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Differential regulation of AKT, MAPK and GSK3β during C_2-ceramide-induced neuronal death

Gonzalo Arboleda [a,b,*], Yolanda Cárdenas [a], Yeldy Rodríguez [a], Luis Carlos Morales [a], Luisa Matheus [d], Humberto Arboleda [a,c]

[a] Grupo de Neurociencias, Facultad de Medicina, Universidad Nacional de Colombia, Colombia
[b] Departamento de Patología, Facultad de Medicina, Universidad Nacional de Colombia, Colombia
[c] Departamento de Pediatría e Instituto de Genética, Facultad de Medicina, Universidad Nacional de Colombia, Colombia
[d] Grupo de investigación en Ciencias Básicas Médicas, Facultad de Ciencias Naturales y Matemáticas, Universidad del Rosario, Colombia

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ABSTRACT

Evidence has implicated apoptosis as a mechanism underlying cell demise in diverse neurodegenerative diseases including Parkinson's disease (PD). Endogenous toxins and other stress signals activate the sphingomyelin pathway increasing the levels of ceramide, an important regulator of cell death.

In the present paper we have analysed the contribution of PI3K/AKT-GSK3β and MAPK (ERK and JNK) pathways to cell death in a catecholaminergic cell line following exposure to C_2-ceramide. We also explored the potential neuroprotective action of insulin-like growth factor-1 (IGF-1) and neurotrophin-3 (NT3).

We demonstrated that C_2-ceramide-induced cell death is associated to an early decrease in phosphorylation (inhibition) of PI3K/AKT and ERK, followed by phosphorylation (activation) of JNK and de-phosphorylation (activation) of glycogen synthase kinase-3 beta (GSK3β). NT3 and IGF-1 increased survival at early time points, but only IGF-1 is capable to attenuate C_2-ceramide-mediated neuronal death, and this neuroprotection is associated to strong and permanent activation of AKT and inhibition of GSK3β.

In conclusion, C_2-ceramide initiates a series of events including an early inactivation of PI3K/AKT and ERK pathways followed by activation of JNK and activation of GSK3β and neuronal death, changes that are counteracted by IGF-1.

1. Introduction

The etiology of neuronal death in most neurodegenerative disease remains controversial, including dopaminergic cell death in Parkinsońs disease (PD) (Levy et al., 2009). Recently, the identification of biochemical pathways that regulate the initiation and execution of apoptosis has become an area of intensive research, and the identification of diverse neurotoxic paradigms, both endogenous and exogenous, have been evaluated as potential mediators of apoptosis in dopaminergic neurons (Bras et al., 2008). Increased levels of ceramide have been associated with diverse chronic and acute neurodegenerative conditions, including PD, and with several cellular stress signals such as cytokines (TNFα, interleukin 1β, Fas ligand), growth factor withdrawal, nitric oxide (NO), cytotoxic agents, environmental stress (heat shock, ionizing and ultraviolet radiation, serum withdrawal) (Bras et al., 2008; France-Lanord et al., 1997). Ceramide is a sphingosine-based lipid second messenger involved in apoptosis through direct or indirect regulation of pro-apoptotic mediators and the inhibition of pro-survival pathways (Hannun and Obeid, 2008). However the mechanisms involved in the activation/inhibition of these pathways, and the mode they interact to execute cell death in cell-specific context remains poorly understood.

Growth factors such as the insulin-like growth factor-1 (IGF-1) and neurotrophin-3 (NT-3) activate tyrosine kinase receptors (TrkR) that directs the activation of AKT, a central player in the regulation of neuronal survival, proliferation, differentiation and metabolism by means of controlling the diverse mediators involved in those processes (Brunet et al., 2001). Specifically through phosphorylation of GSK3β at serine 9 (pSer9-GSK3β), AKT inhibits its activity, which has been associated to diverse aspects of neuronal function and neurodegenerative diseases including PD and AD (Grimes and Jope, 2001; Nair and Olanow, 2008).

* Corresponding author at: Departamento de Patología, Facultad de Medicina, Universidad Nacional de Colombia, Cr 30 No 45-03 Bogotá, Colombia.
Tel.: +57 1 316 5000x11613; fax: +57 1 316 5000x11613.
E-mail addresses: gharboledab@unal.edu.co, garboled@hotmail.com (G. Arboleda).

