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ORIGINAL ARTICLE

Nitric oxide and potassium channels mediate GM1 ganglioside-induced vasorelaxation

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Abstract Monosialotetrahexosylganglioside (GM1) is a glycosphingolipid present in most cell membranes that displays antioxidant and neuroprotective properties. It has been recently described that GM1 induces pial vessel vasodilation and increases NO_x content in cerebral cortex, which are fully prevented by the nitric oxide synthase inhibitor N^G-nitro-*l*-arginine methyl ester (L-NAME). However, it is not known whether GM1 relaxes larger vessels, as well as the mechanisms by which GM1 causes vasorelaxation. In this study, we demonstrate that GM1 (10, 30, 100, 300 μM, 1 and 3 mM) induces vascular relaxation determined by isometric tension studies in rat mesenteric artery rings contracted with 1 μM phenylephrine. The vasorelaxation induced by GM1 was abolished by endothelium removal, by incubation with L-NAME (1 μM), and partially inhibited by the blockade of potassium channels by 1 mM tetraethylammonium, 10 μM glibenclamide, by the soluble guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo [4,3-alpha]quinoxalin-1-one (10 μM), and by 50 nM charybdotoxin, a blocker of large and intermediate conductance calcium-activated potassium channels. Moreover, GM1-induced relaxation was not affected by apamin (50 nM), a small conductance calcium-activated potassium channel blocker. The results indicate that direct and indirect nitric oxide pathways play a pivotal role in vasorelaxation induced by GM1, which is mediated mainly by potassium channels activation. We suggest that vasodilation may underlie some of the biological effects of exogenous GM1 ganglioside.

Keywords Mesenteric artery · Potassium channel · Nitric oxide · Guanylate cyclase · Endothelium

Abbreviations

ACh	Acetylcholine
ANOVA	Analysis of variance
cGMP	Cyclic guanosine monophosphate
(+E)	Endothelium intact
(−E)	Endothelium denuded
eNOS	Endothelial nitric oxide synthase
GLB	Glibenclamide
GM1	Monosialotetrahexosylganglioside
K_{ATP}	ATP-sensitive potassium channel
K_{Ca}	Calcium-activated potassium channel
K_V	Voltage-sensitive potassium channel
L-NAME	N^G-nitro-*l*-arginine methyl ester
NO	Nitric oxide

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NOS	Nitric oxide synthase
ODQ	1H-[1,2,4]oxadiazolo[4,3-alpha] quinoxalin-1-one
Phe	Phenylephrine
sGC	Guanylate cyclase soluble
TEA	Tetraethylammonium
ChTX	Charybdotoxin

Introduction

Gangliosides constitute a heterogeneous family of sialic acid-containing glycosphingolipids that are components of most cell membranes. They are particularly abundant in the brain, where they represent the major lipid constituent of the neuronal surface (Ledeen and Yu 1982; Tettamanti 2004). Administration of monosialotetrahexosylganglioside (GM1) has been reported to protect the central nervous system against various neurotoxic agents or conditions, such as methylmalonic acid (Fighera et al. 2003), pentylenetetrazol and glutaric acid exposure (Fighera et al. 2006), anoxia (Carolei et al. 1991; Tan et al. 1993) and ischemia (Carolei et al. 1991; Kwak et al. 2005), lead-induced neurotoxicity (She et al. 2009), Parkinson's (Schneider 1998) and Alzheimer's diseases (Svennerholm 1994; Yanagisawa 2007), traumatic brain injury (Chen et al. 2003), and spinal cord injury (Geisler et al. 2001) accompanied by an apparent absence of side effects in animals. However, caution is warranted because of reports of sporadic cases of Guillain–Barré syndrome after ganglioside therapy (Yuki 1998; Govoni et al. 2003; Komagamine and Yuki 2006).

Several neurochemical mechanisms have been proposed for GM1-induced neuroprotection. It has been proposed that GM1 interacts with neurotrophic factors and their receptors in vivo and in situ (Duchemin et al. 1997, 1998, 2002; Rabin et al. 2002), activating mitogen-activated protein kinase (Duchemin et al. 2002; Mo et al. 2005), and PI3-kinase/Akt survival pathways (Duchemin et al. 2008). In addition, there are studies showing that GM1 presents antioxidant activity, both in situ and in vivo (Avrova et al. 1994, 1998), and that its systemic administration increases the striatal ascorbic acid content (Fighera et al. 2003) and catalase activity in homogenates of the cerebral cortex (Fighera et al. 2004). In fact, it has been demonstrated that vasodilation underlies the GM1-induced increase of catalase content in the brain and may be responsible, at least in part, for the neuroprotection induced by this ganglioside (Furian et al. 2007).

