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Amyloid-β induced toxicity involves ganglioside expression and is sensitive to GM1 neuroprotective action

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ABSTRACT

The effect of Aβ25–35 peptide, in its fibrillar and non-fibrillar forms, on ganglioside expression in organotypic hippocampal slice cultures was investigated. Gangliosides were endogenously labeled with D-[1-C^{14}] galactose and results showed that Aβ25–35 affected ganglioside expression, depending on the peptide aggregation state, that is, fibrillar Aβ25–35 caused an increase in GM3 labeling and a reduction in GD1b labeling, whereas the non-fibrillar form was able to enhance GM1 expression. Interestingly, GM1 exhibited a neuroprotective effect in this organotypic model, since pre-treatment of the hippocampal slices with GM1 10 μM was able to prevent the toxicity triggered by the fibrillar Aβ25–35, when measured by propidium iodide uptake protocol. With the purpose of further investigating a possible mechanism of action, we analyzed the effect of GM1 treatment (1, 6, 12 and 24 h) upon the Aβ-induced alterations on GSK3β dephosphorylation/activation state. Results demonstrated an important effect after 24-h incubation, with GM1 preventing the Aβ-induced dephosphorylation (activation) of GSK3β, a signaling pathway involved in apoptosis triggering and neuronal death in models of Alzheimer's disease. Taken together, present results provide a new and important support for ganglioside participation in development of Alzheimer's disease experimental models and suggest a protective role for GM1 in Aβ-induced toxicity. This may be useful for designing new therapeutic strategies for Alzheimer's treatment.

1. Introduction

Gangliosides are a large family of glycosphingolipids, structurally characterized by a ceramide hydrophobic core linked to an oligosaccharide chain, which usually contains at least one sialic acid residue. They are synthesized in the Golgi apparatus through sequential glycosylation and sialylation of a glucosylceramide moiety (Tettamanti, 2004).

Gangliosides amount to 10% of the brain membrane lipid content and act as the functional lipid component of the membrane rafts; they play important biochemical roles in cell biology, taking part in some processes like cell differentiation and maturation, synaptogenesis, intercellular communication, neuronal plasticity, and cell death/survival processes. This plethora of effects depends on the expressed ganglioside profile, the localization and organization of these lipids inside and across the membrane and on the biological moment during which they are expressed (Svennerholm, 1980; Vyas and Schnaar, 2001; Mocchetti, 2005; Ichikawa et al., 2009; Lopez and Schnaar, 2009).

It has been reported that little modifications in ganglioside profile and/or distribution could affect cellular biology, and therefore it is possible to hypothesize that gangliosides are involved in the development and evolution of several diseases. Alterations in ganglioside profile and/or distribution in models of hypoxia ischemia (Trindade et al., 2001; Ramirez et al., 2003), organic acidurias (Trindade et al., 2002), hypermethioninemia (Stefanello et al., 2007) and hyperprolinemia (Vianna et al., 2008) have been previously demonstrated. Several other studies have attributed the participation of gangliosides in the development of neurodegenerative disorders like Alzheimer's disease (Yanagisawa, 2007; Ariga et al., 2008; Zhang et al., 2009; Eckert et al., 2010; Harris and Milton, 2010; Haughey et al., 2010). Nevertheless, the exact role of such lipids in disease outcome remains poorly understood.

Alzheimer's disease is a neurodegenerative disorder characterized by a progressive and still irreversible cognitive loss. Although it was firstly described in 1906, little is known about its

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pathogenesis. One of the main hypotheses is that of the amyloid cascade, which consists of the production and extracellular deposition of an amyloid β-peptide (Aβ). The produced peptide may remain in a soluble form (monomer, dimmer or oligomer) or follow on an aggregation process which involves the formation of peptide insoluble fibril forms. Although the fibrils represent the preferential form of Aβ deposition and are considered the main component of the senile plaques (a classic histopathology marker of Alzheimer's disease), both insoluble and soluble forms of the peptide are potentially neurotoxic. However, the exact mechanisms regulating Aβ formation, as well as those involved in the cellular response against this peptide, remain unclear (Suh and Checler, 2002; Pimplikar, 2009; Walsh and Selkoe, 2007). The natural Aβ peptides are composed of 39–43 amino acid residues. Nevertheless, their shorter synthetic analog, Aβ25–35, which contains the amino acid sequence 25–35 of its natural counterparts, seems to trigger similar toxicity mechanisms (El Khoury et al., 1996; Yan et al., 1996; Guan et al., 2001; Qi et al., 2005; Frozza et al., 2009) and, just as the natural Aβ peptides, is able to aggregate into fibrils (Kowall et al., 1992). Consequently, Aβ25–35 is a convenient tool for the investigation of neurotoxic mechanisms involved in Alzheimer's disease.

Since the production of a Aβ peptide occurs in the biological membranes, and taking into account the occurrence of an interaction between Aβ and GM1 ganglioside, which in turn could facilitate or even accelerate the fibril formation, a positive relationship between GM1 expression and the amyloid cascade processing has been proposed (Matsuzaki, 2007; Yanagisawa, 2007; Mikhalyov et al., 2010). On the other hand, many studies suggest a neuroprotective role for GM1 in several disease models (Krajnc et al., 1994; Lazzaro et al., 1994; Augustinsson et al., 1997; Svennerholm et al., 2002; Sokolova et al., 2007).

Several studies have addressed a pivotal role for GSK3β signaling pathway in neuronal death and disease development observed in Alzheimer's (Hooper et al., 2008; Hernández et al., 2009a,b). An amyloid induced activation (dephosphorylation) of GSK3β has been shown in some experimental models, and a correlation between its activity and the neurotoxicity triggered by this peptide. Koh et al. (2008) proposed the analysis of GSK3β phosphorylation as a biochemical parameter in the investigation of possible neuroprotective drugs.

Organotypic hippocampal slice cultures are a considerable alternative to animal model experiments. Cultured slices maintain the cell architecture and interneuronal connections, allowing for a long *in vitro* survival period (Stoppini et al., 1991; Tavares et al., 2001). They have been used to investigate molecular mechanisms involved in cytotoxicity, such as the ones that are determined by oxygen and glucose deprivation (Valentim et al., 2003; Cimarosti et al., 2005; Zamin et al., 2006; Horn et al., 2005, 2009) and Aβ toxicity (Ito et al., 2003; Nassif et al., 2007; Frozza et al., 2009). This methodology has also been used for neuroprotection strategy evaluations (Cimarosti et al., 2006; Simão et al., 2009; Bernardi et al., 2010; Hoppe et al., 2010).

The aim of this study was to examine the effect of Aβ treatment to organotypic hippocampal slice cultures on ganglioside expression, as well as the GM1 effect on Aβ-induced toxicity, as assessed by cellular death and GSK3β phosphorylation.