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The mitogen activated protein kinase (MAPK) family of proteins (extracellular signal-regulated kinase (ERK), the c-Jun N-terminal kinase (JNK) and p38 kinase) are sequentially activated by diverse upstream kinases that are partially specific; once activated they regulate a number of cellular functions, including survival, growth, differentiation and apoptosis (Davis, 2000; Harper and LoGrasso, 2001; Hommes et al., 2003). In neurons, the ERK pathway together with the PI3K/AKT pathway, are important mediators of cell growth, survival and differentiation (Willaime-Morawek et al., 2005; Frebel and Wiese, 2006). The JNK and p38 pathways are particularly associated with diverse apoptotic paradigms, mediated by transcription dependent and independent mechanisms (Davis, 2000; Dhanasekaran and Reddy, 2008).

Ceramide has been shown to inhibit the PI3K/AKT pathway and ERK kinase; it also has been associated with the activation of JNK and P38 pathways during ceramide-induced apoptosis in diverse cellular models, but little is known about the contribution of these pathways in ceramide-induced neuronal death (Bourbon et al., 2001; Stoica et al., 2005; Willaime-Morawek et al., 2003; Zhou et al., 1998).

Based in the above observations, ceramide might be considered therefore, as a potential endogenous neurotoxin, whose fine regulation could be important in the pathogenesis of disorders characterized by abnormal apoptosis and may represent a novel target for the regulation of neuronal death in neurodegenerative diseases.

In the present study we demonstrate a differential regulation of PI3K/AKT-GSK3β and MAPK pathways in C_2-ceramide-induced cell death of catecholaminergic neurons of mesencephalic origin, and how this modulation is important in decision to cell death/survival.

2. Materials and methods

2.1. CAD cell culture

CAD cells were a kind gift from Dr. Dona M. Chikaraishi (Department of Neurobiology, Duke University Medical Center, Durham, NC, USA). CAD cells were grown as previously described by Arboleda et al. (2005). Briefly, CAD cell were grown in DMEM-F12-HAM Mixture 1:1 medium (Sigma–Aldrich, St. Louis, MO, USA) supplemented with 10% foetal calf serum (FCS) (Invitrogen, Carlsbad, CA, USA), 1% penicillin/streptomycin (10,000 U/ml penicillin G sodium, 10,000 μg/ml streptomycin sulphate), 1% glutamine (2 mM). Cells were grown at 37 °C in a humidified 5% CO_2 incubator. CAD cells were seeded at a density of 5×10^6 for 75 cm^2 flasks, 2.5×10^4 per well for 6 well plates and 7×10^3 per well for 96 well plates. After allowing overnight attachment of cells in serum plus medium (SPM), CAD cells were switched to serum free medium (SFM) supplemented with penicillin/streptomycin, glutamine and sodium selenite (50 ng/ml) to achieve neuronal-like differentiation. Complete differentiation status was obtained after 4 days in SFM.

2.2. Quantification of relative cell survival by MTT assay

Cells were seeded at a density of 7×10^3 in 200 μl of SFM per well in a 96 well plate. Differentiated cells were treated with 25 μM of C_2-ceramide (Sigma–Aldrich, St. Louis, MO, USA) for different times (15 min, 2, 6 and 24 h). After removing 50 μl of medium, 25 μl of MTT stock solution (5 mg/ml in PBS) was added to each well, followed by incubation for 2 h at 37 °C. Subsequently the media was removed, the cells lysed and the purple formazan product solubilized by the addition of 200 μl of MTT lysis buffer (20%, w/v SDS, 50%, v/v dimethylformamide in distilled water, pH 4.7). Plates were heated at 100 °C for 20 min and the absorbance read at 570 nm. Untreated cells were used as a positive control. Cell survival was calculated as: % survival = 100 (X/control), where X is the average reading of MTT metabolism in a single treatment group.

The nuclear morphology of the cells was analysed with the membrane permeable, DNA intercalating dye Hoescht 33258 (Sigma–Aldrich, St. Louis, MO, USA) and viewed under fluorescence (excitation 353 nm and emission 460 nm).

2.3. Western blotting analysis

Following diverse treatments the cells were scrapped, pelleted and lysed at 4 °C for 30 min using lysis buffer (10 μg/ml aprotinin, 5 mM benzamidine, 50 μg/ml trypsin inhibitor, 1 mM PMSF, 1 mM sodium vanadate, 0,25 M sodium fluoride). Lysates were centrifugated at 13000 rpm for 10 min at 4 °C, and the protein concentration of the supernatant was determined using the BCA protein assay kit (Pierce, USA) with bovine serum albumin (BSA) as standard. Twenty microgram of protein samples was run in polyacrylamide gel at 100 V. After electrophoresis, proteins were transferred to a nitrocellulose membrane (Hybond C extra, Amersham-Pharmacia), incubated in blocking buffer (5% powder milk in TBS-Tween 20) for 1 h at 4 °C until probed. The nitrocellulose membrane was then incubated with 5 ml of monoclonal rabbit anti-mouse primary antibodies [anti-ERK p44/p42, anti-phospho(Thr202)/Tyr(204) ERK p44/p42, anti-phospho(Ser473) AKT, anti-total AKT and anti-phospho(Ser9) GSK3β were from Cell Signalling (Danvers, MA, USA); anti-phospho(Thr183/Tyr185) SAPK/JNK was from Santa Cruz (Santa Cruz, CA, USA)] diluted 1:1000 in blocking buffer for 2 h at room temperature or overnight at 4 °C, washed 3 times in TBS-Tween (5 min/wash), followed by incubation with peroxidase conjugated secondary antibody for 1 h (1:2000) and washed 3 times with TBS-Tween. Bound antibody was detected using the ECL system (Thermo Scientific, Rockford, IL, USA).

