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Insulin-like growth factor-1 provides protection against psychosine-induced apoptosis in cultured mouse oligodendrocyte progenitor cells using primarily the PI3K/Akt pathway

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Psychosine (galactosylsphingosine) is a toxic metabolite that accumulates in globoid cell leukodystrophy (GLD) due to the deficiency of galactocerebrosidase (GALC) activity. This results in subsequent programmed cell death of oligodendrocytes and demyelination in human patients and animal models. We investigated the potential role of insulin-like growth factor-1 (IGF-1) in modifying the apoptotic effect of psychosine in cultured mouse oligodendrocyte progenitor cells (OLP-II). We show that psychosine inhibits the phosphorylation of Akt and Erk1/Erk2 (Erk1/2), which are the main anti-apoptotic pathways of the IGF-1 receptor (IGF-1R). Although IGF-1 sustained phosphorylation of both of these pathways, it provided maximum protection to OLP-II cells from psychosine-induced cell death in a PI3K/Akt-dependent manner. The effects of IGF-1 were dose-dependent and resulted in increased IGF-1R autophosphorylation levels. Although relatively high concentrations of IGF-1 also resulted in the activation of the insulin receptor (IR), its effect was more significant on the IGF-1R.

Introduction

Globoid cell leukodystrophy (GLD), also known as Krabbe disease, is a metabolic disease caused by a deficiency of the galactocerebrosidase (GALC) activity (Suzuki and Suzuki, 1970). GALC is the lysosomal enzyme which catalyzes the degradation of galactose from several glycosphingolipids, including galactocerebroside (gal-cer) and galactosylsphingosine (psychosine) (reviewed in Wenger et al., 2001). Despite the GALC deficiency, gal-cer does not dramatically increase in the brain of patients with Krabbe disease. However, psychosine accumulates in the brain (Hideki and Suzuki, 1984; Miyatake and Suzuki, 1972; Svennerholm et al., 1980; Vanier and Svennerholm, 1976), causing death of myelin-generating cells (Suzuki, 1998; Tanaka and Webster, 1993). Psychosine is generated by the galactosylation of sphingosine by UDP-galactose:ceramide galactosyltransferase (CGT) (Mitsuo et al., 1989; Svennerholm et al., 1980), and its synthesis is limited to myelinating cells, oligodendrocytes (OL) in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS). A small amount of psychosine is generated even in normal brain during active myelination (Svennerholm et al., 1980). When GALC activity is present, psychosine is degraded, however, it accumulates to levels of 10 times normal in the white matter of humans and the different animal models with GLD, including the twitcher mouse (Suzuki, 1998; Svennerholm et al., 1980; Wenger et al., 2001).

Several studies have suggested that an apoptotic process plays an important role in neural demyelination and OL disappearance in this disease (Hannun and Bell, 1987; LeVine and Brown, 1997; Taniike et al., 1999; Tohyama et al., 2001; Suzuki, 1998). The cytotoxicity of psychosine in several neural cell lines in vitro was described in previous studies (Jatana et al., 2002; Sugama et al., 1990; Sueyoshi et al., 2001; Tanaka and Webster, 1993; Tohyama et al., 2001). Moreover, studies demonstrating apoptosis in cultured mature OL and OL progenitor cells (Haq et al., 2003; Zaka and Wenger, 2004) and in the brains of patients with GLD and twitcher mice (Jatana et al., 2002; Taniike et al., 1999) document an important role for psychosine in OL apoptotic cell death. While a few studies have defined the mechanism of psychosine-induced apoptotic cell death in OL, the specific regulatory events have not been completely elucidated.

The therapeutic potential of insulin-like growth factor-1 (IGF-1) in the treatment of demyelinating conditions has been previously investigated (Komoly et al., 1992; Mason et al., 2000a,b, 2003, Yao et al., 1995; Ye et al., 2002), yet a role for IGF-1 protection in the treatment of GLD has not been demonstrated. A number of studies have shown that IGF-1 accelerates the differentiation of OL precursors into mature OL, and it prevents apoptotic cell death in cells having the IGF-1 receptor (IGF-1R) (Dubois-Dalcq and Murray, 2000; Mason et al., 2003; Mozell and McMorris, 1988; Zumkeller, 1997). IGF-1R-

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containing cells are widely present in the brain, and they play an important role in the growth, development and survival of the nervous system. A wide range of studies have shown that the IGF-1R has been implicated in preventing cell death (Liu et al., 1998; Mason et al., 2003; Sell et al., 1995; Singleton et al., 1996). The IGF-1R has specific anti-apoptotic signaling capacity (Kulik and Weber, 1998), which enables survival stimuli such as IGF-1 to mediate intracellular signaling. IGF-1 binding to IGF-1R causes autophosphorylation of the receptor on tyrosine residues (Baserga, 2000). This leads to the sequential activation of signal transduction pathways which then, by several possible mechanisms, can block apoptosis and promote cell survival. The action of IGF-1 is mediated by the IGF-1R by activating two major pathways, including Akt, also known as protein kinase B (PKB) and mitogen-activated protein kinase (MAPK)/Erk1/2 (Datta et al., 1999). The phosphatidylinositol-3 kinase (PI3K) signaling pathway appears to be required for the survival of a number of cell types (Campana et al., 1999; Flores et al., 2000; Zheng et al., 2000), including OL and OL progenitor cells (Vemuri and McMorris, 1996). The motifs involved in PI3K activation are found in the intracellular domain of the IGF-1R tyrosine kinase. PI3K and their phospholipid products are implicated in promoting survival downstream of extracellular stimuli. The Akt pathway originates with the interaction of the IGF-1R with one of its major substrates, insulin receptor substrate-1 (IRS-1) (Myers et al., 1993) that activates PI3K, which in turn activates Akt.