2. Experimental procedures

2.1. Materials

Acrylamide, bisacrylamide, SDS and β-mercaptoethanol used in sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS–PAGE) were obtained from Sigma (St. Louis, MO, USA) as well as Aβ25–35, Aβ35–25, propidium iodide (PI), standard glycolipids and the ganglioside GM1 used in culture incubation. Polyclonal antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-rabbit IgG peroxidase-conjugated and reagents to detect chemiluminescence (ECL) were purchased from Amersham Pharmacia Biotech (Piscataway, NJ, USA). Millicell culture inserts (Millicell®-CM, 0.4 μm) were obtained from Millipore (Millipore®, Bedford, MA, USA), 6-well culture plates were from TPP (Tissue culture test plates TPP®, Switzerland). Culture medium, HBSS, fungizone and heat inactivated horse serum were obtained from GIBCO (Grand Island, NY, USA). Gentamicin was from Schering–Plough (Rio de Janeiro, Brazil). D-[1-C^{14}] galactose (57 mCi/mmol) was obtained from Amersham Life Science (Buckinghamshire, UK). Silicagel high performance thin layer chromatography (HPTLC) plates were supplied by Merck (Darmstadt, Germany). All other chemicals and solvents used were of analytical grade.

2.2. Organotypic hippocampal slice cultures

All animal procedures were approved by local Animal Care Committee and are in accordance with the NIH Guide for the care and use of laboratory animals. Organotypic hippocampal slice cultures were prepared according to the method of Stoppini et al. (1991), with modifications (Valentim et al., 2003; Cimarosti et al., 2005; Horn et al., 2005; Frozza et al., 2009). Briefly, 400-μm-thick hippocampal slices were prepared from 6 to 8-day-old male Wistar rats using a McIlwain tissue chopper and separated in ice-cold Hank's balanced salt solution (HBSS) composed of (mM): glucose 36, $CaCl_2$ 1.26, KCl 5.36, NaCl 136.89, KH_2PO_4 0.44, Na_2HPO_4 0.34, $MgCl_2$ 0.49, $MgSO_4$ 0.44, HEPES 25; fungizone 1% and gentamicin 0.1 mg/mL, pH 7.2. The slices were placed on Millicell culture insert and the inserts were transferred to a 6-well culture plate. Each well contained 1 mL of tissue culture medium consisting of 50% minimum essential medium, 25% HBSS, 25% heat inactivated horse serum supplemented with (mM, final concentration): glucose 36, HEPES 25 and $NaHCO_3$ 4; fungizone 1% and gentamicin 0.1 mg/mL, pH 7.3. Organotypic cultures were maintained in a humidified incubator gasified with 5% CO_2 atmosphere at 37 °C for 30 days. Culture medium was changed three times a week.

2.3. Aβ25–35 peptide preparation and culture treatments

Aβ25–35 and Aβ35–25 (reverse peptide) stock solutions (675 μM) were prepared in sterile distilled water and stored at −20 °C. To obtain the fibrillar form of Aβ25−35 peptide, an aliquot of the stock solution was incubated under 37 °C during the 4 days preceding its use in culture (Casal et al., 2004). The so-called non-fibrillar Aβ corresponds to the peptide that was not subjected to the aforementioned activation process and was therefore added to the culture directly from stock solution. On the 28th *in vitro* day, the medium was replaced by a serum reduced medium (2.5%) into which 25 μM of fibrillar/non-fibrillar Aβ25–35 or Aβ35–25 was added or not (control slices). Previous experiments showed that this concentration (25 μM) of Aβ25–35 had the most toxic effect (data not shown), at least for the fibrillar peptide form.

2.4. Quantification of cell death

Cellular damage was assessed by fluorescent image analysis of propidium iodide (PI) uptake (Noraberg et al., 1999). One hour before the end of the treatments, which means after 47 h of Aβ25–35 or Aβ35–25 exposure, 7.5 μM of PI was added to the medium and incubated for 1 h. PI uptake is indicative of significant membrane injury (Macklis and Madison, 1990). Cultures were observed with an inverted microscope (Nikon Eclipse TE 300) using a standard rhodamine filter set. Images were captured and then

analyzed using Scion Image software (http://www.scioncorp.com). After capture of images, the area where PI fluorescence (transformed in pixels) was detectable above the background was analyzed using the "density slice" option of Scioncorp Software through the division of PI fluorescence by the total area of the slice (Valentim et al., 2003). The PI intensity, meaning cell death, was expressed as a percentage of fluorescence:

$$\text{Celldeath } (\%) = F_d/F_0 \times 100$$

where F_d is the PI uptake fluorescence of dead area of hippocampal slices and F_0 is the total area of each hippocampal slice.

2.5. Metabolic ganglioside labeling

On the 29th *in-vitro* day, D-[1-C^{14}] galactose was added to the serum reduced (2.5%) culture medium, to a final concentration of 1 μCi/ml, and the slices were maintained incubated during the last 24 h of culture. Subsequent to the death analysis, the slices were removed from the plates, washed three times with PBS buffer, and submitted to lipid extraction protocol.

2.6. Lipid extraction

Each of the two washed slices were submitted to lipid extraction using sequentially the mixture of chloroform:methanol (C:M 2:1, v/v) and chloroform:methanol (C:M 1:2, v/v). The C:M extracts were combined and this pool was directly freed from water-soluble contaminants by passing through a Sephadex G-25 column equilibrated in C:M:Water (60:30:4.5) (Andrade et al., 2003).

2.7. Chromatography and fluorography

The purified lipid extracts (±3000 cpm) were evaporated under N_2 and run on HPTLC silica gel 60 plates (Merck), with two successive solvent systems: first, chloroform/methanol (4:1, v/v) and second, chloroform/methanol/0.25%aqueous $CaCl_2$ (60:36:8, v/v). The second migration was run in a TLC tank designed by Nores et al. (1994). Radioactive glycosphingolipids were visualized by exposure to a radiographic film (Kodak X-Omat AR) at −80 °C, usually for 3 weeks, and their relative contribution was determined by densitometric scanning of the X-ray film in a Geliance 600 Image System (PerkinElmer, USA). Standard gangliosides were visualized by exposure to resorcinol–HCl (Svennerholm, 1957; Lake and Goodwin, 1976).

2.8. GM1 ganglioside culture treatments

GM1 solution was prepared in a sterile saline buffer. In order to investigate the effect of this ganglioside on the Aβ-induced toxicity, a volume of this solution was added to the medium (at a final concentration of 10 μM) 48 h before adding Aβ25–35 peptide, and again at the moment of Aβ25−35 incubation (Ghidoni et al., 1989). Forty-eight hours after the peptide incubation, slices were submitted to death analysis by IP uptake. For Western-blot analysis of signaling proteins, culture slices were treated with GM1 (10 μM) and/or fibrillar Aβ25–35 (25 μM) for 1, 6, 12, or 24 h.

2.9. Western blotting assay

After obtaining the fluorescent images for cell death analysis, slices were homogenized in lyses buffer (4% sodium dodecylsulfate, 2 mM EDTA, 50 mM Tris). Aliquots were taken for protein determination and β-mercaptoethanol was added to a final concentration of 5% in order to prevent protein oxidation. Samples containing 50 μg of protein were resolved by 10% SDS–PAGE. Proteins were electro transferred to nitrocellulose membranes using a semi-dry transfer apparatus (Bio-Rad, Trans-Blot SD). After 1-h incubation at 4 °C in blocking solution containing 5% non-fat milk and 0.1% Tween-20 in Tris–buffered saline (TBS; 50 mM Tris–HCl, 1.5% NaCl, pH 7.4), membranes were incubated overnight with the appropriate primary antibody diluted in the same blocking solution. Primary antibodies against the following proteins were used: anti-phospho GSK-3β (Ser9) (pGSK-3β, 1:1000), anti-GSK-3β (1:1000), and anti-β-actin (1:1000). The membranes were then incubated with horseradish peroxidase-conjugated anti-rabbit antibody (1:1000). The chemioluminescence (ECL) was detected using X-ray films (Kodak X-Omat). Films were scanned and the percentage of band intensity was analyzed using Optiquant software (Packard Instrument). For each experiment, the test groups (treated with GM1, fibrillar Aβ25–35, or simultaneously treated with both GM1 and fibrillar Aβ25–35), were compared to control cultures (exposed neither to Aβ25–35 nor to GM1), which were considered 100%, thus assuring the same signal intensity for control and test groups. Data are expressed as percentage of phosphorylated protein for GSK3β, which was obtained by the ratio of the phospho-protein (pGSK-3β) with its whole amount (GSK-3β) (Frozza et al., 2009). Protein contents were measured by the method of Peterson (1977). In order to normalize the value of protein, we detected β-actin in the same analysis.