Nitric oxide (NO) is a short-lived gas, involved in several cellular functions, particularly in the brain, and has been recognized as a critical physiological mediator in the regulation of the vascular tone (Moncada and Higgs 1991). NO is synthesized from L-arginine by the members of the nitric oxide synthase (NOS) family of proteins endothelial (eNOS), neuronal, and inducible (Calabrese et al. 2007). NO synthesized in endothelial cells diffuses to smooth muscle cells where it activates soluble guanylate cyclase (Dudzinski and Michel 2007), resulting in cyclic guanylate monophosphate-dependent vasodilation (Furchgott and Zawadzki 1980; Katsuki et al. 1977; Palmer et al. 1987), an important process in the homeostasis of blood flow. Cyclic GMP (cGMP) causes hyperpolarization by activating K^+ channels, resulting in smooth muscle relaxation (Jackson 1998). In addition, it has also been reported that NO causes direct activation of K^+ channels in smooth muscle cells, causing vasodilation (Bolotina et al. 1994). In summary, accumulating evidence suggests that NO regulates blood vessel tonus through direct or indirect activation of K^+ channels, but the role of these channels on GM1-induced vasodilation is unknown. Therefore, since (1) GM1 increases NO content, (2) N^G-nitro-*l*-arginine methyl ester (L-NAME) prevents GM1-induced vasodilation (Furian et al. 2008), and (3) guanylate cyclase soluble (sGC) and K^+ channels play a key role in the NO-mediated regulation of blood vessel tonus, we hypothesized that these downstream effectors of nitric oxide pathways (guanylate cyclase and K^+ channels) are involved in GM1-induced vasorelaxation.

Experimental procedures

Animals

Adult male Wistar rats (250–280 g) maintained on a 12-h light/dark cycle and with free access to tap water and standard laboratory chow (Nuvital®, Brazil) were used. All experimental protocols (including statistical evaluation) were designed aiming to keep the number of animals used to a minimum, as well as their suffering. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The Institutional Ethics Committee of the Federal University of Paraná approved all procedures adopted in this study.

Drugs

Phenylephrine (Phe) hydrochloride, acetylcholine (ACh) chloride, L-NAME, 1H-[1,2,4]oxadiazolo[4,3-alpha] quinoxalin-1-one (ODQ), tetraethylammonium (TEA), glibenclamide (GLB), apamin, and charybdotoxin (ChTX) were purchased from Sigma (St. Louis, MO, USA). GLB was dissolved in dimethyl sulfoxide. All other reagents were of the highest grade, and solutions were prepared in fresh type I

ultrapure water. GM1 ganglioside was kindly donated by TRB Pharma Laboratories, São Paulo, Brazil.

Preparation of rat mesenteric rings

The rat superior mesenteric artery was identified, and mesenteric rings were prepared. Briefly, segments of the mesenteric artery were excised, cleaned of adhering tissue, cut into 2-mm-long rings, and transferred to a dish filled with Krebs–Henseleit buffer (pH 7.4; composition in mM: NaCl 115.3, KCl 4.9, $CaCl_2 \cdot 2H_2O$ 1.46, KH_2PO_4 1.2, $MgSO_4$ 1.2, D-glucose 11.1, $NaHCO_3$ 25). The rings were suspended in organ baths containing the physiological solution bubbled with carbogen (5% CO_2/95% O_2) at 37°C. Isometric force transducers (Letica Scientific Instruments, Barcelona, Spain) coupled to a MacLab® recording system and its application program (Chart, v 3.3) from ADInstruments (Castle Hill, Australia) were used to record contractions and relaxations. Preparations were maintained at a basal tension of 5 mN and allowed to stabilize for 1 h before drugs were added, and during this time, the solution was changed every 15 min. In some experiments, endothelium was removed by gently rubbing inside the vessel with a small wire. The integrity of the endothelial layer was verified by the ability of ACh (1 μM) to fully relax vessels precontracted with Phe (1 μM; Rattmann et al. 2009).

Measurement of vascular relaxation

The ability of cumulative concentrations of GM1 (10, 30, 100, 300 nM, 1, 3, 10, 30, 100, 300 μM, 1 and 3 mM) to relax endothelium-intact and endothelium-denuded mesenteric rings under sustained contraction elicited with Phe (1 μM) was determined. Only intact endothelium mesenteric rings were used in the experiments designed to evaluate the involvement of K^+ channels, NOS, and sGC in the vasorelaxing effect of GM1.