The MAPK (p44/p42), also known as Erk1 and Erk2 (Erk1/2), is another anti-apoptotic pathway of the IGF-1R system. So far, three branches of the MAPK pathway have been described, including the MAPK/Erk1/2, the c-*jun* N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and the p38 protein kinase. These three branches of the MAPK pathway have been implicated in the regulation of signaling pathways leading to apoptosis, depending on the various models. The MAPK/Erk1/2 pathway is activated by mitogenic signals, DNA damage, or oxidative stress (Seger and Krebs, 1995; Woodgett et al., 1996; Xia et al., 1995). Furthermore, this particular pathway has been implicated in the protection from apoptosis by IGF-1R (Peruzzi et al., 1999).

Although recent studies document psychosine-induced cell death in cultured OL (Haq et al., 2003; Zaka and Wenger, 2004), the molecular signaling mechanism(s) regulating this process remains unclear. Here, we report that psychosine induces apoptotic cell death by inhibiting the phosphorylation of the Akt and Erk1/2 regulatory pathways. In addition, IGF-1 was found to sustain the activation of these anti-apoptotic pathways and inhibit the activation of caspase-3. These effects of IGF-1 were dose-dependent. While data suggest that both pathways are involved in IGF-1-mediated survival of OLP-II cells against psychosine-induced apoptosis, the PI3K/Akt is necessary for the protective effect of IGF-1.

Results

IGF-1 provides protection on OLP-II cells from psychosine-induced apoptosis in a dose-dependent manner

Previously, we have shown a role for psychosine in OLP-II cell death via an apoptotic mechanism that involves the activation of caspases (Zaka and Wenger, 2004). To determine the time course of cell death and the ability of IGF-1 to provide protection of OLP-II cells against psychosine, the percentage of cells that exhibited apoptotic cell death was determined between 0 and 24 h in the absence or presence of 50 μM psychosine with or without the addition of 100 ng/ml IGF-1. As shown in Fig. 1A, 100 ng/ml IGF-1 provided a small amount of protection to cells at 2, 5, 10 and 24 h. To evaluate whether increasing IGF-1 concentrations would have a more significant survival effect, we chose the 16 h time point. This particular time was chosen because the toxic effect of psychosine was relatively low at 10 h (approximately 22% dead cells) and high at 24 h (approximately 70% dead cells) (Fig. 1A). Increasing IGF-1 concentrations to 400 ng/ml and 1 μg/ml decreased the percentage of dead cells to 18% and 7%, respectively, compared to psychosine treatment alone (33% dead cells) at 16 h (Fig. 1B). Cells cultured for 24 h in the presence of 50 μM psychosine (Fig. 1C,b) exhibited the characteristics of apoptotic cells as compared with untreated controls (Fig. 1C,a). The condensed fragmented nuclei incorporated the fluorescein-labeled dUTPs, indicating the presence of the labeled 3′-OH ends characteristic of apoptosis. These data show that IGF-1 provides protection against psychosine-induced apoptosis in a dose-dependent manner (Figs. 1C,c–e).

Effects of inhibitors LY and PD on treated and untreated OLP-II cells

To investigate the effect of increasing IGF-1 dosages on the induction of caspase activation, we studied the proteolytic cleavage of caspase-3 with time by Western blot analysis. As shown in Fig. 2A, treatment of OLP-II cells with 50 μM of psychosine resulted in processing of caspase-3 to its cleaved product p17. One μg/ml IGF-1 completely inhibited the processing of caspase-3 at 24 h (Fig. 2A) and greatly reduced it at 48 h (Fig. 2B). Previous studies have shown that the survival effect of IGF-1 is mediated through the activation of the PI3K/Akt and MAPK/Erk1/2 pathways in multiple cell types (Dudek et al., 1997; Kulik and Weber, 1998). Therefore, we investigated whether the PI3K inhibitor LY-294002 could block the protective effect of IGF-1 on OLP-II cells treated with psychosine. Addition of this inhibitor to cells treated with psychosine plus 1 μg/ml IGF-1 resulted in the activation of caspase-3 comparable to the levels detected in cells treated with psychosine alone after 24 h (Fig. 2C). Similarly, the addition of PD-98059, a selective inhibitor of the MAPK-activating enzyme, MAPK/Erk kinase (MEK), resulted in the activation of caspase-3 in cultured cells treated with psychosine plus 1 μg/ml IGF-1 (Fig. 2C). These data suggest that 1 μg/ml IGF-1 inhibits the proteolytic cleavage of caspase-3 in psychosine-treated cells by utilizing the PI3K/Akt and MAPK/Erk1/2 pathways.

