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GM1 ganglioside modulates prostaglandin E1 stimulated adenylyl cyclase in neuro-2A cells

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This study demonstrates modulation by GM1 ganglioside of prostaglandin E1 (PGE1)-induced cAMP formation in Neuro-2a neuroblastoma cells. Pretreatment of the cells with neuraminidase, an enzyme that increases cell surface GM1, resulted in significant elevation of PGE1-induced cAMP formation, as did preincubation of the cells with nmolar concentrations of GM1. Pretreatment with brain ganglioside mixture lacking GM1 had no effect. Cholera toxin B subunit, a specific GM1-binding ligand, inhibited adenylyl cyclase. When the concentration of exogenous GM1 in which the cells were preincubated was increased from nmolar to μmolar levels there was a dose-responsive fall off in cAMP elevation, attributed to progressive inhibition of adenylyl cyclase by increasing GM1. These results are interpreted as indicating modulation of this PGE1 receptor in Neuro-2a cells by plasma membrane-localized GM1 in a structure-specific manner.

Keywords: GM1 ganglioside, prostaglandin receptor, adenylyl cyclase, cyclic AMP, cholera toxin B subunit, neuraminidase, gangliosides, GM1 as receptor modulator

Abbreviations: PGE1, prostaglandin E1; Ctx B, B subunit of cholera toxin; BBG, bovine brain ganglioside mixture; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; IBMX, 3-isobutyl-1-methylxanthine; N'ase, neuraminidase; D-PBS, Dulbecco's phosphate-buffered saline.

Introduction

Ever since their discovery some 60 years ago gangliosides have posed an intriguing enigma to neuroscientists attempting to understand their biological raison d'etre. Through the painstaking efforts of many laboratories a coherent picture is now emerging according to which the primary function of glycolipids generally, including the subgroup containing sialic acid (gangliosides), is modulation of a wide variety of receptors, enzymes, ion channels, cell adhesion molecules, and perhaps other membrane-localized proteins. This model would rationalize the large and still growing family of reported glycolipid structures (approximately 90 gangliosides [1] and 300 glycosphingolipids of all kinds [2]), assuming each glycolipid is designed for optimal interaction with a specific protein structure. Early observations along this line included ganglioside interactions with receptors for thyrotropin [3], chorionic gonadotropin [4], and luteinizing hormone [5].

Several important contributions to this paradigm of ganglioside function have been made over the years by Dr Sen-itiroh Hakomori and coworkers, whose ideas have been summarized in recent reviews [6–8]. In proposing that gangliosides function not as receptors themselves but through interaction with specific protein receptors in a manner that modifies signal transduction [9], Bremer and Hakomori demonstrated that the proliferative effects of epidermal- and platelet-derived growth factors on Swiss 3T3 cells were significantly inhibited by GM1 and GM3. Subsequently, Hakomori and coworkers showed that tyrosine kinase associated with the epidermal growth factor receptor was specifically inhibited by GM3 [10], while tyrosine kinase associated with the receptor for platelet-derived growth factor was inhibited by both GM3 and GM1 [11]. Recent work has suggested that the mechanism involves inhibition of receptor dimerization/oligomerization, thus accounting for inhibition of tyrosine kinase and associated cell proliferation [8, 12]. Insulin receptor-associated tyrosine kinase activity was recently shown to be modulated by 2,3-sialosylparagloboside [13].

In addition to these examples of tyrosine kinase-associated receptors, a number of G protein-linked

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receptors are now known to be modulated by gangliosides, some in a rather striking fashion. Thus, opioid receptors operating in the excitatory mode are potently modulated by endogenous as well as exogenously applied GM1, resulting in adenylyl cyclase stimulation as well as ion channel changes [14]. The present study demonstrates a similar phenomenon with respect to the prostaglandin E1 (PGE1) receptor in Neuro-2a neuroblastoma cells. The ability of this ligand-receptor interaction to activate adenylyl cyclase is significantly enhanced or inhibited by manipulations which increase or decrease, respectively, the plasma membrane GM1 level. We show in addition a direct inhibitory effect of this exogenously applied ganglioside on adenylyl cyclase, this inhibition being sufficient to offset receptor activation at high GM1 concentrations.