2.4. Statistical analyses

All data are presented as mean ± SEM of triplicates of at least three independent experiments. Statistical analysis was performed using one-way ANOVA followed by Tukey–Kramer multiple comparison test when appropriate. The level of significance was set at $P < 0.05$.

3. Results

3.1. C_2-ceramide induces apoptosis of CAD: neuroprotection by IGF-1

Treatment of CAD cells with C_2-ceramide (25 μM), a cell-permeable and biological active analogue of ceramide, caused a decrease in cell survival (Fig. 1), showing several apoptotic characteristics such as cellular detachment, rounding, cytoplasm shrinkage, chromatin condensation and DNA fragmentation (Fig. 1C and D) (Arboleda et al., 2005).

Pre-treatment of CAD cells with NT3 and IGF-1 significantly increased relative survival after 6 h following 25 μM C_2-ceramide; only IGF-1 attenuated C_2-ceramide-mediated neuronal death after 24 h (Fig. 1A and B).

3.2. C_2-ceramide induces de-phosphorylation of ERK and phosphorylation of JNK during apoptosis of CAD cells

To evaluate the contribution of JNK to C_2-ceramide-induced apoptosis in CAD cells, activation of JNK was analysed by western blot. Transcription and translation of JNK genes (JNK 1–3) gives rise

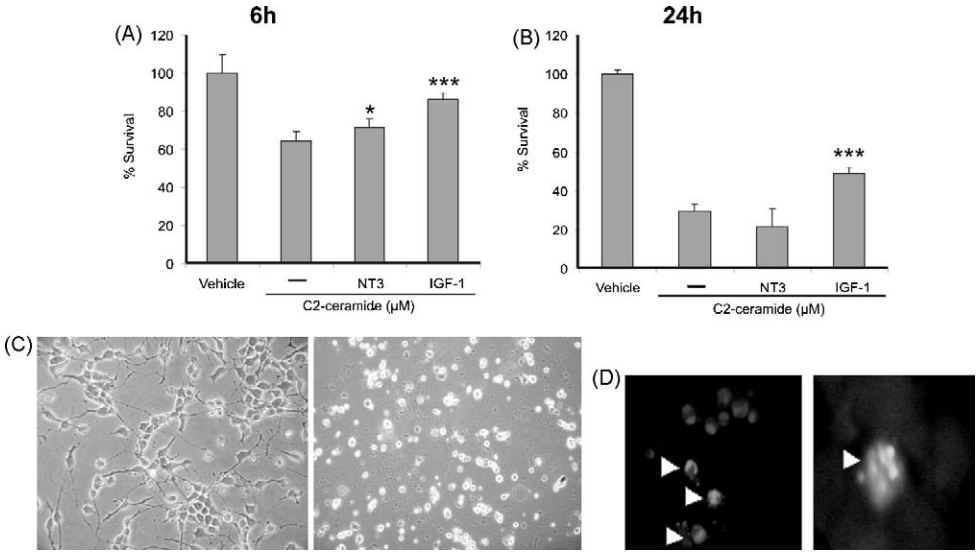

Fig. 1. C_2-ceramide-induced CAD cell death: neuroprotection conferred by IGF-1 and NT-3. CAD cells were treated for 6 h (A) or 24 h (B) with C_2-ceramide (25 μM) either alone or following pre-treatment with IGF-1 (100 ng/ml) or NT3 (100 ng/ml), and the relative survival assessed by MTT assay. Data are presented as mean ± SEM. $^{*}P < 0.05$, $^{***}P < 0.001$, compared to C_2-ceramide treated cells using one-way ANOVA followed by Tukey test [$n = 4$]. (C) Morphology of cells was analysed by light microscopy for changes associated to apoptosis. Left panel: normal and differentiated CAD cells. Right panel: apoptotic morphology of cells after 6 h of C_2-ceramide treatment. (D) Representative fluorescence microscopy of nuclei following Hoescht 33258 staining: normal cells display a diffuse chromatin while apoptotic cells showed condense and fragmented chromatin (arrowheads). Results are representative of three repetitions.