2.10. Statistical analysis

Data are expressed as mean ± S.D. One-way or two-way analysis of variance (ANOVA) was applied to the means to determine statistical differences between experimental groups. *Post hoc* comparisons were performed using the Tukey test for multiple comparisons. Differences between mean values were considered significant when $p < 0.05$.

3. Results

3.1. Toxicity induced by the different Aβ25–35 peptide forms in organotypic hippocampal slice cultures

Culture exposure to fibrillar Aβ25–35 (25 μM) caused marked fluorescence in hippocampal slices after 48 h of treatment, indicating a high incorporation of PI, which in turn means peptide-induced cellular death. On the other hand, the non-fibrillar form of Aβ25–35 (25 μM) caused no significant cellular death to the hippocampal slices, as observed in Fig. 1A. The quantification of PI incorporation is shown in Fig. 1B. We did not observe any increase in fluorescence in hippocampal slices exposed to the reverse sequence of peptides (Aβ35–25) at 25 μM (data not shown).

3.2. Aβ25–35 peptide effects upon ganglioside expression

Although neither the fibrillar nor the non-fibrillar β-amyloid forms were able to cause any change to total radiolabeling (Fig. 2A), chromatographic and densitometric analysis revealed that they exerted distinct effects on the profile and distribution of expressed gangliosides. While non-fibrillar Aβ caused a significant increase in GM1 expression ($p < 0.05$), the fibrillar form induced an increase in GM3 ($p < 0.05$) and a decrease in GD1b ($p < 0.05$) metabolic labeling (Fig. 2B and C). We did not observe any effect of the reverse sequence of peptides (Aβ35–25) upon ganglioside expression (data not shown).

3.3. Analysis of GM1 effect on Aβ25–35-induced cell death

To test for a possible GM1 neuroprotective effect in organotypic hippocampal slice cultures, we challenged the fibrillar Aβ-induced

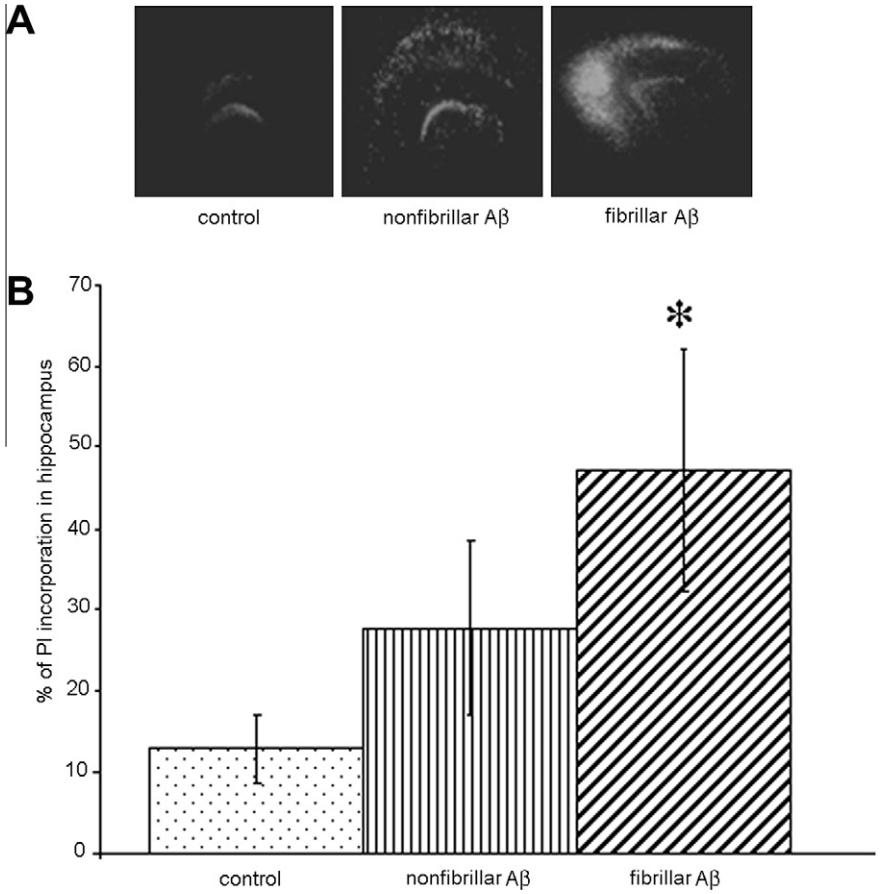

Fig. 1. Toxicity induced after 48-h exposure of organotypic hippocampal cultures to non-fibrillar and fibrillar Aβ25–35 peptide (25 μM): (A) representative photomicrographs of slices stained with PI at the end of treatment; (B) quantitative analysis of hippocampal damage just after each treatment with non-fibrillar Aβ25–35 (non-fibrillar Aβ) and with fibrillar Aβ25–35 (fibrillar Aβ), respectively. Bars represent the mean + S.D., $n = 8$. *Significantly different from control cultures (one-way ANOVA followed by Tukey test, $p < 0.05$).

toxicity above described (Fig. 1). As shown in Fig.3, the treatment of culture slices with 10 μM GM1 (48 h prior to Aβ exposure and during the 48 h of this peptide incubation) was able to significantly prevent the peptide-induced cell death.

3.4. Effect of GM1 upon fibrillar Aβ-induced alteration of GSK3β phosphorylation state

To further investigate one of the possible mechanisms involved on neuroprotective effect of GM1 just reported, we analyzed GM1 effect upon Aβ induced alteration in GSK3β phosphorylation after 1, 6, 12 and 24 h. Results show no effect of GM1 or fibrillar Aβ25–35 treatment after 1 h of treatment. Nevertheless, 6 h of co-treatment with GM1 and Aβ25–35 caused a significant increase in GSK3β phosphorylation. After 12 h of GM1 treatment we observed a decrease ($p < 0.05$) in GSK3β phosphorylation, and after 24 h of treatment it was shown that GM1 was able to augment GSK3β phosphorylation; moreover the co-treatment with GM1 and Aβ25–35 was able to prevent β-amyloid-induced reduction in GSK3β phosphorylation state (Fig. 4).