Mesenteric rings were subjected to a 15-min incubation with L-NAME (1 μM; a nonselective nitric oxide synthase inhibitor), or ODQ (10 μM; a soluble guanylate cyclase inhibitor), or TEA (1 mM; a nonselective K^+ channel blocker), or GLB (10 μM; an ATP-sensitive K^+ channel blocker), or apamin (50 nM; a small conductance calcium-activated K^+ channel blocker), or ChTX (50 nM; a large and intermediate-conductance calcium-activated K^+ channel blocker) and contracted with Phe (1 μM). After sustained contraction elicited by Phe to the incubation medium, GM1 (10, 30, 100, 300 nM, 1, 3, 10, 30, 100, 300 μM, 1 and 3 mM) was added and the relaxation response measured, as described in "Preparation of rat mesenteric rings" section. EC50 values were calculated with GraphPad Prism 4.0 software, by using a nonlinear regression, which employ a sigmoidal logarithmic function to estimate the plateau.

Statistical analysis

Variations in the tension of mesenteric rings induced by GM1 were analyzed by a two-way analysis of variance, with the concentrations treated as a within-subject factor. Post hoc analysis was carried out by the Student–Newman–Keuls test. A probability of $P<0.05$ was considered significant. All data are reported as mean±standard error of the mean (SEM).

Results

Figure 1 shows the effect of increasing cumulative concentrations of GM1 (10, 30, 100, 300 nM, 1, 3, 10, 30, 100, 300 μM, 1 and 3 mM) on the tonus of endothelium-intact (+E) and in endothelium-denuded (−E) mesenteric rings contracted with Phe (1 μM). GM1 induced vascular relaxation only in endothelium-intact rings ($P<0.001$; Fig. 1). The EC_{50} was 43 (22–88) μM. Post hoc analysis revealed that the minimal effective relaxing concentration of GM1 ganglioside was 10 μM. Figure 1c shows the representative trace showing the contracting and relaxing effects of Phe (1 μM) and ACh (1 μM), on mesenteric rings, and the relaxing effect after the exposure to cumulative concentrations of GM1.

Since there is evidence suggesting the involvement of nitric oxide on GM1-induced vasodilation (Furian et al. 2008), we investigated whether L-NAME alters GM1-induced vascular relaxation. L-NAME (1 μM) completely inhibited GM1-induced relaxation in rat mesenteric artery rings ($P<0.001$; Fig. 2a, b). This finding is in agreement with previous studies which have reported an inhibitory effect of L-NAME on GM1-induced vasodilation (Furian et al. 2008). In addition, the guanylate cyclase inhibitor ODQ (10 μM) attenuated the vasorelaxation induced by 3 mM GM1, further supporting a role for NO and the coupled guanylate cyclase system in the currently described effect of GM1 ($P<0.001$; Fig. 2c, d).

It was well established that potassium channels are involved in the control of smooth muscle contractility and vascular tone. The incubation with nonselective potassium channel blocker TEA (1 mM; $P<0.001$; Fig. 3a, b) reduced GM1-induced vasorelaxation, suggesting the involvement of potassium channels in this effect. In addition, ATP-sensitive potassium channel blocker GLB (10 μM) completely inhibited the vasorelaxation induced only by 3 mM of GM1 ($P<0.001$; Fig. 3c, d), providing additional experimental evidence for the involvement of K^+ channels in GM1-induced vasodilation.

Furthermore, apamin (50 nM), a small conductance calcium-activated potassium channel blocker, had no effect on GM1-induced vasorelaxation ($P=0.999$; Fig. 4a, b),