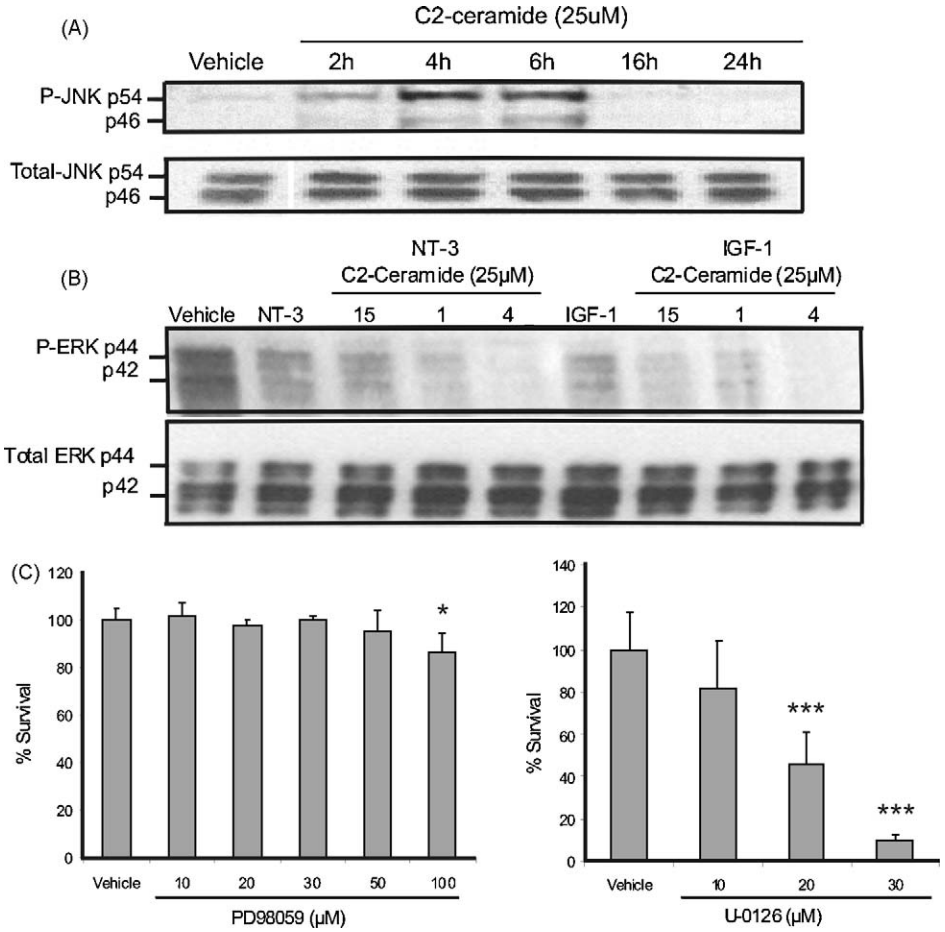

Fig. 2. C_2-ceramide exposure-induced changes in ERK and JNK. (A) CAD cells were exposed to C_2-ceramide (25 μM) for 2–24 h, harvested and cell lysates (20 μg of protein) were subject to western blot and probed with antibodies against total (total p54 and total p46) and phosphorylated (Phos-p54 and P-p46) forms of JNK. The blots shown are representative of three independent experiments; (B) CAD cells were exposed to NT-3 (100 ng/ml) and IGF-1 (100 ng/ml) alone or in conjunction with C_2-ceramide (25 μM) for 15 min, 1, 4 h, harvested and cell lysates (20 μg of protein) were subject to western blot and probed with antibodies against total (total p44 and total p42) and phosphorylated (P-p44 and P-p42) ERK. Blots shown are representative of three independent experiments. (C) Effect of the MEK inhibitors PD98059 (10–100 μM) and U0126 (10–30 μM) on survival of CAD cells after 24 h exposure. Relative survival was assessed using MTT assay. Data represent mean ± SEM. $^{*}P < 0.05$, $^{***}P < 0.001$. Results are representative of three repetitions.