Psychosine inhibits the phosphorylation of Akt and Erk1/2

To investigate the effect of psychosine on Akt and Erk1/2, the phosphorylation of these pathways was analyzed in cells following treatment with different dosages of psychosine. As shown in Figs. 3A and B, psychosine inhibited the phosphorylated state of Akt in a dose-dependent manner with 50 μM psychosine, almost completely inhibiting the phosphorylation of Akt at 24 h. Similarly, psychosine inhibited the phosphorylated state of Erk1/2 in a dose-dependent manner (Figs. 4A, B). These data indicate that one possible signaling mechanism by which psychosine exerts its

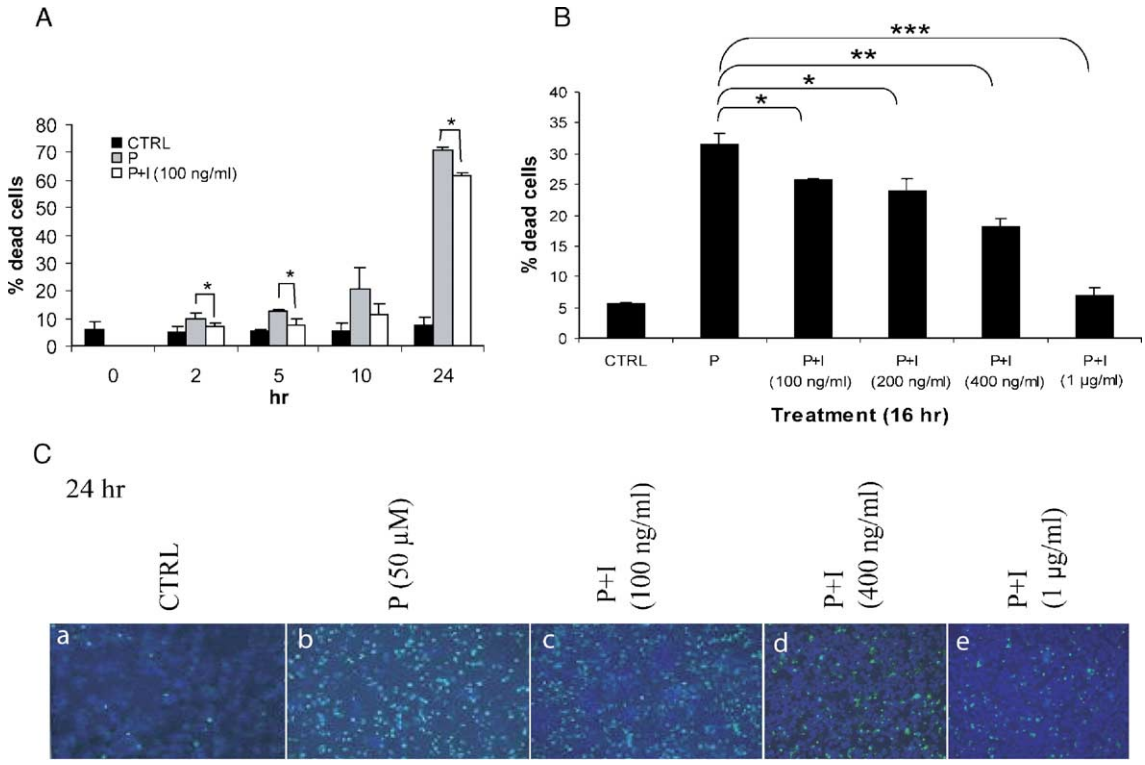

Fig. 1. Effects of psychosine and IGF-1 on OLP-II cells. (A) Cells were incubated in the presence of 50 μM psychosine (P) without or with 100 ng/ml IGF-1 (I), and TUNEL positive cells (dead cells) were counted at 0, 2, 5, 10 and 24 h. (B) Cells were incubated in the presence of 50 μM psychosine without or with 100, 200, 400 ng/ml or 1 μg/ml IGF-1, and TUNEL positive cells (dead cells) were counted at 16 h. (C) Aliquots of cell suspensions were untreated (a), treated with 50 μM psychosine (b), 50 μM psychosine plus 100 ng/ml IGF-1 (c), 50 μM psychosine plus 400 ng/ml IGF-1 (d) or 50 μM psychosine plus 1 μg/ml IGF-1 (e) for 24 h. Cells were then processed using the TUNEL assay (green) and counterstained with DAPI (blue) to detect changes in nuclear morphology. Data shown are the means and standard deviations for three separate experiments. Statistical analysis was performed using pair-wise Student's *t* test (the signs *, ** and *** denote $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively).

cytotoxic effect is through its ability to dephosphorylate Akt and Erk1/2.