4. Discussion

Organotypic cultures, in spite of some limitations, are a good alternative to *in vivo* models, since they provide a good experimental access to mimic pathophysiological pathways in living tissues, and because they preserve the cell organization and tissue architecture (Stoppini et al., 1991; Tavares et al., 2001; Holopainen, 2005; Cimarosti et al., 2006; Horn et al., 2009; Simão et al., 2009; Hoppe et al., 2010). Using this model, we could observe that the Aβ induced death depended on its aggregation state, since the non-fibrillar peptide form was unable to trigger toxicity, or at least the toxicity as measured by PI uptake protocols (Fig. 1). Even though the main limitation observed in this *in vitro* technique is the variation, which is inherent in this model, we believe in the reliability of our results, since we performed the experiments comparing the effect of Aβ-peptide and/or the effect of GM1, using slice culture of the same animal. Nevertheless our results showed strong toxic effect of Aβ and a notable neuroprotective effect of GM1.

Taking into account a considerable number of studies suggesting a role of gangliosides and membrane lipid dynamics in the amyloid cascade modulation, as well as a participation of these lipids in the toxicity mechanisms triggered by amyloid peptide, the present study has investigated the effect of Aβ25–35, in its fibrillar or non-fibrillar forms, upon ganglioside expression in a model of hippocampal organotypic cultures (Yanagisawa, 2007; Ariga et al., 2008; Zhang et al., 2009; Eckert et al., 2010; Harris and Milton, 2010; Haughey et al., 2010).

Our results firstly demonstrate an Aβ25–35 effect on ganglioside expression, which seemed to depend on the peptide aggregation state. Whereas fibrillar Aβ25–35 caused an increase in GM3 and a decrease in GD1b metabolic labeling, its non-fibrillar form was able to enhance GM1 expression (Fig. 2B and C).

Considering that GM3 is a ganglioside usually associated with apoptotic mechanisms, at least when expressed in mature neuro-

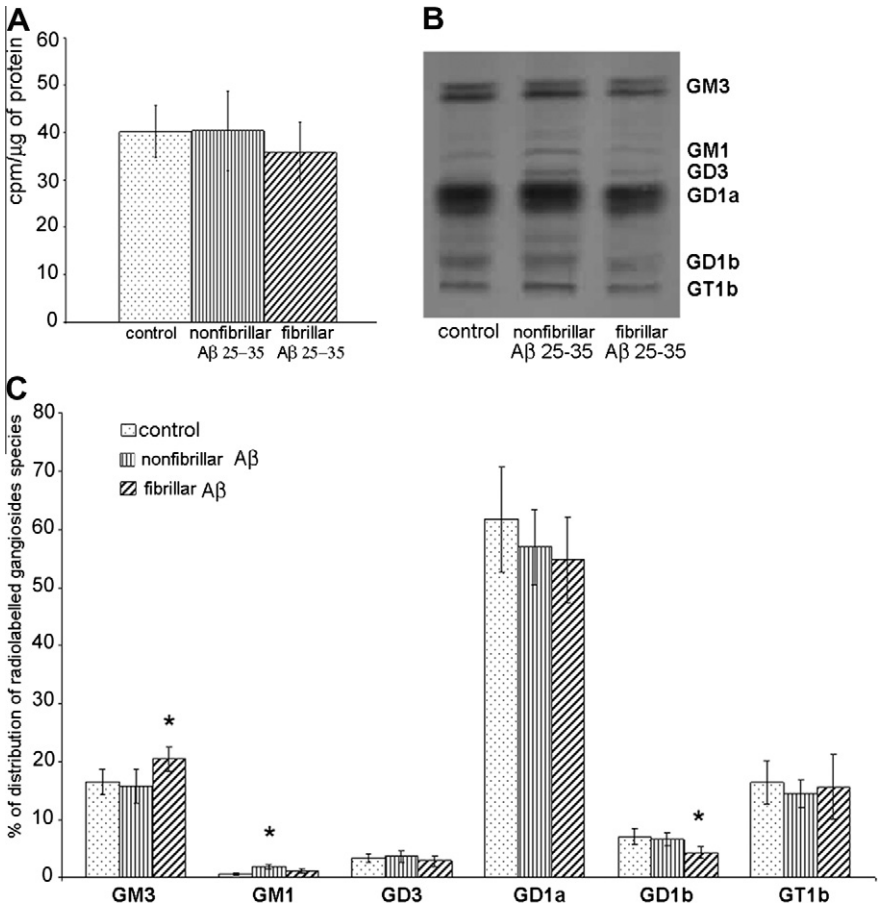

Fig. 2. Effect of non-fibrillar and fibrillar forms Aβ25–35 on the profile and distribution of radiolabeled ganglioside species: (A) quantification of total ganglioside radiolabeling (measured as cpm/µg protein) obtained from organotypic hippocampal slice cultures exposed either to non-fibrillar Aβ25–35 (non-fibrillar Aβ) or fibrillar Aβ25–35 (Fibrillar Aβ). Gangliosides were extracted and purified with sephadex G-25 as described in Material and Methods. Bars represent the mean + S.D., $n = 8$. (one-way ANOVA followed by Tukey test, $p < 0.05$); (B) high performance thin layer-autoradiography of D-[1-C^{14}] galactose labeled endogenous gangliosides from organotypic hippocampal slice cultures exposed to non-fibrillar Aβ, or fibrillar Aβ, or not exposed (control). Gangliosides were extracted, purified with sephadex G-25 and analyzed by HPTLC, as described in experimental procedures. The position of co-chromatographed ganglioside standards is indicated; present results are representative of eight independent experiments; (C) ganglioside distribution in organotypic hippocampal slice cultures exposed to non-fibrillar Aβ, or fibrillar Aβ, or not exposed (control). Results correspond to densitometric analysis of HPLC-autoradiographies as shown in Fig. 2A. Bars express individual ganglioside as a percentage of the total radioactivity incorporated into all ganglioside bands. Results show mean percentage + S.D. *Significantly different from control cultures (one-way ANOVA followed by Tukey test, $p < 0.05$).

nal cells (Sohn et al., 2006; Valaperta et al., 2006), and taking into account an anti-apoptotic effect attributed to GD1b (Chen et al., 2005), the enhancement in GM3 expression as well as the decrease in GD1b expression, as a consequent of fibrillar Aβ25–35 incubation, can be suggested to take part in the toxic mechanisms triggered by this peptide.

GM1, in turn, is a ganglioside usually associated with neuroprotective effects. The exact mechanism involved in its neuroprotective action is not completely understood, however GM1 is able to enhance/potentiate neurotrophin release and action (Rabin et al., 2002; Mocchetti, 2005), to exert antioxidant effects (Fighera et al., 2004; Furian et al., 2007; Gavella et al., 2007), to prevent/revert glutamate induced excitotoxicity (Cunha et al., 1999), and to modulate some signaling pathways involved in death/survival processes (Mutoh et al., 1995; Pitto et al., 1998; Lili et al., 2005; Duchemin et al., 2002, 2008).

On the other hand, several studies have attributed a participation in the mechanisms of Aβ aggregation to GM1 since the interaction of the peptide with this ganglioside could act as a seed for the aggregation process, accelerating or even potentiating its fibrillation on membrane surfaces. This effect, however, seems to depend on a clustering of this ganglioside into membrane microdomains (lipid rafts) (Matsuzaki, 2007; Yanagisawa, 2007), as well as on the pH and ionic concentration of the medium (McLaurin et al., 1998). Besides that, other studies have suggested a participation of GM1 ganglioside in maturation of Amyloid Precursor Protein (APP), affecting its localization on membrane surface, and therefore, positively modulating Aβ production (Ehehalt et al., 2003; Zha et al., 2004; Zhang et al., 2009).

