 ng/mL PDGF-BB. After 24 hours, cells were trypsinized, and cell counts were determined as described in Methods (mean±SD of 3 separate independent experiments, each performed in triplicate). *P<0.05 for GM1 or GM2 effect vs control; **P<0.05 for PD 098,059+GM1 or PD 098,059+GM2 effect vs GM1 or GM2 effect, respectively.
2) and 15 minutes (Figures 3 and 7) among different experiments. This is a normal observation obtained by several laboratories and probably depends on several experimental conditions, such as strain and age of animal, cultivation conditions, number of passages, cell density, and culture time and even on the individual animals. In this context, it is established that the proliferation of VSMCs in response to various growth factors also differs and depends on the same experimental conditions.

Activation of ERK1/2 by classic growth factors is assumed to occur via a cascade that requires the activation of p210GTPase–activating protein, Raf-1 kinase (a 74-kDa protein kinase encoded by the proto-oncogene raf-1), and MEK1. It is believed that activation of Raf-1 kinase occurs via phosphorylation of several serine and threonine residues of the molecule by the activated c-Ras. The ERK1/2-activating cascade may also be stimulated by G proteins via activation of MEK kinase (MEKK) independently of the activating cascade. Other than Raf and MEKK, c-Mos kinase activation of MEK kinase (MEKK) independently of the activating cascade may also be stimulated by G proteins via activation of MEK kinase (MEKK) independently of the Raf-1 cascade. Other than Raf and MEKK, c-Mos kinase is also capable of stimulating MEK1. On the basis of the fact that we did not observe an activation of Raf-1 kinase and that the farnesyl transferase blocker of c-Ras did not inhibit the phosphorylation of ERK1/2, it can be suggested that the activation of ERK1/2 by GM1 and GM2 occurs via a Ras/Raf-1 kinase–independent pathway. Therefore, we may assume that probably c-Mos kinase or MEKK is involved in the ganglioside GM1/2-induced activation of ERK1/2. In this context, we showed that treatment of the cells with the MEK1 inhibitor PD 098,059 prevented activation of ERK1/2 and inhibited the proliferative effects of GM1 and GM2. From these findings, we suggest that GM1 and GM2 promote proliferation of VSMCs by ERK1/2 activation. However, because PD 098,059 reversed the activation of both ERK1/2 and JNK, it cannot be excluded that JNK stimulation also contributes to the proliferative effects of both gangliosides.

Furthermore, because treatment of VSMCs with PTX abolished the GM1- and GM2-induced activation of ERK1/2, it can be concluded that the proliferative effects of GM1 and GM2 on VSMC growth are mediated by a G1-coupled receptor. This could be of significant clinical relevance, inasmuch as there is evidence that activation of the GPCR signaling pathway can markedly amplify the responses produced by a separate coincident activation of other receptors. It should be pointed out that other sphingolipids also activate the mitogenic intracellular signal transduction pathway via a G1-coupled receptor. In this context, it has been established that the sphingolipids, sphingosine-1-phosphate and sphingosylphosphorylcholine, are potent VSMC mitogens acting through a PTX-sensitive activation of ERK1/2. Because GM1 and GM2 contain the same sphingolipid moiety (ceramide) as sphingosine-1-phosphate and sphingo-lysylphosphorylcholine, it may be suggested that the sphingo- lipid moiety of gangliosides is essential for their mitogenic intracellular transduction pathway. Ceramide has been known to interact with the ERK1/2 and the JNK signaling system. In particular, ceramide regulates the ERK1/2 pathway in a variety of cell types, including human umbilical vein endothelial cells, in which it activates Raf-1, MEK, and ERK1/2. Ceramide may lead to JNK activation by 2 potential mechanisms, either via transforming growth factor-β–activated kinase or via the small G protein Rac-1. However, because GM3 contains the same ceramide moiety as GM1 and GM2, it is rather unlikely that ceramide is responsible for the growth-promoting effects of the gangliosides. Gangliosides have a water-soluble carbohydrate head group as well as a lipophilic tail. There is a potential for enormous structural diversity in each portion of the molecule. Differences in the type of oligosaccharide moieties as well as the number, position, and linkage of sialic acid residues, combined with variations in ceramide structure and fatty acyl hydroxylation, lead to a wide range of biological activities. Indeed, there are differences between GM1, GM2, and GM3 in the carbohydrate head group structure. GM2 contains 1 N-acetylgalactosamine molecule more than GM3, whereas GM1 contains 1 N-acetylgalactosamine molecule and 1 galactose molecule more than GM3. Therefore, it may be postulated that differences between GM1 or GM2 and GM3 regarding their ability to stimulate proliferation of VSMCs and early intracellular events may be due to differences in their carbohydrate head groups. In this context, we may speculate that the carbohydrate head group of GM1 and GM2 is essential for the stimulation of the putative ganglioside G1-coupled receptor, resulting in stimulation of ERK1/2 and proliferation of VSMCs.

Gangliosides interact with a variety of cell surface receptors, including integrins and galectins. Integrins and galectins, a widely expressed group of mammalian lectins, are known to be involved in the regulation of cell adhesion, immune function, cell proliferation, and apoptosis. Recently, it has been shown that galectin-1, present on the surface of human neuroblastoma cells, is the major receptor for ganglioside GM1. Interestingly, GM3 cannot bind to the galectin-1 receptor.

More recently, there has been accumulating evidence that direct stimulation of different mammalian cells, such as U-1242 human glioma cells and microglia cells (brain resident macrophages), with gangliosides results in a stimulation of MAPK normally induced by growth factors. Our results are in agreement with the findings showing that gangliosides are able to stimulate ERK1/2 and JNK in brain microglia. Also, stimulation of ERK1/2 and DNA synthesis by GM1 has been reported in quiescent U-1242 human glioma cells. Van Brocklyn et al have demonstrated that PD 098,059 prevents the GM1-induced stimulation of ERK1/2 and the GM1-induced proliferation of the human glioma cells.

It is known that VSMC proliferation plays an important role in the pathogenesis of cardiovascular diseases, such as atherosclerosis and hypertension. Although there is some evidence from the literature indicating that gangliosides may be involved in the development of atherosclerosis and hypertension, only little is known concerning the role of gangliosides in the development of hypertension. In this context, it is established that structural changes of small arteries are associated with hypertension. These changes occur because of an increase of the medial thickness and decreased luminal diameter, thus resulting in an increased media/lumen ratio. According to the hypertrophic remodeling
hypothesis, the media/lumen ratio is increased as a consequence of proliferation of the smooth muscle cells induced by growth factors, such as angiotensin II and endothelin.1,2,52 In this context, it is established that the classic growth factors mainly promote proliferation of VSMCs via activation of the ERK1/2 pathway. Our findings provide evidence for the first time that GM1 and GM2, like classic growth factors, directly promote VSMC proliferation via activation of the ERK1/2 pathway and may, therefore, play an important role in the pathogenesis of hypertension. However, further clinical studies are required to elucidate the role of gangliosides in the development of cardiovascular disease.

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References


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