Materials and methods

Materials

The following substances were obtained from the sources indicated: Dulbecco's modified Eagle's medium (DMEM), Dulbecco's phosphate buffered saline (D-PBS), fetal bovine serum (FBS) from GIBCO Laboratories (Grand Island, NY); neuraminidase (N'ase, type V from C. perfringens), PGE1, forskolin, IBMX, and chemicals for cAMP assay from SIGMA (St Louis, MO); cholera toxin B subunit (Ctx B) from List Biological Laboratories (Campbell, CA). GM1 ganglioside and bovine brain ganglioside mixture (BBG) were gifts from Fidia Research Laboratories (Abano Terme, Italy). Stock solutions of PGE1 (10 mM), forskolin (10 mM), and IBMX (100 mM) were prepared in dimethylsulfoxide, and stocks of N'ase (5 U ml^{-1}), Ctx B (0.5 mg ml^{-1}), GM1 (1 mM), and BBG lacking GM1 (BBG-GM1) (1 mM) were prepared in medium. BBG-GM1 was prepared as described [15], with modification: BBG dissolved in methanol was applied to a DEAE-Sephadex column (acetate form); monosialogangliosides were eluted with 0.012 M ammonium acetate in methanol, and the remainder (BBG-GM1) with 0.25 M of same. Salt was removed by dialysis and the sample lyophilized to dryness.

Cell culture

Neuro-2a neuroblastoma cells from American Type Culture Collection (CCL 131, Rockville, MD) were grown routinely in T-25 Falcon tissue culture flasks containing 5 ml of DMEM supplemented with 10% FBS (v/v) plus antibiotics in 5% CO_2/95% humidified air at 37 °C. Cells were passaged three times per week.

cAMP formation and assay

Cells grown as above were transferred for 2 h at 37 °C to fresh DMEM (3 ml per flask) containing HEPES (25 mM, pH 7.2) and one of the following: N'ase (0.5 U ml^{-1}), GM1 (0.01–100 μM), or BBG-GM1 (0.01–100 μM). IBMX (500 μM) was applied during the final 30 min of incubation. After removing the medium, cells were treated for 1 h with varied amounts of PGE1 or forskolin with or without Ctx B (5 μg ml^{-1}) in DMEM/HEPES supplemented with the same amount of IBMX. The reaction was stopped by removing medium and rinsing twice with 3 ml cold D-PBS. Cells were harvested by scraping twice with 0.3 ml of 5% trichloroacetic acid solution. Cyclic-AMP was extracted and assayed as previously described [16].

Results

Treatment of Neuro-2a cells with PGE1 caused intracellular accumulation of cAMP, maximum response being achieved at 1 μM PGE1. These cells were found to bind cholera toxin B subunit (Ctx B), consistent with previous demonstration that GM1 ganglioside (and some of its oligosialo analogues) occur in the plasma membrane [17]. Application of Ctx B concurrently with PGE1 resulted in 30–50% less cAMP formation than with PGE1 alone (Fig. 1A). On the other hand, elevation of plasma membrane GM1 by N'ase pretreatment [18] significantly potentiated PGE1-stimulated cAMP formation (Fig. 1B). In this case the increase of PGE1-stimulated cAMP formation was blocked by coapplication of Ctx B (Fig. 1C). N'ase or Ctx B alone or in combination did not change basal levels of cAMP.

Preincubation of the cells in GM1-containing medium for 2 h, a procedure known to elevate plasma membrane GM1, had similar potentiating effect as N'ase provided GM1 concentration was in the nmolar to low μmolar range (Fig. 2A). While 10 μM was marginally effective, 100 μM GM1 had no effect. When the mixture of bovine brain gangliosides lacking GM1 (BBG-GM1) was applied over a similar concentration range, no significant effects were observed (Fig. 2B). Ganglioside treatment alone did not elevate basal levels of cAMP.

The apparent inhibitory effect of the higher GM1 preincubation concentrations was further investigated by examining the effects of such concentrations on forskolin-stimulated adenylyl cyclase. While no significant inhibition was seen up to 1 μM GM1, 10 μM and 100 μM GM1 caused progressively greater inhibition (Fig. 3). Ctx B had no effect on this reaction, consistent with the fact that forskolin in acting directly on the enzyme by-passes the receptor-mediated reaction (and effects of associated GM1).