To further investigate the role of this ganglioside (neuroprotective or not) in the present model, we performed experiments consisting in the treatment of slices cultures with a saline GM1 solution, in order to assess a possible effect of this ganglioside upon the Aβ25–35 induced toxicity. Considering that just fibrillar Aβ25–35 was able to trigger toxicity in our model, we have chosen this peptide form to perform the neuroprotective investigation. The pretreatment of slices with 10 µM GM1, 48 h previous to Aβ25–35 addition, was able to significantly prevent the amyloid toxicity measured after 48 h of amyloid incubation, as the PI uptake experiments have demonstrated (Fig. 3).

Several studies have indicated the existence of a link among Aβ toxicity, progression of Alzheimer's disease, and the activation of the GSK3β signaling pathway. This signaling pathway exerts an important effect on neurons, triggering the activation of cell death processes that could include oxidative stress induction and apoptosis response activation. In models of Alzheimer's disease, GSK3β

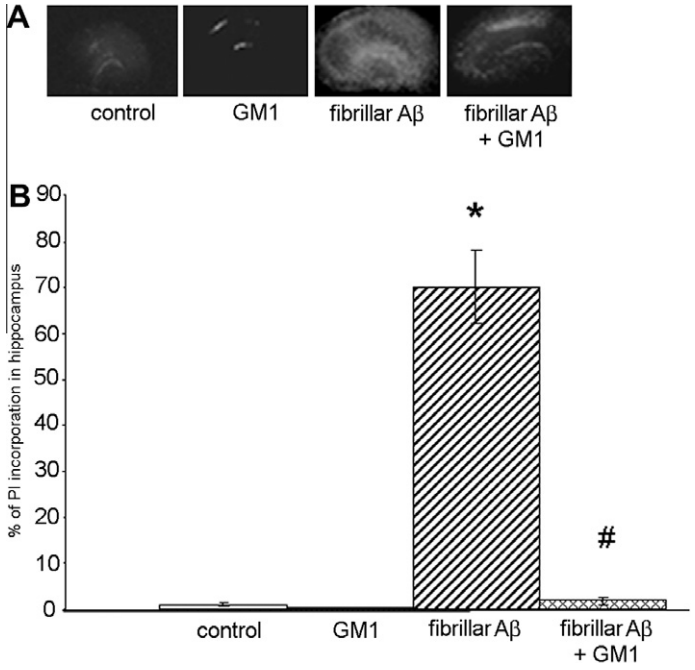

Fig. 3. Effect of GM1 treatment on fibrillar Aβ25–35-induced cell death: Slices were treated or not with 10 μM GM1 from the 26th *in vitro* day; on the 28th *in vitro* day, fibrillar Aβ25–35 was added to the medium, and cell death was analyzed after 48 h of its incubation by PI uptake. Control cultured slices received neither GM1 nor amyloid–peptide. (A) Representative photomicrographs of slices stained with PI at the end of treatment; (B) quantitative analysis of hippocampal damage. Bars represent mean + S.D. of cell death, $n = 5$. (*) Significantly different from control cultures; (#) significantly different from Aβ25–35 treated slices (two-way ANOVA followed by Tukey test, $p < 0.05$).

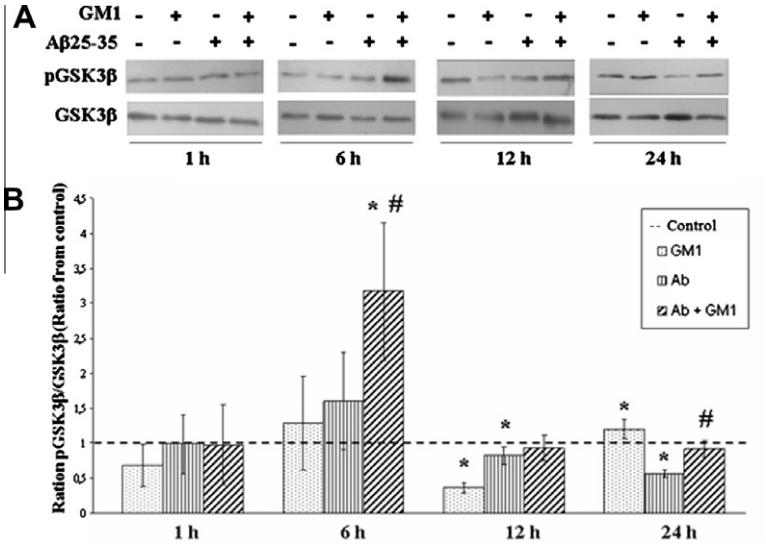

Fig. 4. Effect of 10 μM GM1 treatment on fibrillar Aβ25–35-induced alterations of GSK3β phosphorylation state: (A) representative Western blotting of phospho-GSK3β and GSK3β after each treatment; (B) histograms representing the quantitative Western blotting analysis of GSK3β phosphorylation state. Densitometric values obtained to phospho- and total-GSK3β were first normalized to their respective controls; control values are shown as the horizontal clotted line. Data are expressed as a ratio of the normalized phospho-GSK3β/GSK3β relation and bars represent the mean + S.D., $n = 3$ for 1, 6 and 12-h treatments, and $n = 5$ for 24-h treatment. (*) Significantly different from respective control culture; (#) significantly different from respective fibrillar Aβ25–35 treated group (two-way ANOVA followed by Tukey test, $p < 0.05$).

signaling pathway, besides its effect on apoptotic triggering, could represent a link between Aβ production/aggregation and the posterior hyper-phosphorylation of *tau* protein, which in turn involves the development of other important histopathology marker of Alzheimer's disease, the neurofibrillary tangles (Hooper et al., 2008; Hernández et al., 2009a,b). Also, there is evidence that GSK3β activation, as measured by its phosphorylation state, could be useful as a biochemical marker for the study of neuroprotective drugs, i.e., drugs able to inhibit the Aβ-induced activation of GSK3β could be considered as potential neuroprotective ones (Koh et al., 2008; Avila et al., 2010).

Thus, in order to further analyze the neuroprotective potential of GM1 in our model, and to propose a possible mechanism by which this ganglioside could trigger its neuroprotective action, we investigated the effect of GM1, in a 10 μM concentration, upon the Aβ-induced alterations of GSK3β phosphorylation state (Fig. 4).

Although after 1 h of incubation no alteration was observed in GSK3β phosphorylation, neither with GM1 nor Aβ25–35, a longer period of incubation (6 h) revealed that the co-treatment with GM1 and Aβ25–35 was able to increase GSK3β phosphorylation. After 12 h of GM1 treatment, a decrease in GSK3β phosphorylation was verified. Most importantly, however, it was observed that GM1 was able to reverse the dephosphorylation/activation of GSK3β ($p < 0.05$) detected after 24 h of Aβ25–35 incubation.

Our results demonstrate a potential neuroprotective effect of GM1 ganglioside, which suggests that the Aβ-induced alterations in ganglioside expression could affect the tissue response against the peptide induced cell death (via GSK3β more phosphorylated and less active). Although the GM1 concentration used in this study favors micelle formation and thereby facilitates its incorporation into plasma membranes, such inclusion is still small (Rauvala, 1979; Ulrich-Bott and Wiegandt, 1984; Schwarzmann, 2001), so that the neuroprotective effects here observed should be understood as a result of exogenous administration of a bioactive molecule, and not necessarily as a result of lipid content manipulation of neural membranes. More studies are needed to investigate the actual biological effect of ganglioside metabolism modulation (especially GM1) triggered by Aβ. If an increase of endogenous GM1 content could result, like its exogenous administration, in modulation o GSK3β and neuroprotection, on the other hand we cannot rule out the hypothesis that a long-term change in neural membrane content of this lipid could accelerate fibrillogenesis.