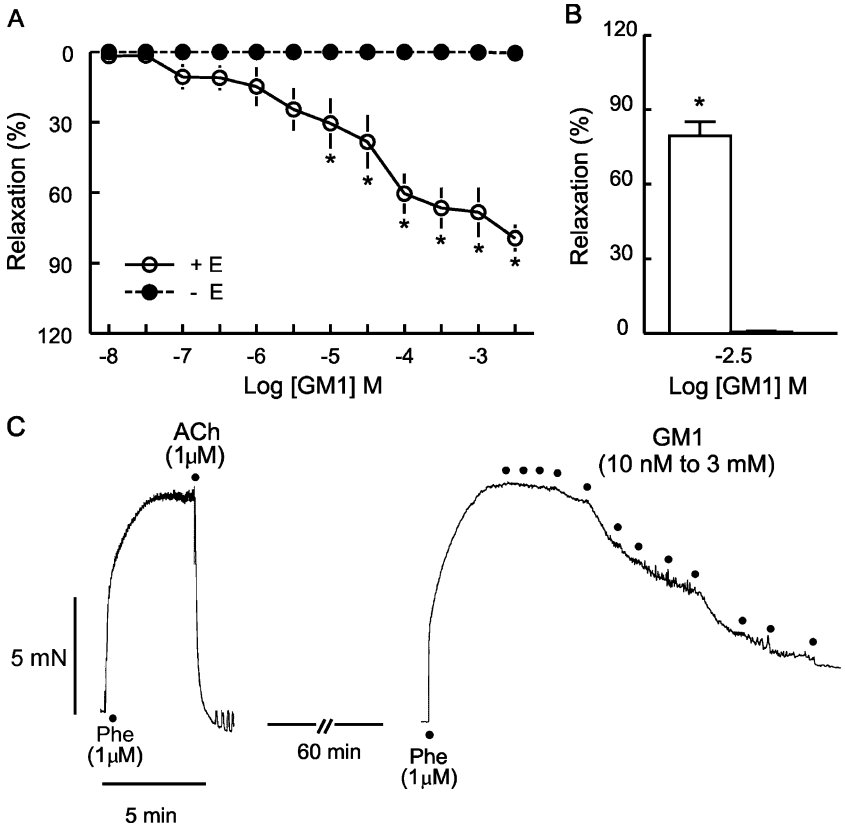

Fig. 1 GM1 induces endothelium-dependent relaxation in rat mesenteric rings. **a** Relaxing effect of GM1 (10, 30, 100, 300 nM; 1, 3, 10, 30, 100, 300 μM; 1 and 3 mM) in endothelium-intact (indicated by $+E$) and endothelium-denuded (indicated by $-E$) mesenteric rings. **b** Relaxing effect of GM1 (3 mM) in the presence and absence of endothelium. Data are mean±SEM, $n=6$. *$P<0.05$ when compared to the respective control. **c** Representative trace showing the contracting and relaxing effects of Phe (1 μM) and ACh (1 μM), respectively, on mesenteric rings maintained at a basal tension of 5 mN. After 60 min, mesenteric rings were exposed to cumulative concentrations of GM1. *Dots* indicate the addition of the drugs

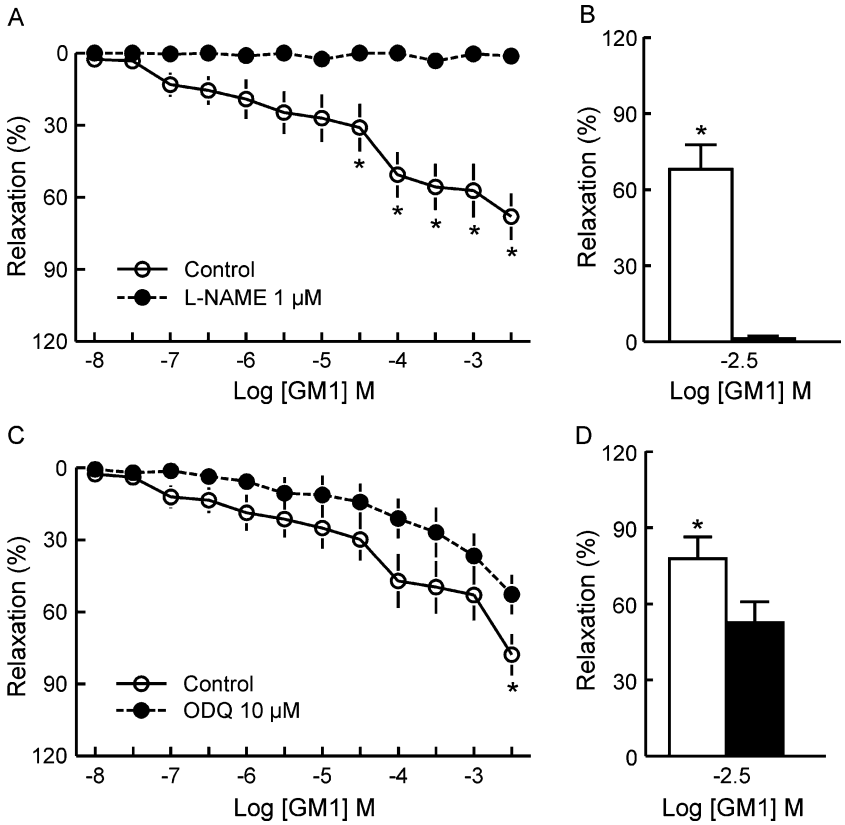

Fig. 2 GM1 induces NO-/sGC-/cGMP-dependent relaxation in rat mesenteric rings. **a** The NOS inhibitor L-NAME (1 μM) prevents, and **c** the guanylate cyclase inhibitor ODQ attenuates GM1-induced vasorelaxation. **b**, **d** The effect of L-NAME and ODQ on the vasorelaxant effects of GM1 (3 mM). Data are mean±SEM. *$P<0.05$ when compared to the respective control ($n=6$–7 per group)