IGF-1 sustains the phosphorylation of Akt and Erk1/2

To investigate whether IGF-1 can prevent psychosine-mediated dephosphorylation of Akt and Erk1/2 in cultured cells, phosphorylation of these pathways was analyzed in OLP-II cells following treatment with 50 μM psychosine in the absence or presence of 100, 200, 400 ng/ml or 1 μg/ml IGF-1. IGF-1 inhibited the dephosphorylation of Akt in a dose-dependent manner, with 1 μg/ml showing phosphorylation of Akt comparable to the untreated control cells (Figs. 5A, B). Furthermore, to determine whether IGF-1 was utilizing the PI3K pathway to provide sustained activation of Akt, cells were cultured in the presence of the PI3K inhibitor LY-294002. Cells treated with 50 μM psychosine, 1 μg/ml IGF-1 and 30 μM LY-294002 resulted in the inhibition of phospho-Akt production compared to cells treated with psychosine plus IGF-1 (Figs. 5C, D). In contrast, inhibition of the MAPK/Erk1/2 pathway by 50 μM PD-98059 had no statistical effect on Akt phosphorylation by IGF-1 in the presence of psychosine (Figs. 5C, D). The ability of IGF-1 to sustain Akt phosphorylation was sustained for 48 h (Figs. 5C, D).

To evaluate the effect of IGF-1 on the Erk pathway, cells were treated with psychosine and increasing concentrations of IGF-1 for 24 h as described above, and Erk1/2 phosphorylation was evaluated by Western blotting. IGF-1 suppressed psychosine-mediated dephosphorylation of Erk1/2 in a dose-dependent manner, with 400 ng/ml and 1 μg/ml IGF-1 showing the highest phosphorylation of Erk1/2 (Figs. 6A, B). Treatment of cells with 50 μM PD-98059 resulted in the inhibition of phosphorylated Erk1/2 (Figs. 6C, D). In contrast, there was no inhibition of Erk1/2 phosphorylation by LY-294002 (Figs. 6C, D). Cells treated with psychosine and IGF-1 did not show statistically significant phosphorylation levels of Erk1/2 at 48 h (Figs. 6C, D). These data indicate that IGF-1 suppresses psychosine-mediated dephosphorylation of Akt and Erk1/2, with the effect being greater on Akt than Erk1/2 (Figs. 5,6).

PI3K/Akt, not MAPK/Erk1/2, is required in IGF-1-mediated protection of cells

To assess the involvement of Akt and Erk1/2 in IGF-1-mediated cell survival using the MTT reduction assay, OLP-II cells were treated with 50 μM psychosine and 1 μg/ml IGF-1 in the absence or presence of the inhibitors LY-294002 or PD-98059 for 20 h. As shown in Fig. 7C, a significant decrease in cell viability was obtained with 30 μM LY-294002, abrogating the protective effect of IGF-1 on psychosine-treated cell. Cell viability value is similar to that obtained when cells were treated with psychosine alone. In contrast, 50 μM PD-98059 did not have a significant effect on cells treated with IGF-1 and psychosine. The effects of these drugs on cell viability were tested in untreated cells (Fig. 7A) and cells treated with IGF-1 alone (Fig. 7B). There was a small but

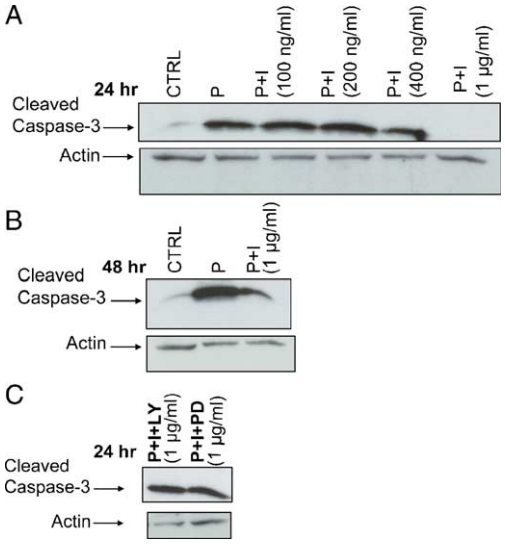

Fig. 2. Effects of psychosine, IGF-1 and LY-294002 (LY) and PD-98059 (PD) on caspase-3 activation. (A) Cells were either untreated (CTRL), treated with 50 μM psychosine or 50 μM psychosine plus 100, 200, 400 ng/ml or 1 μg/ml IGF-1 and harvested at 24 h. (B) Cells were untreated or treated with 50 μM psychosine or 50 μM psychosine plus 1 μg/ml IGF-1 for 48 h. (C) Cells were treated with 50 μM psychosine and 1 μg/ml IGF-1 and with either the PI3K/Akt inhibitor (LY) or the MEK inhibitor (PD) for 24 h. Cultures were lysed in sample buffer, and the isolated protein was analyzed for activated caspase-3 (17 kDa) by Western blot analysis. Blots were stripped and analyzed for actin to control for protein loading.

significant decrease in viable cells compared to cells that were not treated with the inhibitors. While these results demonstrate that Akt and Erk1/2 are both involved in OLP-II cell survival, the data indicate that the PI3K/Akt pathway is crucial for IGF-1-mediated protection of OLP-II cells from psychosine-induced toxicity.