At any rate, our work demonstrates the effect of Aβ on the ganglioside expression, and although the interpretation of the role of these alterations in AD has a still speculative nature, our data on the GM1 neuroprotective effect reinforce the hypothesis that these lipid changes may have an important biological significance, rekindling the interest in investigating the clinical use of GM1, or its synthetic analogs, in the treatment of Alzheimer's disease (Biraboneye et al., 2009; Avila et al., 2010).

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References

Andrade, C.M.B., Trindade, V.M.T., Cardoso, C.C.A., Ziulkoski, A.L., Trugo, L.C., Guaragna, R.M., Borojevic, R., Guma, F.C.R., 2003. Changes of sphingolipid species in the phenotype conversion from myofibroblasts to lipocytes in hepaticstellate cells. J. Cell. Biochem. 88, 533–544.

Ariga, T., McDonald, M.P., Yu, R.K., 2008. Role of ganglioside metabolism in the pathogenesis of Alzheimer's disease – a review. J. Lipid Res. 49, 1157–1175.

Augustinsson, L.E., Blennow, K., Blomstrand, C., Brane, G., Ekman, R., Fredman, P., Karlsson, I., Kihlgren, M., Lehmann, W., Lekman, A., 1997. Intracerebroventricular administration of GM1 ganglioside to presenile Alzheimer patients. Dementia Geriatr. Cognit. Dis. 8, 26–33.

Avila, J., Wandosell, F., Hernández, F., 2010. Role of glycogen synthase kinase-3 in Alzheimer's disease pathogenesis and glycogen synthase kinase-3 inhibitors. Expert Rev. Neurother. 10, 703–710.

Bernardi, A., Frozza, R.L., Horn, A.P., Campos, M.M., Calixto, J.B., Salbego, C., Pohlmann, A.R., Guterres, S.S., Battastini, A.M., 2010. Protective effects of indomethacin-loaded nanocapsules against oxygen–glucose deprivation in organotypic hippocampal slice cultures: involvement of neuroinflammation. Neurochem. Int. 57, 629–636.

Biraboneye, A.C., Madonna, S., Laras, Y., Krantic, S., Maher, P., Kraus, J.L., 2009. Potential neuroprotective drugs in cerebral ischemia: new saturated and polyunsaturated lipids coupled to hydrophilic moieties: synthesis and biological activity. J. Med. Chem. 52, 4358–4369.

Casal, C., Serratosa, J., Tussel, J.M., 2004. Effects of β-AP peptides on activation of the trasncription factor NF-kappaB and in cell proliferation in glial cell cultures. Neurosci. Res. 24, 233–238.

Chen, X., Chi, S., Liu, M., Yang, W., Wei, T., Qi, Z., Yang, F., 2005. Inhibitory effect of ganglioside GD1b on K^+ current in hippocampal neurons and its involvement in apoptosis suppression. J. Lipid Res. 46, 2580–2585.

Cimarosti, H., Zamin, L.L., Frozza, R., Nassif, M., Horn, A.P., Tavares, A., Netto, C.A., Salbego, C., 2005. Estradiol protects against oxygen and glucose deprivation in rat hippocampal organotypic cultures and activates Akt and inactivates GSK-3β. Neurochem. Res. 30, 191–199.

Cimarosti, H., O'Shea, R.D., Jones, N.M., Horn, A.P., Simão, F., Zamin, L.L., Nassif, M., Frozza, R., Netto, C.A., Beart, P.M., Salbego, C., 2006. The effects of estradiol on estrogen receptor and glutamate transporter expression in organotypic hippocampal cultures exposed to oxygen–glucose deprivation. Neurochem. Res. 31, 483–490.

Cunha, G.M., Moraes, R.A., Moraes, G.A., França Jr., M.C., Moraes, M.O., Viana, G.S., 1999. Nerve growth factor, ganglioside and vitamin E reverse glutamate cytotoxicity in hippocampal cells. Eur. J. Pharmacol. 367, 107–112.

Duchemin, A.M., Qun, R., Lili, M., Norton, H.N., Hadjiconstantinou, M., 2002. GM1 ganglioside induces phosphorylation and activation of Trk and Erk in brain. J. Neurochem. 81, 696–707.

Duchemin, A.M., Qun, R., Norton, H.N., Hadjiconstantinou, M., 2008. GM1-induced activation of phosphatidylinositol 3-kinase: involvement of Trk receptors. J. Neurochem. 104, 1466–1477.

Eckert, G.P., Wood, W.G., Müller, W.E., 2010. Lipid membranes and beta-amyloid: a harmful connection. Curr. Protein Pept. Sci. 11, 319–325.

Ehehalt, R., Keller, P., Haass, C., Thiele, C., Simons, K., 2003. Amyloidogenic processing of the Alzheimer-amyloid precursor protein depends on lipid rafts. J. Cell Biol. 160, 113–123.

El Khoury, J., Hickman, S.E., Thomas, C.A., Cao, L., Silverstein, S.C., Loike, J.D., 1996. Scavenger receptor-mediated adhesion of microglia to beta amyloid fibrils. Nature 382, 716–719.

Fighera, M.R., Bonini, J.S., Frussa-Filho, R., Dutra-Filho, C.S., Hagen, M.E., Rubin, M.A., Mello, C.F., 2004. Monosialoganglioside increases catalase activity in cerebral cortex of rats. Free Radic. Res. 38, 495–500.

Frozza, R.L., Horn, A.P., Hoppe, J.B., Simão, F., Gerhardt, D., Comiran, R.A., Salbego, C.G., 2009. A comparative study of β-amyloid peptides Aβ1–42 and Aβ25–35 toxicity in organotypic hippocampal slice cultures. Neurochem. Res. 34, 295–303.

Furian, A.F., Oliveira, M.S., Royes, L.F., Fiorenza, N.G., Fighera, M.R., Myskiw, J.C., Weiblen, R., Rubin, M.A., Frussa-Filho, R., Mello, C.F., 2007. GM1 ganglioside induces vasodilation and increases catalase content in the brain. Free Radic. Biol. Med. 43, 924–932.

Gavella, M., Kveder, M., Lipovac, V., Jurasin, D., Filipovi-Vincekovic, N., 2007. Antioxidant properties of ganglioside micelles. Free Radic. Res. 41, 1143–1150.

Ghidoni, R., Fiorilli, A., Trinchera, M., Venerando, B., Chigorno, V., Tettamanti, G., 1989. Uptake, cell penetration and metabolic processing of exogenously administered GM1 ganglioside in rat brain. Neurochem. Int. 15, 455–465.

Guan, Z.Z., Miao, H., Tian, J.Y., Unger, C., Nordberg, A., Zhang, X., 2001. Suppressed expression of nicotinic acetylcholine receptors by nano molar beta-amyloid peptides in PC12 cells. J. Neural Transm. 108, 1417–1433.