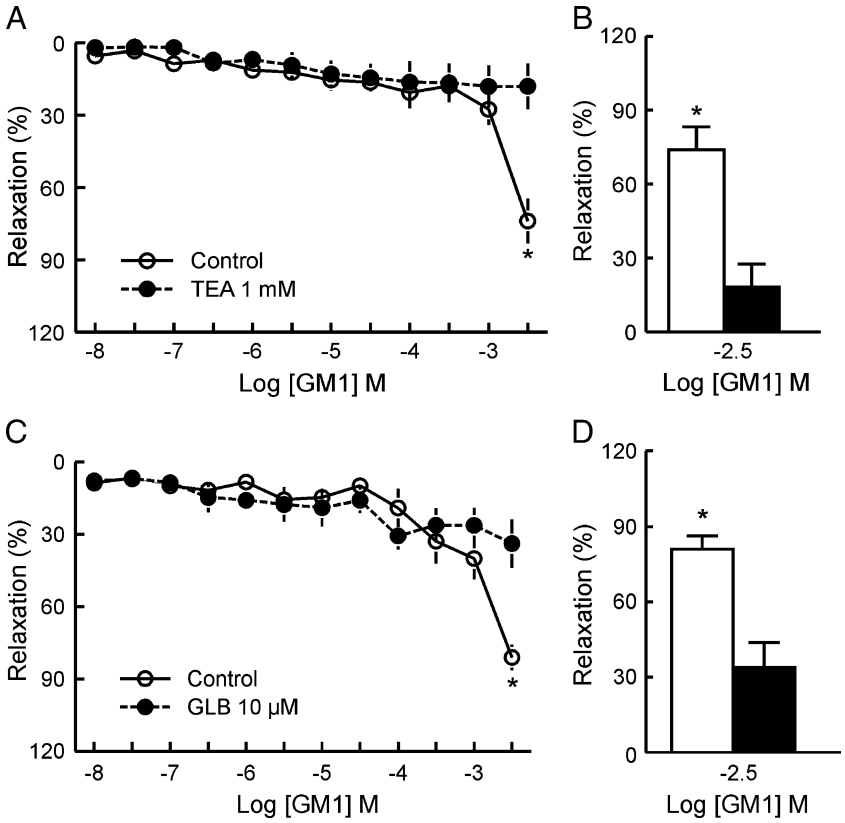

Fig. 3 Potassium channel blockers inhibit GM1-induced mesenteric relaxation. Effect of GM1 in the absence (*control curve*) or presence of **a** tetraethylammonium (*TEA*; $n=7$), **c** glibenclamide (*GLB*; $n=6$). **b**, **d** The effect of GM1 3 mM in the presence an absence of potassium channels blockers. Data are mean±SEM. *$P<0.05$ when compared to the respective control