Discussion

This study has revealed a powerful modulatory effect of GM1 ganglioside on the PGE1 receptor of Neuro-2a cells, resulting in significant enhancement of PGE1-stimulated

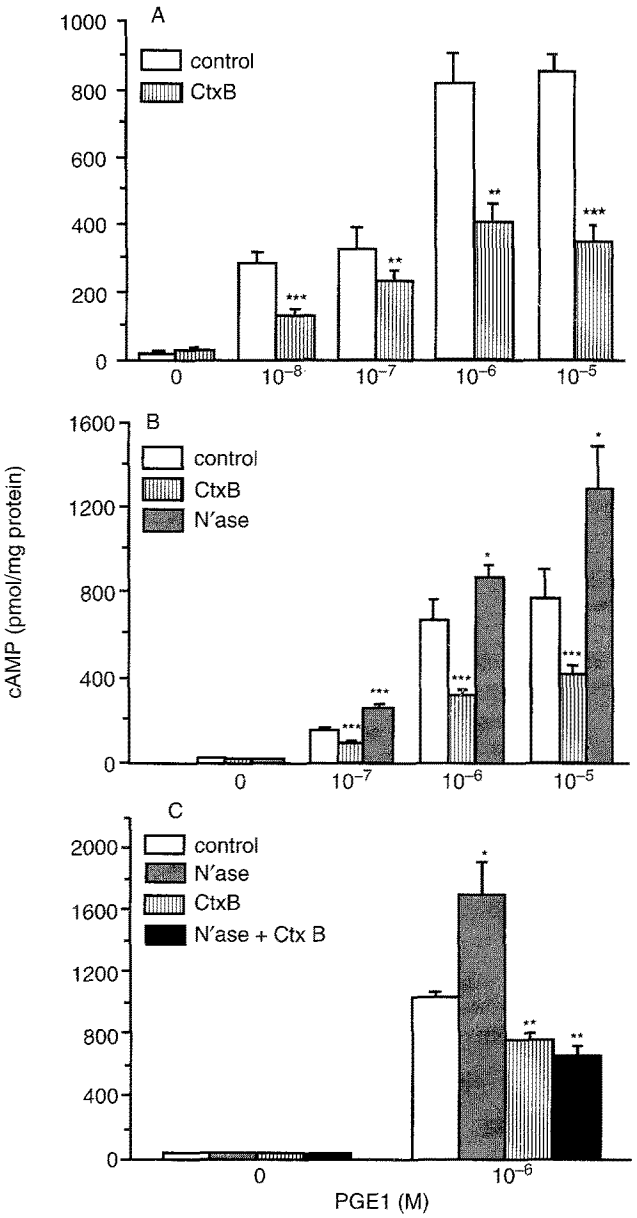

Figure 1. Effect of endogenous GM1 on PGE1-stimulated cAMP formation. Cells were treated with PGE1 (0.01–10 μM) at 37 °C for 1 h, resulting in accumulation of cAMP. Application of Ctx B together with PGE1 caused reduction of cAMP. Increase of GM1 by pretreatment of cells with N'ase (0.2 U ml^{-1}) resulted in an increase in PGE1-stimulated cAMP formation which was blocked by Ctx B; the latter also inhibited adenylyl cyclase stimulated by PGE1 alone. Neither Ctx B, N'ase, or a combination of these affected basal levels of cAMP. Data are means ± SEM ($n = 6$ in panel A, $n = 5$ in panels B and C) from one of two representative experiments. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ (relative to control at same concentration).