Harris, J.R., Milton, N.G., 2010. Cholesterol in Alzheimer's disease and other amyloidogenic disorders. Subcell. Biochem. 51, 47–75.

Haughey, N.J., Bandaru, V.V., Bae, M., Mattson, M.P., 2010. Roles for dysfunctional sphingolipid metabolism in Alzheimer's disease neuropathogenesis. Biochim. Biophys. Acta 1801, 878–886.

Hernández, F., de Barreda, E.G., Fuster-Matanzo, A., Goñi-Oliver, P., Lucas, J.J., Avila, J., 2009a. The role of GSK3 in Alzheimer disease. Brain Res. Bull. 80, 248–250.

Hernández, F., Barreda, E.G., Fuster-Matanzo, A., Lucas, J.J., Avila, J., 2009b. GSK3: a possible link between beta amyloid peptide and tau protein. Exp. Neurol. 223, 322–325.

Holopainen, I.E., 2005. Organotypic hippocampal slice cultures: a model system to study basic cellular and molecular mechanisms of neuronal cell death, neuroprotection, and synaptic plasticity. Neurochem. Res. 30, 1521–1528.

Hooper, C., Killick, R., Lovestone, S., 2008. The GSK3 hypothesis of Alzheimer's disease. J. Neurochem. 104, 1433–1439 (Minireview).

Hoppe, J.B., Frozza, R.L., Horn, A.P., Comiran, R.A., Bernardi, A., Campos, M.M., Battastini, A.M., Salbego, C., 2010. Amyloid-beta neurotoxicity in organotypic culture is attenuated by melatonin: involvement of GSK-3beta, tau and neuroinflammation. J. Pineal Res. 48, 230–238.

Horn, A.P., Gerhardt, D., Geyer, A.B., Valentim, L., Cimarosti, H., Tavares, A., Horn, F., Lenz, G., Salbego, C., 2005. Cellular death in hippocampus in response to PI3-K pathway inhibition and oxygen and glucose deprivation. Neurochem. Res. 30, 355–361.

Horn, A.P., Frozza, R.L., Grudzinski, P.B., Gerhardt, D., Hoppe, J.B., Bruno, A.N., Chagastelles, P., Nardi, N.B., Lenz, G., Salbego, C., 2009. Conditioned medium from mesenchymal stem cells induces cell death in organotypic cultures of rat hippocampus and aggravates lesion in a model of oxygen and glucose deprivation. Neurosci. Res. 63, 35–41.

Ichikawa, N., Iwabuchi, K., Kurihara, H., Ishii, K., Kobayashi, T., Sasaki, T., Hattori, N., Mizuno, Y., Hozumi, K., Yamada, Y., Arikawa-Hirasawa, E., 2009. Binding of laminin-1 to monosialoganglioside GM1 in lipid rafts is crucial for neurite outgrowth. J. Cell Sci. 122, 2892–2899.

Ito, Y., Ito, M., Takagi, N., Saito, H., Ishige, K., 2003. Neurotoxicity induced by amyloid beta-peptide and ibotenic acid in organotypic hippocampal cultures: protection by S-allyl-L-cysteine, a garlic compound. Brain Res. 985, 98–107.