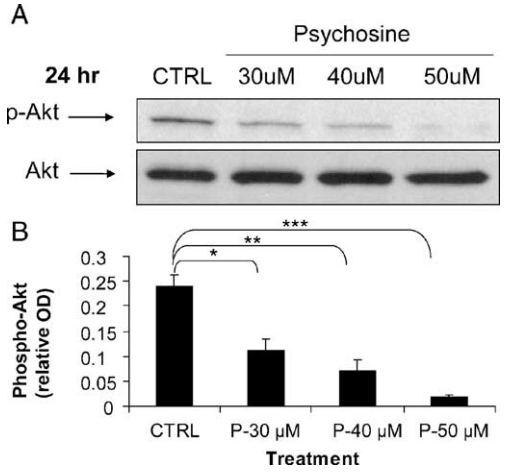

Fig. 3. Effect of psychosine on Akt. (A) Cells were either untreated or treated for 24 h with 30, 40 or 50 μM psychosine, and phosphorylation of Akt was assessed by Western blotting. Blots were stripped and used for total Akt analysis. (B) Quantification of band density was expressed as the ratio of the optical density (OD) of phosphorylated Akt to the OD of total Akt. Graph represents data from three separate experiments. Statistical analysis was performed using pair-wise Student's *t* test (the signs *, ** and *** denote $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively).

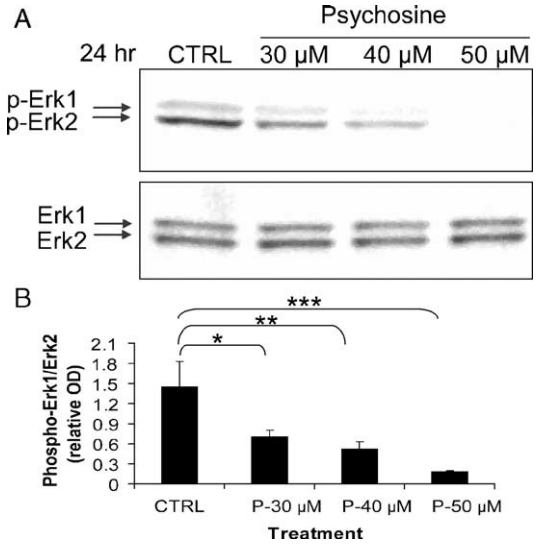

Fig. 4. Effect of psychosine on Erk1/2. (A) Cells were treated for 24 h with 30, 40 or 50 μM psychosine, and phosphorylation of Erk1/2 was assessed by Western blot analysis. Blots were stripped and used for total Erk1/2 analysis. (B) Quantification of band density was expressed as the ratio of the OD of phosphorylated Erk1/2 to the OD of total Erk1/2. Graph represents data from three separate experiments. Statistical analysis was performed using pair-wise Student's *t* test (the signs *, ** and *** denote $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively).

IGF-1 treatment results in autophosphorylation of the IGF-1 receptor

To understand the mechanism of IGF-1-mediated protection by the IGF-1R, we analyzed the expression of phosphorylated and total IGF-1R in IGF-1-treated OLP-II cells. Cells were stimulated with 100 ng/ml or 1 μg/ml IGF-1 for 24 h, and IGF-1R immunoprecipitates were assayed for tyrosine autophosphorylation of the IGF-1R. The levels of IGF-1R phosphorylation were significantly increased in the IGF-1-treated cells compared to the untreated control cells with 1 μg/ml IGF-1 showing more phosphorylation than 100 ng/ml (Figs. 8A, B). Membranes were then stripped and reblotted with an anti-IGF-1R total antibody to confirm that similar amounts of protein were immunoprecipitated in all samples.

IGF-1 treatment phosphorylates the insulin receptor

Previous studies have indicated that high concentrations of IGF-1 result in activation of the insulin receptor (IR) (Myers et al., 1993; Prisco et al., 1999). To examine the effect of increasing IGF-1 concentration on the activation of the IR, we investigated the expression of phosphorylated and total IR following IGF-1 treatment. Figs. 9A and B show that 1 μg/ml IGF-1 can give a significant increase in phosphorylated IR, although the response was considerably less than the IGF-1R (Figs. 8,9). Using 100 ng/ml IGF-1, a lower level of phosphorylation of the IR was detected (Figs. 9A, B).

Discussion

GLD is caused by mutations in the GALC gene (reviewed in Wenger et al., 2001). In the absence of GALC activity, psychosine