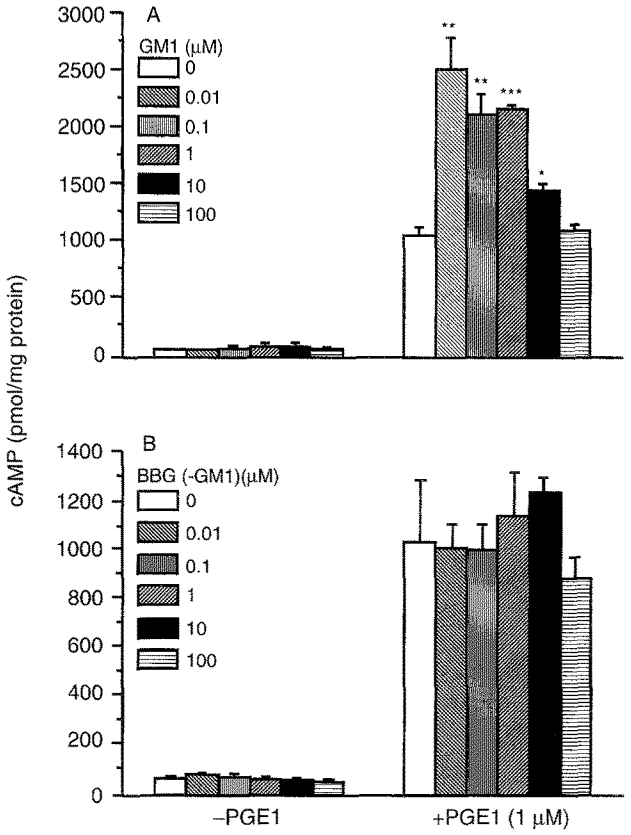

Figure 2. Effect of exogenous gangliosides on PGE1-stimulated cAMP formation. Cells preincubated with low concentrations of GM1 (≤ 1 μM) and treated with PGE1 as above showed elevated cAMP, while higher concentrations of GM1 showed progressively less effect (A). Preincubation of cells with BBG-GM1 did not effect cAMP (B). Gangliosides alone had no effect on basal adenylyl cyclase. Data are means ± SEM ($n = 5$ in A, $n = 4$ in B) from one of two representative experiments. $*p < 0.05$; $**p < 0.01$, $***p < 0.001$ (relative to zero GM1).

adenylyl cyclase. That the cell's endogenous pool of GM1 may have such a physiological role seems plausible in view of the substantial inhibitory effect of Ctx B, a specific GM1-binding ligand of high affinity [19], and the potentiation induced by N'ase, an enzyme previously shown to increase endogenous GM1 on the surface of Neuro-2a cells [18]. This conclusion is strengthened by the similar enhancement caused by preincubation of the cells in nmolar concentrations of GM1, known to result in plasma membrane insertion of the exogenously applied ganglioside [20, 21]. The effect showed molecular specificity, based on failure of brain gangliosides other than GM1 (BBG-GM1) to influence the reaction.

Although gangliosides are now seen as modulators of a variety of membrane receptors (for reviews see [6, 7, 22]), only a few have been found to involve GM1 specifically. Inhibitory as well as stimulatory effects have been reported, depending on receptor class, cell type, and interactions with other signalling systems. An example of the former effect is the structure-specific inhibition by GM1 of the isoproterenol-activated human β_1-adrenergic receptor expressed in Sf9 insect cells [23]. An example