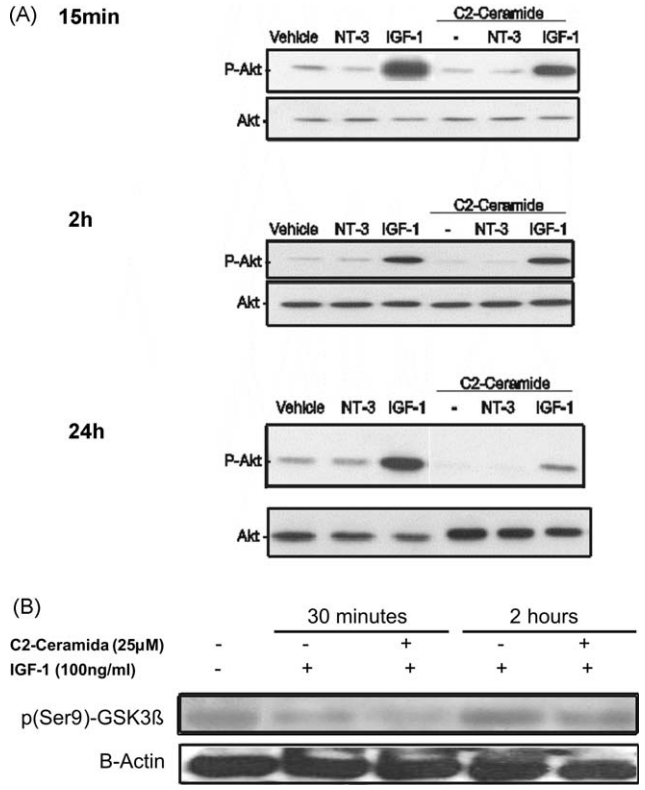

Fig. 3. Time course of C_2-ceramide-mediated changes in the phosphorylation status of AKT and GSK3β. (A) CAD cells were exposed to NT-3 (100 ng/ml) or IGF-1 (100 ng/ml) alone or together with C_2-ceramide (25 μM) for 15 min, 2 or 24 h. Cells were harvested and cell lysates (20 μg of protein) were subject to Western blot analysis and probed with antibodies against total and phosphorylated (serine 473) AKT. Blots shown are representative of three independent experiments. (B) Analysis of GSK3β phosphorylation at serine 9 upon exposure to IGF-1 and/or C_2-ceramide. Cells were pretreated with C_2-ceramide (25 μM) followed by IGF-1 (100 ng/ml) for 30 min or 2 h, and GSK3β phosphorylation at serine 9 was analysed by western blotting.

to two major proteins of 54 and 46 kDa (p54 and p46), respectively. Dual phosphorylation of these proteins at Thr183 and Tyr185 (P-p54 and P-p46) is needed for JNK activation (Gupta et al., 1996). C_2-ceramide (25 μM) exposure-induced phosphorylation of both proteins. The levels of P-p54 and P-p46 increased after 2 h of C_2-ceramide treatment, reached a peak of phosphorylation after 4–6 h and then declined to control levels after 16 h. These changes in levels of phosphorylated proteins are the result of phosphorylation rather than change in JNK expression, as the total levels of JNK did not change significantly during C_2-ceramide treatment (Fig. 2A).

ERK proteins (p44 and p42) are phosphorylated by MEK1 and MEK2 on Thr202 and Tyr204 of ERK1 (P-p44), and Thr183 and Tyr185 of ERK2 (P-p42) (Willaime et al., 2001). In CAD cells ERK was basally phosphorylated, and growth factors (NT-3 or IGF-1) decrease ERK phosphorylation (Fig. 2B). C_2-ceramide (25 μM) exposure further decreases phosphorylation of ERK (Fig. 2B). In addition, inhibition of ERK using chemical inhibitors (PD98059 and U0126) was associated to decrease survival of CAD cells (Fig. 2C).

3.3. C_2-ceramide induces de-phosphorylation of AKT

Ceramide has recently been described to inhibit the PI3K/AKT pathway. Thus, ceramide may act by altering the balance between anti-apoptotic and pro-apoptotic signalling.

CAD cells show low basal phosphorylation of AKT, and phosphorylation is increased strongly by IGF-1 but not by NT-3, at all the times examined (Fig. 3A). C_2-ceramide (25 μM) decreased the basal and growth factor-mediated phosphorylation of AKT, such that after 24 h of treatment, phosphorylation was undetectable in cells treated with vehicle or NT-3 plus C_2-ceramide, and was significantly reduced but remain present in cells treated with IGF-1 plus C_2-ceramide (Fig. 3A). The total level of AKT was not affected by C_2-ceramide.