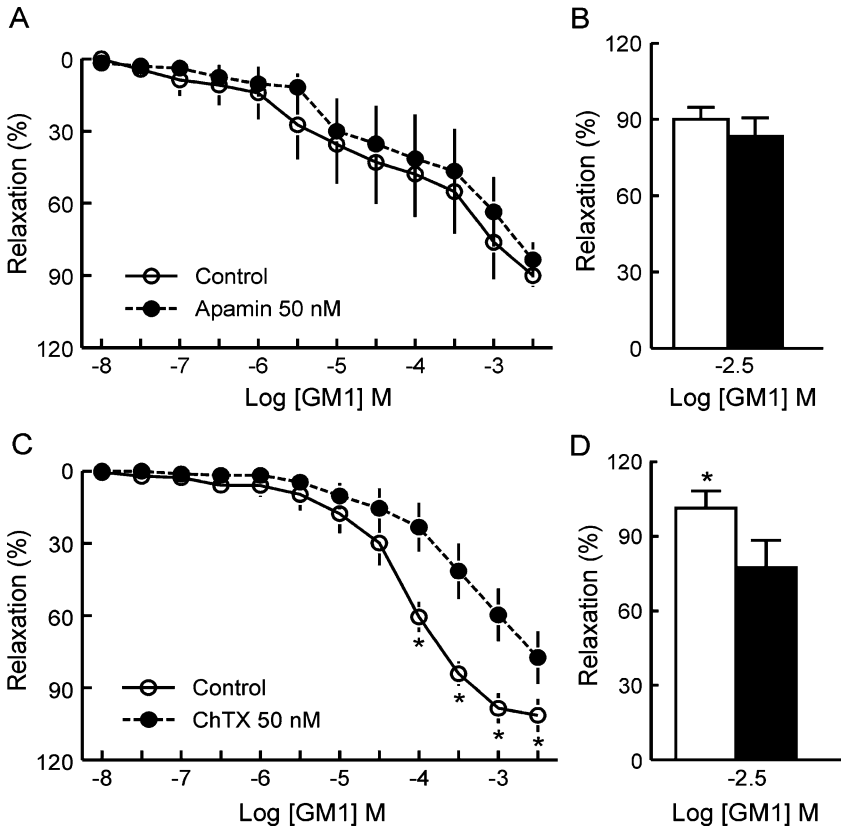

Fig. 4 Inhibition of GM1-induced mesenteric relaxation by conductance calcium-activated potassium channel blockers. Effect of GM1 in the absence (*control curve*) or presence of **a** apamin ($n=5$) and **c** charybdotoxin (*ChTX*; $n=5$). **b**, **d** The effect of GM1 3 mM in the presence and absence of apamin and ChTX. Data are mean±SEM. *$P<0.05$ when compared to the respective control

whereas ChTX (50 nM), a blocker of large and intermediate conductance calcium-activated potassium channels, induced a concentration-related right shift in cumulative concentration–effect curve for GM1 ($P<0.001$; Fig. 4c, d) increasing the EC_{50} from 114 (86–151) μM to 1 mM (668 μM–1 mM).

We also measured tensions in controls after the incubation with enzyme inhibitors and channel blockers in the presence of Phe. Statistical analyses (paired *T* test) revealed that L-NAME (3.9 to 5.5 mN), ODQ (2.6 to 4.1 mN), and apamin (3.6 to 4 mN) significantly increased Phe-induced contraction. On the other hand, TEA, ChTX, and GLB did not change Phe-induced contraction (data not shown).

Discussion

In this report, we demonstrate that GM1 induces endothelium-dependent vasorelaxation in rat superior mesenteric arteries. In addition, we gathered pharmacological evidence supporting the involvement of the NO/sGC/cGMP pathway and of K_{Ca} and K_{ATP} channels in GM1-induced vasorelaxation.

There is some evidence suggesting that GM1 causes vasodilation (Tanaka et al. 1986; Svennerholm et al. 2002). Accordingly, it has been shown that GM1 (30 mg/kg, i.v.) significantly restores local cerebral blood flow and glucose metabolism in animals subjected to arterial occlusion (Tanaka et al. 1986) and improves neurological status and cerebral blood flow in Alzheimer's disease patients (Svennerholm et al. 2002), suggesting that vasodilation and better perfusion may underlie some pharmacological effects of GM1 administration. In addition, we have recently demonstrated that GM1 increases catalase content in brain samples by causing vasodilation (Furian et al. 2007), providing a possible convergent mechanism for the neuroprotective action of GM1 (Furian et al. 2007). Furthermore, a role for NO in GM1-induced vasodilation has been proposed, since L-NAME prevents GM1-induced NO_x increase in cerebral slices and fully inhibits GM1-induced vasodilation in pial vessels (Furian et al. 2008). Interestingly, the concentration of GM1 that caused a 60% relaxation of the mesenteric artery in the current study (100 μM; Fig. 1) increased by 50% NO_x content in slices of cerebral cortex in a previous study (Furian et al. 2008).

In order to determine whether the vasorelaxing effect of GM1 involved NOS activation, mesenteric rings were incubated with the nonselective NOS inhibitor L-NAME (1 μM) and then exposed to cumulative concentrations of GM1. L-NAME fully prevented the vascular relaxation induced by GM1, suggesting that NOS plays a crucial role in the vascular effects of GM1, as previously suggested for pial vessels. In addition, GM1-induced relaxation depended on the endothelium integrity, reinforcing the role of endothelium-derived relaxing factors, particularly nitric oxide, in GM1-induced vasodilation.

Endothelial nitric oxide synthase is a dually acylated peripheral membrane protein that targets to the Golgi region and caveolae of endothelial cells and can coprecipitate with caveolin-1, the structural protein of caveolae (Garcia-Cardena et al. 1997). This protein, in some instances, regulates the activity of other proteins targeted to caveolae, as potassium channels and calcium regulatory proteins (Saliez et al. 2008). These elements play an important functional role in the modulation of cell signal transduction pathways involved in eNOS activity. It has been demonstrated that caveolin-1 KO mice have increased NO-mediated relaxation in superior mesenteric arteries and that increased vascular flow (Rizzo et al. 1998) promotes eNOS dissociation from caveolin and association with calmodulin to activate the enzyme (Saliez et al. 2008). Sphingolipids are the major components of caveolae structure. Therefore, one might suggest that GM1, a sphingolipid, can promote eNOS dissociation from caveolin, by binding to CaM-binding domain of eNOS, and determine the membrane association of the enzyme. However, this discussion is speculative in nature, and further studies are necessary to elucidate this point.