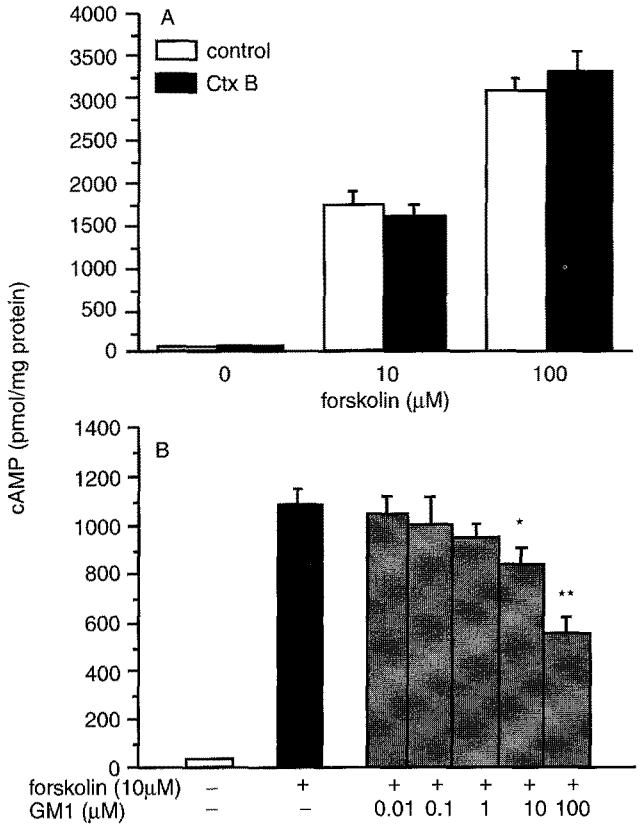

Figure 3. Effect of GM1 on forskolin-induced cAMP formation. Cells treated 1 h at 37° with 10 or 100 μM forskolin showed increased levels of cAMP. Application of Ctx B (5 $\mu g\,ml^{-1}$) had no effect on the forskolin-induced reaction (A). Preincubation of cells with higher concentrations of GM1 ($\geq$ 10 μM) resulted in decrease of forskolin-stimulated cAMP (B). Data are means $\pm$ SEM ($n = 5$ in A, $n = 10$ in B) from one of two representative experiments. $*p < 0.05$; $**p < 0.001$ (relative to forskolin-treated, GM1 untreated sample = solid bar in B).

of structure-specific potentiation, very similar to that observed here, was the enhancement by GM1 of the excitatory effect of opioids on sensory neurons in cultures of dorsal root ganglia [14, 24]. Pretreatment of those cells with Ctx B caused selective blockade of the opioid-induced prolongation of the Ca^{2+} component of the action potential duration, an effect mediated by opioid receptors in the excitatory mode. As with the PGE1 receptor, preincubation of those opioid receptors with nmolar concentrations of GM1 potentiated the effect in a structure specific manner [14]. Opioid receptors also interact with adenylyl cyclase, and it was of some interest that a recently cloned δ-opioid receptor, stably expressed in a non-neuronal cell line, was converted from inhibitory to excitatory modulator of this enzyme by preincubation of the cells with GM1 [25]. As an example of structural nonspecificity, GM1 and other gangliosides enhanced serotonin-induced elevation of cAMP and produced a 10-fold increase in receptor affinity for serotonin in NCB-20 cells [26].

Prostaglandins are present in the nervous system and their receptors have been identified as belonging to the G protein-associated family [27, 28]. PGE1 and PGE2 exert similar effects with most of the receptor subtypes that have been identified, one prominent effect being modulation of adenylyl cyclase. The EP2 receptor, which was recently cloned [29–31], was shown to mediate enhancement of adenylyl cyclase and is possibly the type present in Neuro-2a cells. An illuminating study which reveals the correlative effect between GM1 interaction with prostaglandin- and opioid receptors showed that chronic exposure of NG108-15 cells to opioid enhanced the coupling of the prostaglandin receptor to Gs with increased cAMP formation [32]. We believe this can be attributed to elevation of membrane GM1, which we recently showed occurs with neural-derived cells during chronic exposure to opioids [16].

The seeming paradox in our observation of reduced activation with higher GM1 concentrations is explained by the direct, dose-response inhibition by GM1 of the forskolin-stimulated enzyme (Fig. 3). This is consistent with the previously reported inhibitory effect of exogenous ganglioside on basal, thyrotropin-induced, and fluoride-induced adenylyl cyclase in thyroid membranes, suggested as resulting from action on the enzyme effector rather than receptor [33]. Another report, however, showed significant increase in basal adenylyl cyclase activity on addition of mixed brain gangliosides to cerebral cortex membranes [34]. These divergent effects might now be understood on the basis of the multiplicity of G protein-regulated adenylyl cyclases with their distinct patterns of regulation [35].

This study adds to the growing list of G protein-regulated receptors that are modulated by GM1 or related molecule(s). A ganglioside of the latter type, migrating behind GM1 on TLC but reactive toward Ctx B, was shown to be associated with the thyrotropin receptor during its isolation [36]. It is clear that not all G protein-associated receptors are modulated in this manner in view of the absence of ganglioside effect on the β_2-adrenergic receptor expressed in Sf9 cells [23]. The earlier finding that thyrotropin receptor contains an amino acid sequence homologous to Ctx B (37) suggests that this may be the source of the difference and that it may be profitable to seek evidence of similar homology in other receptors of this family that are responsive to GM1.

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