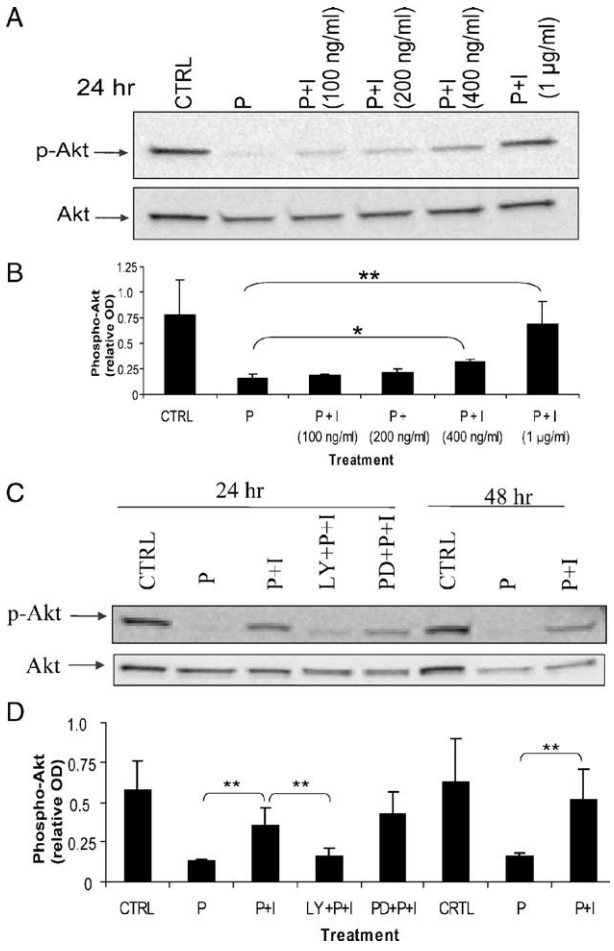

Fig. 5. Effects of IGF-1 and the inhibitors LY and PD on phospho-Akt in psychosine-treated cells. (A) Cells were incubated for 24 h in the presence of psychosine with or without the addition of increasing IGF-1, and phosphorylation of Akt was determined. Blots were stripped and used for total Akt analysis. (B) Band density was quantified and expressed as the ratio of the OD of phosphorylated Akt to the OD of total Akt. (C) Cells were treated for 24 h with 50 μM psychosine with and without the addition of 1 μg/ml IGF-1 and the inhibitors LY or PD. To show that IGF-1 sustains the phosphorylation of Akt for a longer period, total lysates were prepared from untreated cells and cells treated with 50 μM psychosine or psychosine plus 1 μg/ml IGF-1 for 48 h. Blots were stripped and used for total Akt analysis. (D) Quantification of band density was expressed as the ratio of the OD of phosphorylated Akt to the OD of total Akt. Each graph represents data from three separate experiments. Statistical analysis was performed using pair-wise Student's t test (the signs * and ** denote $P < 0.05$ and $P < 0.01$, respectively).

accumulates, and this appears to account for much of the pathology of GLD, including loss of OL and severe demyelination. This toxic effect of psychosine can be demonstrated in cultured cells. In this report, we show that IGF-1 protects oligodendrocyte progenitor cells from psychosine-induced apoptosis in a dose-dependent manner. Furthermore, IGF-1 suppresses psychosine-mediated dephosphorylation of Akt and Erk1/2. Inhibition of these pathways blocked IGF-1-mediated phosphorylation of Akt and Erk1/2 while resulting in the activation of caspase-3. Furthermore, inhibition of PI3K/Akt prevented IGF-1 from protecting OLP-II cells against psychosine-induced cell death. Finally, we show enhanced autophosphorylation of the IGF-1R with increasing concentrations of IGF-1. These data suggest that the mechanism for IGF-1 protection against psychosine-induced apoptosis is mediated by IGF-1R signaling, and the PI3K/Akt pathway is required for the survival effect of IGF-1.

Although several studies have documented a role for psychosine in apoptotic cell death in GLD (Haq et al., 2003; Jatana et al., 2002; Sueyoshi et al., 2001; Sugama et al., 1990; Tanaka and Webster, 1993; Tohyama et al., 2001; Zaka and Wenger, 2004), the molecular mechanism(s) regulating this process has not been completely elucidated. In particular, published studies showing the signaling pathways activated by IGF-1 and mediated by the IGF-1R have lead to the identification of proteins that are critical mediators of cell survival, including Akt and Erk1/2 (Parrizas et al., 1997; Peruzzi et al., 1999). We show here that psychosine