Koh, S.H., Noh, M.Y., Kim, S.H., 2008. Amyloid-beta-induced neurotoxicity is reduced by inhibition of glycogen synthase kinase-3. Brain Res. 1188, 254–262.
Kowall, N.W., McKee, A.C., Yankner, B.A., Beal, M.F., 1992. In vivo neurotoxicity of beta-amyloid [beta(1–40)] and the beta(25–35) fragment. Neurobiol. Aging 13, 537–542.
Krajnc, D., Wemlinger, T.A., Neff, N.H., Hadjiconstantinou, M., 1994. Neonatal hypoxia: early neurotransmitter responses and the consequences of treatment with GM1 ganglioside. J. Pharmacol. Exp. Ther. 271, 1299–1305.
Lake, B.D., Goodwin, H.J., 1976. Lipids. In: Smith, I., Seakins, J.W.T. (Eds.), Chromatographic and Eletrophoretic. William Heinemann Medical Books Ltd., London, pp. 345–366.
Lazzaro, A., Seren, M.S., Koga, T., Zanoni, T., Schiavo, N., Manev, H., 1994. GM1 reduces infarct volume after focal cerebral ischemia. Exp. Neurol. 125, 278–285.
Lili, M., Qun, R., Duchemin, A.M., Neff, N.H., Hadjiconstantinou, M., 2005. GM1 and ERK signaling in the aged brain. Brain Res. 1054, 125–134.
Lopez, P.H.H., Schnaar, R.L., 2009. Gangliosides in cell recognition and membrane protein regulation. Curr. Opin. Struct. Biol. 19, 549–557.
Macklis, J.D., Madison, R.D., 1990. Progressive incorporation of propidium iodide in cultured mouse neurons correlates with declining electrophysiological status: a fluorescence scale of membrane integrity. J. Neurosci. Method 31, 43–46.
Matsuzaki, K., 2007. Physicochemical interactions of amyloid β-peptide with lipid bilayers. Biochim. Biophys. Acta 1768, 1935–1942 (Review).
McLaurin, J., Franklin, T., Fraser, P.E., Chakrabartty, A., 1998. Structural transitions associated with the interaction of Alzheimer β-amyloid peptides with gangliosides. J. Biol. Chem. 273, 4506–4515.
Mikhalyov, I., Olofsson, A., Grobner, G., Johansson, L.B., 2010. Designed fluorescent probes reveal interactions between amyloid-beta (1–40) peptides and GM1 gangliosides in micelles and lipid vesicles. Biophys. J. 99, 1510–1519.
Mocchetti, I., 2005. Exogenous gangliosides, neuronal plasticity and repair, and the neurotrophins. Cell. Mol. Life Sci. 62, 2283–2294 (Review).
Mutoh, T., Tokuda, A., Miyadai, T., Hamaguchi, M., Fujiki, N., 1995. Ganglioside GM1 binds to the Trk protein and regulates receptor function. Cell Biol. 92, 5087–5091.
Nassif, M., Hoppe, J., Santin, K., Frozza, R., Zamin, L.L., Simão, F., Horn, A.P., Salbego, C., 2007. Beta-amyloid peptide toxicity in organotypic hippocampal slice culture involves Akt/PKB, GSK-3beta, and PTEN. Neurochem. Int. 50, 229–235.
Noraberg, J., Kristensen, B.W., Zimmer, J., 1999. Markers for neuronal degeneration in organotypic slice cultures. Brain Res. Protoc. 3, 278–290.
Nores, G.A., Mitzumari, R.K., Kremer, D.M., 1994. Chromatographic tank designed to obtain highly reproducible highperformance thin-layer chromatograms of gangliosides and neutral glycosphingolipds. J. Chromatogr. 686, 155–157.
Peterson, G.L., 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem. 83, 346–356.
Pimplikar, S.W., 2009. Reassessing the amyloid cascade hypothesis of Alzheimer's disease. Int. J. Biochem. Cell Biol. 41, 1261–1268.
Pitto, M., Mutoh, T., Kuriyama, M., Ferraretto, A., Palestini, P., Masserini, M., 1998. Influence of endogenous GM1 ganglioside on TrkB activity, in cultured neurons. FEBS Lett. 439, 93–96.
Qi, X.L., Xiu, J., Shan, K.R., Xiao, Y., Gu, R., Liu, R.Y., Guan, Z.Z., 2005. Oxidative stress induced by beta-amyloid peptide (1–42) is involved in the altered composition of cellular membrane lipids and the decreased expression of nicotinic receptors in human SH-SY5Y neuroblastoma cells. Neurochem. Int. 46, 613–621.
Rabin, S.J., Bachis, A., Mocchetti, I., 2002. Gangliosides activate Trk receptors by inducing the release of neurotrophins. J. Biol. Chem. 277, 49466–49472.
Ramirez, M.R., Muraro, F., Zylbersztejn, D.S., Abel, C.R., Arteni, N.S., Lavinsky, D., Netto, C.A., Trindade, V.M., 2003. Neonatal hypoxia–ischemia reduces ganglioside, phospholipid and cholesterol contents in the rat hippocampus. Neurosci. Res. 46, 339–347.
Rauvala, H., 1979. Monomer–micelle transition of the ganglioside GM1 and the hydrolysis by *Clostridium perfringes* neuraminidase. Eur. J. Biochem. 97, 555–564.
Schwarzmann, G., 2001. Uptake and metabolism of exogenous glycosphingolipids by cultured cell. Cell Dev. Biol. 12, 163–171.
Simão, F., Zamin, L.L., Frozza, R., Nassif, M., Horn, A.P., Salbego, C.G., 2009. Protective profile of oxcarbazepine against oxygen–glucose deprivation in organotypic hippocampal slice culture could involve PI3K cell signaling pathway. Neurol. Res. 31, 1044–1048.
Sohn, H., Kim, Y.S., Kim, H.T., Kim, C.H., Cho, E.W., Kang, H.Y., Kim, N.S., Kim, C.H., Ryu, S.E., Lee, J.H., Ko, J.H., 2006. Ganglioside GM3 is involved in neuronal cell death. FASEB J. 20, 1248–1250.
Sokolova, T.V., Zakharova, I.O., Furaev, V.V., Rychkova, M.P., Avrova, N.F., 2007. Neuroprotective effect of ganglioside GM1 on the cytotoxic action of hydrogen peroxide and amyloid beta-peptide in PC12 cells. Neurochem. Res. 32, 1302–1313.
Stefanello, F.M., Kreutz, F., Scherer, E.B.S., Breier, A.C., Vianna, L.P., Trindade, V.M.T., Wyse, A.T.S., 2007. Reduction of gangliosides, phospholipids and cholesterol content in cerebral cortex of rats caused by chronic hypermethioninemia. Int. J. Dev. Neurosci. 25, 473–477.
Stoppini, L., Buchs, P.A., Muller, D., 1991. A simple method for organotypic cultures of nervous tissue. J. Neurosci. Methods 37, 173–182.
Suh, Y., Checler, F., 2002. Amyloid precursor protein, presenilins, and α-synuclein: molecular pathogenesis and pharmacological applications in Alzheimer's disease. Pharmacol. Rev. 54, 469–525 (Review).
Svennerholm, L., 1957. Quantitative estimation of sialic acids a colorimetric resorcinol–hydrochloric acid method. Biochim. Biophys. Acta 24, 604–611.
Svennerholm, L., 1980. Gangliosides and synaptic transmission. Adv. Exp. Med. Biol. 125, 533–544.
Svennerholm, L., Brane, G., Darlsson, I., Leckman, A., Ramstrom, I., Wikkelso, C., 2002. Alzheimer disease – effect of continuous intracerebroventricular treatment with GM1 ganglioside and a systematic activation programme. Dementia Geriatr. Cognit. Dis. 14, 128–136.
Tavares, A., Cimarosti, H., Valentim, L., Salbego, C., 2001. Profile of phosphoprotein labelling in organotypic slice cultures of rat hippocampus. NeuroReport 12, 2705–2709.
Tettamanti, G., 2004. Ganglioside/glycosphingolipid turnover: new concepts. Glycoconj. J. 20, 301–317.
Trindade, V.M., Daniotti, J.L., Raimondi, L., Chazan, R., Netto, C.A., Maccioni, H.J., 2001. Effects of neonatal hypoxia/ischemia on ganglioside expression in the rat hippocampus. Neurochem. Res. 26, 591–597.
Trindade, V.M.T., Brusque, A.M., Raasch, J.R., Pettenuzzo, L.F., Rocha, H.P., Wannmacher, C.M.D., Wajner, M., 2002. Ganglioside alterations in the central nervous system of rats chronically injected with methylmalonic and propionic acids. Metab. Brain Dis. 17, 93–102.
Ulrich-Bott, B., Wiegandt, H., 1984. Micelle properties of glycosphingolipids in aqueous media. J. Lipid Res. 25, 1233–1245.
Valaperta, R., Chigorno, V., Basso, L., Prinetti, A., Bresciani, R., Preti, A., Miyagi, T., Sonnino, S., 2006. Plasma membrane production of ceramide from ganglioside GM3 in human fibroblasts. FASEB J. 20, 1227–1229.
Valentim, L.M., Rodnight, R., Geyer, A.B., Horn, A.P., Tavares, A., Cimarosti, H., Netto, C.A., Salbego, C.G., 2003. Changes in heat shock protein 27 phosphorylation and immunocontent in response to preconditioning to oxygen and glucose deprivation in organotypic hippocampal cultures. Neuroscience 118, 379–386.
Vianna, L.P., Delwing, D., Kurek, A.G., Breier, A.C., Kreutz, F., Chiarani, F., Stefanello, F.M., Wyse, A.T., Trindade, V.M., 2008. Effects of chronic proline administration on lipid contents of rat brain. Int. J. Dev. Neurosci. 26, 567–573.
Vyas, A.A., Schnaar, R.L., 2001. Brain gangliosides: functional ligands for myelin stability and the control of nerve regeneration. Biochimie 83, 677–682.
Walsh, D.M., Selkoe, D.J., 2007. Aβ oligomers – a decade of discovery. J. Neurochem. 101, 1172–1184.
Yan, S.D., Chen, X., Fu, J., Chen, M., Zhu, H.J., Roher, A., Slattery, T., Zhao, L., Nagashima, M., Morser, J., Migheli, A., Nawroth, P., Stern, D., Schmidt, A.M., 1996. RAGE and amyloid-β peptide neurotoxicity in Alzheimer's disease. Nature 382, 685–691.
Yanagisawa, K., 2007. Role of gangliosides in Alzheimer's disease. Biochimica et Biophysica Acta 1768, 1943–1951 (Review).
Zamin, L.L., Dillenburg-Pilla, P., Argenta-Comiran, R., Horn, A.P., Simão, F., Nassif, M., Gerhardt, D., Frozza, R.L., Salbego, C., 2006. Protective effect of resveratrol against oxygen–glucose deprivation in organotypic hippocampal slice cultures: involvement of PI3-K pathway. Neurobiol. Dis. 24, 170–182.
Zha, Q., Ruan, Y., Hartmann, K., Beyreuther, D., Zhang, D., 2004. GM1 ganglioside regulates the proteolysis of amyloid precursor protein. Mol. Psychiatry 9, 946–952.
Zhang, H., Ding, J., Tian, W., Wang, L., Huang, L., Ruan, Y., Lu, T., Sha, Y., Zhang, D., 2009. Ganglioside GM1 binding the N-terminus of amyloid precursor protein. Neurobiol. Aging 30, 1245–1253.