It is well known that NO-induced artery relaxation is predominantly mediated by the sequential activation of soluble guanylate cyclase, accumulation of cGMP (Moncada and Higgs 1991), cGMP-dependent protein kinase activation, and subsequent K^+ channels opening (Alioua et al. 1998; Robertson et al. 1993). Accordingly, in order to evaluate the participation of the NO/sGC/cGMP pathway in GM1-induced vasodilation, we incubated the preparations with ODQ, an inhibitor of the NO-sensitive guanylate cyclase (Garthwaite et al. 1995). GM1-induced relaxation was partially blocked by ODQ suggesting that the relaxing response elicited by GM1 involves the L-arginine/NO/sGC/cGMP pathway. The partial effect of ODQ on GM1-induced vascular relaxation markedly contrasted with the complete prevention of GM1-induced relaxation by L-NAME. Such a discrepancy could be explained by a direct stimulation of calcium-activated K^+ channels by NO, without the participation of cGMP (Bolotina et al. 1994), suggesting that cGMP-independent pathways also play a role in the relaxing effect induced by GM1.

Potassium channels are the dominant ion conductive pathways in vascular muscle cells, and their activities contribute to the regulation of muscle contractility and vascular tone (Jackson 2000; Nelson and Quayle 1995). Moreover, it has been reported that endothelium-derived relaxing factors, such as NO, induce vasorelaxation by activating K^+ channels and consequently closing voltage-dependent calcium channels in the vascular smooth muscle (Nelson and Quayle 1995). Smooth muscle cells have been

shown to express at least four different classes of potassium channels: voltage-activated K^+ (K_V), ATP-sensitive K^+ (K_{ATP}), Ca^{2+}-activated K^+ (K_{Ca}), and inward rectifier K^+ (K_{IR}) channels (Jackson 2005). Therefore, in order to identify the K^+ channels involved in the vasorelaxation induced by GM1, we incubated mesenteric ring preparations with K^+ channels blockers TEA, GLB, ChTX, and apamin. The findings that TEA and GLB prevent GM1-induced vasorelaxation suggest that ATP-sensitive potassium channels are important for its relaxing activity (Fig. 3). In addition, the relaxation induced by GM1 was right-shifted by ChTX, a large and intermediate conductance calcium activated potassium channel blocker (Fig. 4c), but not by apamin, a small conductance calcium-activated potassium channel blocker (Fig. 4a). These findings indicate that while large calcium-activated potassium channels are involved in the vascular relaxation induced by GM1, the involvement of small conductance calcium-activated potassium channels is unlikely. These data are in agreement with the view that large conductance channels (BK_{Ca}) are the main K_{Ca} channels in microvascular smooth muscle cells (Jackson 2005). Interestingly, there is evidence suggesting that NO activates BK_{Ca} either directly or indirectly, by activating protein kinases (Archer et al. 1994).

Furthermore, it is worth to pointing out that drug-induced vasorelaxation depends on the tonus of the artery prior to drug exposure. Since inhibition of the NO pathway with NOS or sGC inhibitors and by blockers of K_{Ca} channels could increase the basal tonus of the mesenteric ring, eliciting a stronger contraction with Phe, a functional antagonism may have come into play in these experiments. Therefore, we cannot rule out partial functional antagonism as a cause for the currently described antagonism of GM1-induced vasorelaxation by 1 μM L-NAME, 10 μM ODQ, and 50 nM apamin, even considering the widespread use of these agents at concentrations higher (i.e., 40–300 μM for L-NAME, 0.1–10 μM for ODQ, 100 nM for apamin) than those used in the current study to pharmacologically demonstrate the involvement of the NOS pathway and of K_{Ca} channels in the vasodilating effect of several compounds (Sampson et al. 2001; Capasso et al. 2008; Tirapelli et al. 2008).