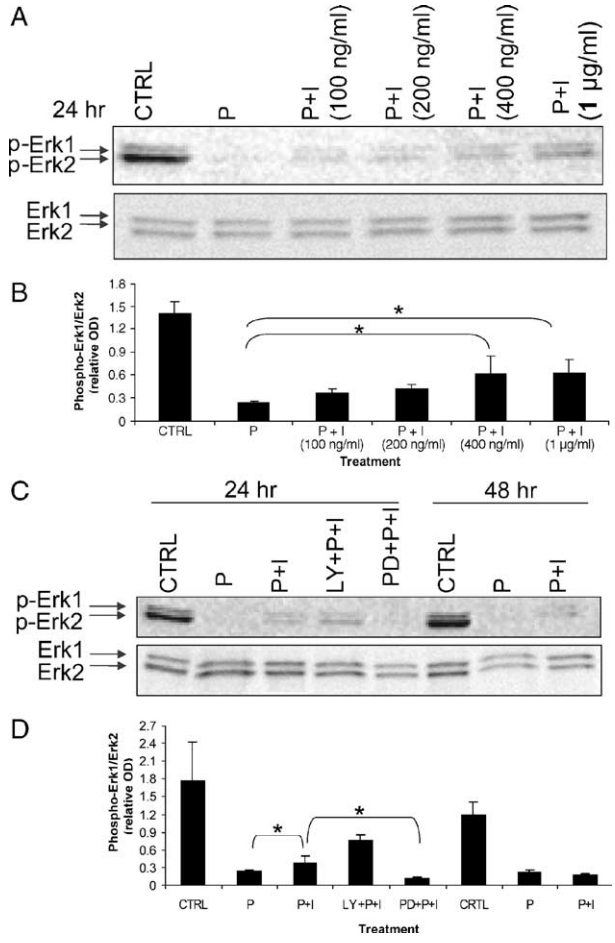

Fig. 6. Effects of IGF-1 and the inhibitors LY and PD on phospho-Erk1/2 in psychosine-treated cells. (A) Cells were incubated for 24 h in the presence of psychosine and increasing concentrations of IGF-1, and phosphorylation of Erk1/2 was determined by Western blotting. Blots were stripped and used for total Erk1/2 analysis. (B) Band density was quantified and expressed as the ratio of the OD of phosphorylated Erk1/2 to the OD of total Erk1/2. (C) Cells were treated for 24 h with 50 μM psychosine with and without the addition of 1 μg/ml IGF-1 and the inhibitors PD or LY. To determine whether IGF-1 sustains the phosphorylation of Erk1/2 through 48 h, total cell lysates were prepared from untreated, 50 μM psychosine or psychosine plus 1 μg/ml IGF-1-treated cultures and analyzed by Western blotting. Blots were stripped and used for total Erk1/2 analysis. (D) Quantification of band density was expressed as the ratio of the OD of phosphorylated Erk1/2 to the OD of total Erk1/2. Each graph represents data from three separate experiments. Statistical analysis was performed using pair-wise Student's t test (the sign * denotes $P < 0.05$).

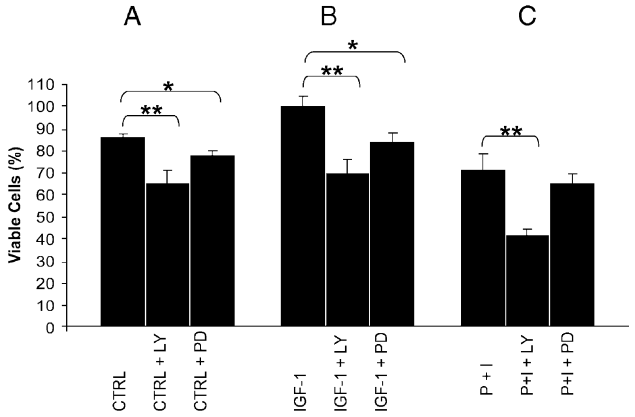

Fig. 7. Effects of LY and PD on survival of OLP-II cells. (A) Control cells (CTRL) were incubated without psychosine or IGF-1, (B) cells were treated with 1 μg/ml IGF-1 and (C) cells were treated with 50 μM psychosine and 1 μg/ml IGF-1 in the presence or absence of the inhibitors LY or PD. Cell survival was determined by the MTT assay. Data shown are the means and standard deviations for two separate experiments performed in triplicate. Statistical analysis was performed using pair-wise Student's *t* test (the signs * and ** denote $P < 0.05$ and $P < 0.01$, respectively).

promotes the dephosphorylation of Akt in a dose-dependent manner (Fig. 3). Evidence from mutational studies indicates that phosphorylation of the Akt residues is required for its activity (Datta et al., 1999). Moreover, analysis of the downregulation of Akt after its activation demonstrates that tensin homolog deleted on chromosome 10 (PTEN), a 3-position lipid phosphatase, and the recently identified carboxyl-terminal modulator protein (CTMP)

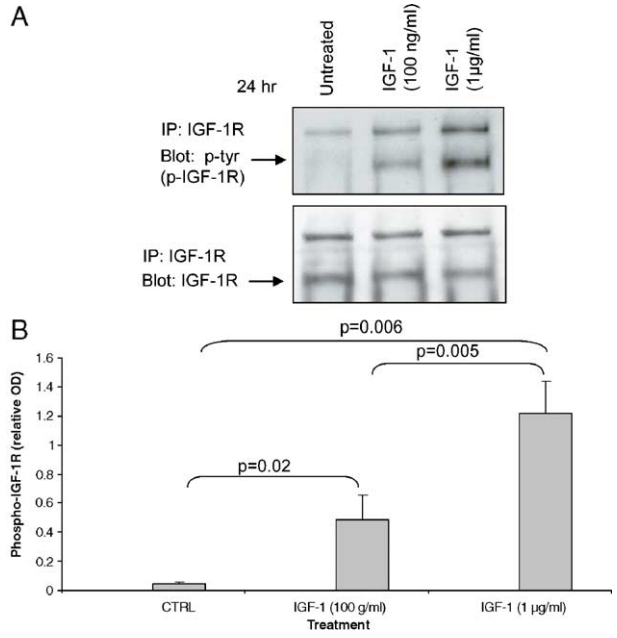

Fig. 8. Effect of IGF-1 on autophosphorylation of IGF-1R. (A) Cells were treated with 100 ng/ml or 1 μg/ml IGF-1. The lysates were harvested, immunoprecipitated (IP), and autophosphorylation of IGF-1R was detected using a phospho-tyrosine antibody. Blots were stripped and probed for analysis of total IGF-1R expression as described in the Experimental methods. (B) Quantification of band density was expressed as the ratio of the OD of phosphorylated IGF-1R to the OD of total IGF-1R. Each graph represents data from three separate experiments. Statistical analysis was performed using pair-wise Student's *t* test.