In order to test the effect of PI3K inhibition in CAD cell survival, relative survival was analysed following exposure to LY294002, a PI3K inhibitor, alone or with C_2-ceramide. LY294002 (10 μM) decreased NT-3 and IGF-1 mediated AKT phosphorylation in a time

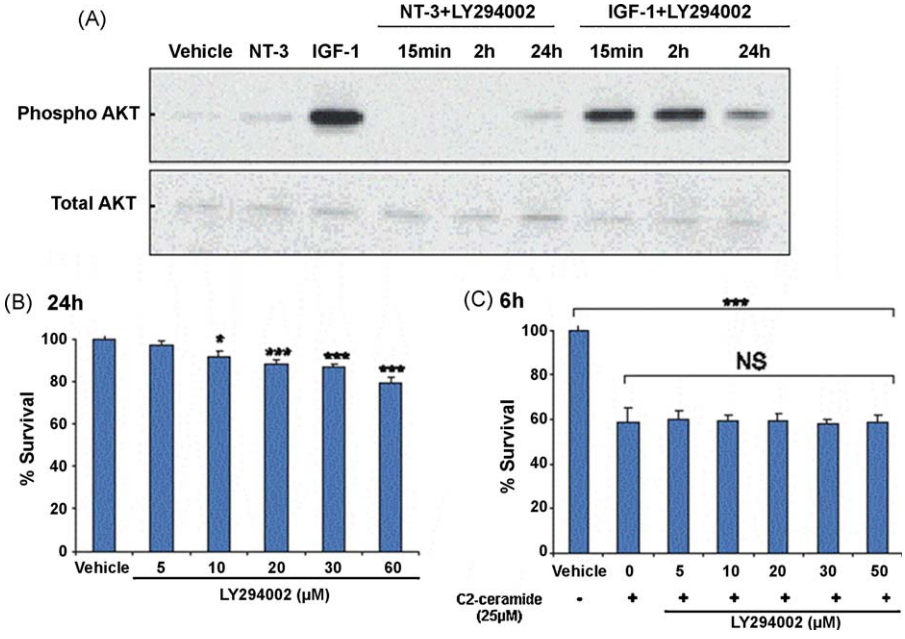

Fig. 4. PI3K inhibition decreased AKT phosphorylation and CAD cell survival. (A) CAD cells were exposed to NT-3 (100 ng/ml) or IGF-1 (100 ng/ml) alone for 15 min or together with the PI3K inhibitor LY294002 (10 μM) for 15 min, 2 or 24 h. Cells were harvested and cell lysates (20 μg of protein) were subject to Western blot analysis and probed with antibodies against total and phosphorylated AKT. Blots shown are representative of three independent experiments. CAD cells were treated with increasing concentrations of LY294002 (5–50 μM) alone for 24 h (B) or in conjunction with C_2-ceramide (C_2-cer: 25 μM) for 6 h (C). Relative survival of CAD cells was measured with the MTT assay. (B) $^*P < 0.05$, $^{***}P < 0.001$ compared to vehicle treated cells. (C) $^{***}P < 0.001$ compared to vehicle; NS: not significant, compared to C_2-cermide [$n = 3$].

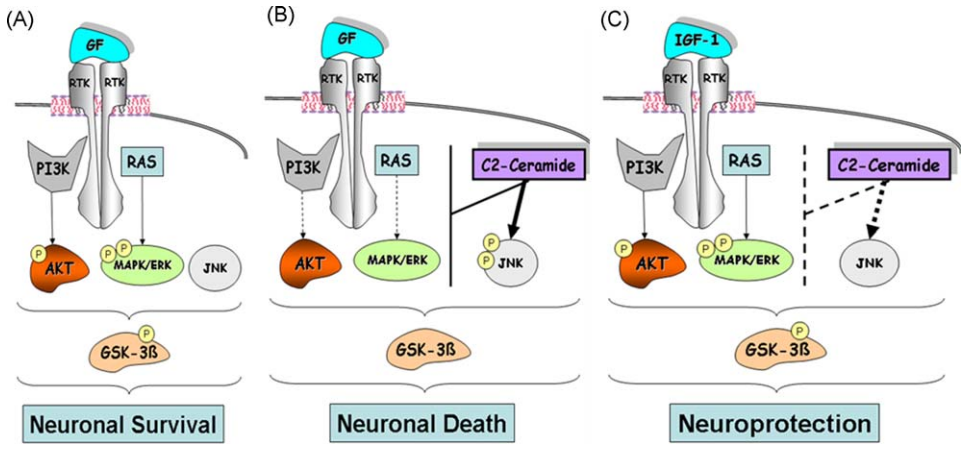

Fig. 5. Squematic model illustrating the modulation of CAD cell death/survival by C_2-ceramide. (A) CAD cell survival is mediated by activation of tyrosine kinase receptors (RTK) by growth factors (GF), such as NT-3, which in turn activates through the PI3K/AKT and MAPK/ERK pathways and inhibits JNK and GSK3β pathways; (B) C_2-ceramide-induced neuronal cell death is associated to a series of events orchestrated in part by an early inactivation/de-phosphorylation of the PI3K/AKT and ERK pathways followed by activation of JNK (phosphorylation) and GSK3β (de-phosphorylation); (C) treatment with IGF-1 protects against C_2-ceramide-induced neuronal cell death, mainly through inhibition of GSK3β and sustained AKT activation.

dependent manner (Fig. 4A), and also decreased CAD cell survival after 24 h in a concentration dependent manner (Fig. 4B). However, LY294002 did not reinforce the level of C_2-ceramide-induced death (Fig. 4C).