Regarding the possible clinical significance of the presently reported vasodilator effect of GM1, one must be aware that the concentrations of GM1 required for cause total relaxation are in the low millimolar range, which could be quite high to reach in the body fluids. However, it should be noted that intermediate concentrations of GM1, from 10 to 100 μM, were able to cause 30–60% of vasorelaxation in the present study. In this regard, a previous clinical study by Svennerholm et al. (2002) revealed that treatment of five Alzheimer's disease patients with GM1 (30 mg/24 h; i.c.v.) increased CSF GM1 concentrations to 40–60 μM, which is close to the range of GM1 concentrations required to cause 30–60% of vasorelaxation in mesenteric rings. In light of these results, we think that the present data may have clinical significance, depending basically on the level of vasorelaxation required to reach therapeutic effects in a given condition. In light of this premise, it is remarkable that a 42% increase in middle cerebral artery diameter by simvastatin treatment significantly ameliorates cerebral vasospasm and reduces neurological deficits resulting from subarachnoid hemorrhage in mice (McGirt et al. 2002).

In conclusion, our results demonstrate that GM1-induced vasorelaxation in mesenteric rings is endothelium dependent and mediated by the activation of the NO/sGC/cGMP pathway and K_{Ca} and K_{ATP} channels. Figure 5 shows an integrative view of the mechanisms which underlie the currently described vasorelaxing effect of GM1. While the mechanisms by which GM1 facilitates NO production are still unknown, our findings constitute strong experimental evidence supporting a role for NO and K^+ channels in

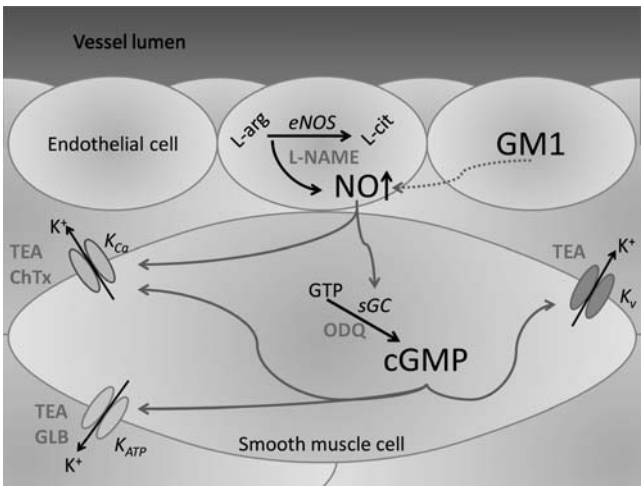

Fig. 5 Representation of the proposed pathways activated by GM1 to induce vasorelaxation in mesenteric artery rings. GM1 increases NO levels, which activate sGC, increasing cGMP levels. cGMP activates K_{Ca}, K_V, and K_{ATP} channels, promoting vasorelaxation. In addition, NO may directly activate K_{Ca}, promoting vasorelaxation. The vasorelaxing effect of GM1 disappeared when the endothelium was not intact and fully prevented in the presence of the NOS inhibitor L-NAME. The sGC inhibitor, ODQ, partially prevented GM1-induced relaxation. Potassium channel blockers, namely TEA and GLB, also prevented the effect of GM1, while the K_{Ca} blocker, ChTX, induced a concentration related right shift in cumulative concentration–effect curve for GM1. Blockers/inhibitors are presented in *gray* and *bold* and enzymes in *italics*. *Dotted* and *solid lines* represent activation of partially and fully known pathways, respectively. *L-NAME* N^G-nitro-*l*-arginine methyl ester, *L-arg* L-arginine, *L-cit* L-citrulline, *eNOS* endothelial nitric oxide synthase, *sGC* guanylate cyclase soluble, *cGMP* cyclic guanosine monophosphate, *ODQ* 1H-[1,2,4]oxadiazolo [4,3-alpha]quinoxalin-1-one, K_{Ca} calcium-activated potassium channel, *TEA* tetraethylammonium, *ChTX* charybdotoxin, K_{ATP} ATP-sensitive potassium channel, *GLB* glibenclamide, K_V voltage-sensitive potassium channel

GM1-induced vasodilation in mesenteric artery rings. As a consequence, this study significantly contributes for the understanding of pharmacological effects of exogenous GM1, a compound that could improve the outcome in several experimental models of disorders associated with perfusional deficits, such as traumatic brain injury (Chen et al. 2003), anoxia (Carolei et al. 1991; Tan et al. 1993), ischemia (Carolei et al. 1991; Kwak et al. 2005), and Parkinson's (Schneider 1998) and Alzheimer's diseases (Svennerholm 1994; Svennerholm et al. 2002; Yanagisawa 2007). On the other hand, a clinical trial with GM1 for ischemic stroke did not show enough evidence to conclude that gangliosides are beneficial in this situation (Candelise and Ciccone 2002). However, factors such as time for initiating treatment, doses, and patient sample size could underestimate potential benefic effects of neuroprotective agents in general (Wahlgren and Ahmed, 2004), and therefore, more clinical studies are needed to further evaluate the value of GM1 treatment for disorders associated with perfusional deficits.

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