Downstream of AKT we analysed the phosphorylation of glycogen synthase kinase-beta at serine 9 (pSer9-GS3Kβ) which induces its inhibition. IGF-1 increases pSer9-GS3Kβ after 2 h but not at 30 min (Fig. 3B), and co-treatment with C_2-ceramide decreases pSer9-GS3Kβ (Fig. 3B).

4. Discussion

The intracellular events associated with ceramide-induced apoptosis in neurons are poorly understood and the comprehension of the mechanisms involved in cell death in neurodegeneration, in particular apoptosis, might give insights into new therapeutic targets that could slow the progression of neuronal loss in neurodegenerative diseases (D'Mello and Chin, 2005; Nicholson, 2000).

C_2-ceramide-induced an early decrease in the phosphorylation of PI3K/AKT and ERK, followed by a pronounced phosphorylation of JNK, de-phosphorylation of GSK3β previous to cell death. The basal activation of AKT and ERK observed in CAD is most probably mediated by autocrine/paracrine production of NT-3 (Horton et al., 2001), and both kinases seem to contribute to survival of CAD cells as demonstrated by using PI3K and ERK inhibitors. Comparison of ERK inhibitors PD98059 (inhibits preferentially MEK1) and U0126 (inhibits MEK1 and MEK2) showed that both MEK1 and MEK 2 are important of CAD cells survival. Importantly, LY294002 did not increase cell death induced by C_2-ceramide alone, suggesting complete inhibition of the PI3K/AKT by C_2-ceramide. In addition, IGF-1 not only protects CAD cells by sustained AKT phosphorylation but also reverses AKT inactivation induced by C_2-ceramide.

How does inhibition of PI3K/AKT and ERK pathways by C_2-ceramide direct downstream signalling toward cell death? Xue et al. (2000) using sympathetic neurons have demonstrated that Ras activates the PI3K/AKT and ERK neuronal survival pathways and that both pathways inhibit apoptosis before death mechanisms converge into mitochondria. The inhibition of ERK has been related to inhibition of NGF-induced neuronal phenotype that is detrimental for neuronal survival (Virdee and Tolkovsky, 1996). Willaime et al. (2001) have shown that C_2-ceramide induces a decrease of both MEK and ERK phosphorylation in cortical neurons, associated with neurite retraction and fragmentation that precedes apoptosis; however, ERK inhibition did not influence cell survival. Ceramide also induces early cell detachment, cleavage of focal adhesion kinase (FAK) (Di Bartolomeo and Spinedi, 2002), and down-regulation of integrins (Panigone et al., 2001). In CAD cells the decreased survival caused by MEK 1/2 inhibition may be also related to alterations in the differentiation process and loss of cell attachment, as those changes occurs soon after C_2-ceramide exposure (data not shown).

Signalling through the PI3K/AKT pathway is critical for survival of neurons (Brunet et al., 2001), and its inhibition by ceramide contributes to neuronal apoptosis (Arboleda et al., 2007). The mechanism of inhibition of AKT by ceramide is still controversial and uncertain. Some studies argue that ceramide inhibits PI3K (Zundel et al., 2000), while others have postulated that it may occur downstream through activation of a phosphatase that regulates PDK2 (Schubert et al., 2000).

Studies have described JNK activation during ceramide-induced apoptosis (Willaime-Morawek et al., 2003; Verheij et al., 1996). The mechanisms by which ceramide controls activation of JNK in neurons and the outcome of its activation is still poorly understood, as JNK signalling response seems to be cell- and context-dependent (Basu and Kolesnick, 1998). In human U937 leukemia cells, C_2-ceramide-induces JNK activation mediated by MKK7 (Kim et al., 2001). Recently it has been demonstrated that activation of JNK by the mixed lineage kinase 3 (MLK-3) is regulated by ceramide, and that inhibition of MLK-3 significantly attenuates ceramide-induced activation of JNK (Sathyanarayana et al., 2002). Furthermore, it has been shown that neuronal apoptosis can be prevented by CEP-1347 a broad-spectrum inhibitor of MLK family (Maroney et al., 2001).

Willaime-Morawek et al. (2003) and Willaime et al. (2001) demonstrated that ceramide-induced neuronal death is associated with activation of p38 and JNK, and that although the independent inhibition of each pathway partially protects against C_2-ceramide, their combined inhibition provided complete protection. Interestingly, this protection was accompanied by activation of ERK, and therefore it was suggest that neuronal apoptosis induced by ceramide probably involves a balance between the three MAPK pathways.

Little is known about how activation of JNK is related to the PI3K/AKT pathway. In our model, inhibition of PI3K/AKT and ERK

preceded the activation of JNK. In sympathetic neurons, NGF deprivation-induced apoptosis has been linked to activation of JNK; however, whether activation of the JNK pathway is the result of inactivation of survival pathways or the result of pro-apoptotic signalling is not yet clear (Ham et al., 2000). In PC12 cells, it was suggested that ERK promotes survival by the inhibiton of JNK and p38 kinases (Xia et al., 1995). However in a different study this relationship was not found (Virdee and Tolkovsky, 1995). Growth factor-mediated signalling through the PI3K/AKT pathway or expression of a constitutively active AKT chronically inhibits the JNK pathway (Levresse et al., 2000), and inhibition of PI3K stimulates JNK phosphorylation prior to apoptosis (Shimoke et al., 1999). The predominant and early role of ceramide as an inhibitor of the PI3K/AKT pathway suggests that activation of JNK in such paradigm may be an indirect consequence of this inhibition, and thus, the ability of AKT signalling to inhibit JNK activation represents a potentially novel site of action for AKT in the regulation of cell viability.

The neuroprotective potential of IGF-1 and NT-3 against C_2-ceramide seems to be mostly associated to the differential regulation of AKT phosphorylation profile (transient or sustain) rather than to ERK activation: IGF-1 had a protective effect, while NT-3 only delayed cell death. This protection was also associated to early phosphorylation (Ser9) and inhibition of GSK3β.

It has been demonstrated that the levels and activation of TrkC decrease in response to continuous exposure to NT-3 (Ness et al., 2002), as may be the case in CAD cells (Horton et al., 2001), which leads to receptor desensitisation (Knusel et al., 1997). In contrast to NT-3, chronic stimulation of IGF-1 receptor does not affect its activation or expression levels (Ness et al., 2002). Although most studies have demonstrated transient activation of AKT by IGF-1 (Cheng et al., 2000), some observed persistent activation of AKT (as in the present study) associated with protection against stress insults (Ness et al., 2002; Gary and Mattson, 2001). Similar to our finding, studies using glia and cerebellar granule neurons, comparing the effect of neurotrophins with IGF-1 on AKT activity, have demonstrated that IGF-1 activates AKT to a higher and more persistant level (Ness and Wood, 2002; Yamagishi et al., 2003), probably through internalization and recycling of the IGF-1 receptor (IGF-1R). Furthermore, IGF-1 allows a sustained phosphorylation of GSK3β up to 24 h (Romanelli et al., 2007).

Previously it has been described that in sensory neurons IGF-1 and NGF induces GSK3β phosphorylation and inhibition after 24 and 48 h respectively (Jones et al., 2003), while others showed decrease in 30% GSK3β activation following 10 min IGF-1 exposure in muscle cells (Cross et al., 1994), and 26% decrease in GSK3β activity upon NGF treatment in sympathetic neurons after 15 min (Crowder and Freeman, 2000). Herein we demonstrate that IGF-1 phosphorylates and inhibits GSK3β in a time dependent manner while C_2-ceramide blocks this inhibition and therefore favours GSK3β activity contributing to the apoptotic changes observed. GSK3β has been involved in the control of apoptosis by regulation of the mitochondrial localization and phosphorylation of Bax (Linseman et al., 2004), activation of caspases 2 and 8 (Lin et al., 2007), induction of activation of p53 and caspase 3 (King et al., 2001), phosphorylation and destabilization of the anti-apoptotic Mcl-1 protein (Maurer et al., 2006), and by regulation of mitochondrial hexokinase II (Gimenez-Cassina et al., 2009).

In conclusion, C_2-ceramide initiates a series of events orchestrated in part by an early inactivation of PI3K/AKT and ERK pathways followed by activation of JNK and GSK3β that ultimately leads to neuronal death. In addition it is suggested that through early inhibition of GSK3β and sustained AKT activation, IGF-1 attenuated C_2-ceramide-mediated neuronal death (Fig. 5). This potential neuroprotective role of IGF-1 offers a plausible therapeutic potential to decrease catecholaminergic cell death.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

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