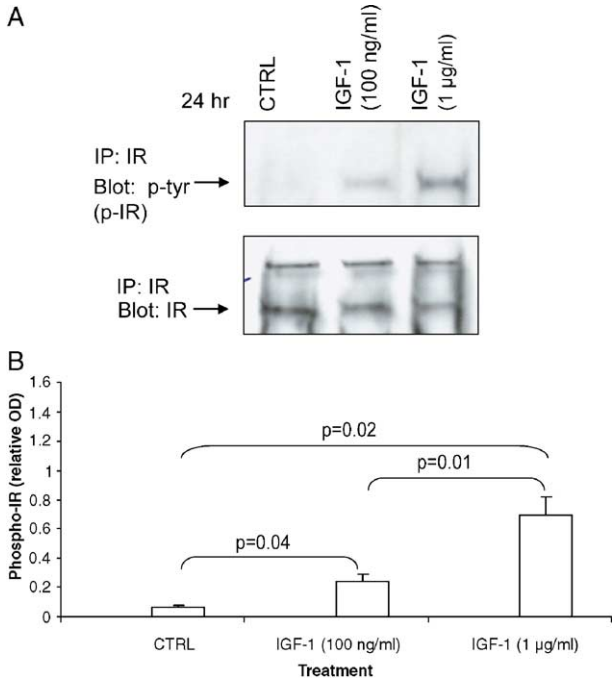

Fig. 9. Effect of IGF-1 on phosphorylation of IR. (A) OLP-II cells were treated with 100 ng/ml or 1 μg/ml IGF-1. The lysates were harvested, immunoprecipated (IP), and phosphorylation of IR was detected using a phospho-tyrosine antibody. Blots were stripped and probed for analysis of total IR expression as described in the Experimental methods. (B) Quantification of band density was expressed as the ratio of the OD of phosphorylated IR to the OD of total IR. Each graph represents data from three separate experiments. Statistical analysis was performed using pair-wise Student's *t* test.

are negative regulators of Akt (Maira et al., 2001; Stambolic et al., 1998). Thus, due to the fact that Akt is constitutively expressed in OLP-II cells, our data could suggest that psychosine promotes dephosphorylation of Akt by activating negative regulators of this pathway.

Similarly, our data indicate that psychosine promoted the dephosphorylation of Erk1/2 (Fig. 4). A potential role for psychosine has been indicated in the inhibition of neurite outgrowth and MAPK activity in PC12 cells (rat neuronal cells) (Sakakura et al., 1996). In addition, Haq et al. (2003) showed that psychosine treatment upregulates the JNK/SAPK pathway and inhibits nuclear factor-kappa B activation, indicating that psychosine upregulates the MAPK stress signal transduction pathway and also inhibits the survival signals.

While the importance of IGF-1 in promoting survival of oligodendrocyte progenitor cells has been indicated by several in vivo and in vitro studies (Barres et al., 1992, 1993; Cui et al., 2005; Mason et al., 2000a; Ye et al., 2000), the mechanism of IGF-1 protection against psychosine-induced cell death has not previously been demonstrated. We observed that high IGF-1 concentrations significantly protected OLP-II cells from psychosine-mediated apoptosis as detected by the TUNEL assay. Relatively high concentrations of IGF-1 have been utilized in other studies to demonstrate different functions of IGF-1. For example, it was shown that IGF-1 protects Rat-1 fibroblasts from UV-induced apoptosis in a dose-dependent fashion, with maximum protection occurring around 1 μg/ml (Kulik et al., 1997). In addition, a recent study indicated that relatively high IGF-1 concentrations

(500–1000 ng/ml) were required to stimulate the differentiation of multipotent rat neural progenitor cells into oligodendrocytes (Hsieh et al., 2004). While previous studies have shown that psychosine toxicity against mature rat OL is blocked by 10 and 100 ng/ml IGF-1 (Cho et al., 1997), there are considerable differences in the test systems utilized. These differences include the properties of the cell lines used, the growth conditions, the source of IGF-1 utilized and the purity of psychosine used. Adult OL that have been in the quiescent and terminally differentiated stage for a long time are less susceptible to cytokines (Yu et al., 2000). As described herein, OLP-II cells proliferate continuously in response to the B104-conditioned medium, undergo maximum cell death in the absence of this medium and could not be induced to differentiate into mature OL in vitro (unpublished data).

We observed that IGF-1 prevented psychosine-mediated dephosphorylation of Akt and Erk1/2, which were reversed by the use of inhibitors of these pathways (Figs. 5,6). Although similar results were obtained for both pathways, the effect of IGF-1 was more significant on Akt compared to Erk1/2. This variance may derive from differences in the affinities and/or concentrations of the relevant IGF-1R substrates and possible cross-talk between the IGF-1R and IR. While inhibition of both pathways clearly blocked IGF-1 from suppressing the proteolytic cleavage of caspase-3 (Fig. 2), only PI3K/Akt was indispensable for IGF-1-mediated protection of OLP-II cells against psychosine-induced cytotoxicity as determined by the cell viability assay (Fig. 7C). These data could suggest that inhibition of the MAPK/Erk1/2 pathway can be compensated by PI3K/Akt signaling. Our results are similar to the reported effect of PI3K/Akt on neuronal cell survival in the CNS (Rodgers and Theibert, 2002) and to studies demonstrating that this pathway is crucial for survival of oligodendrocyte progenitors (Cui et al., 2005; Ebner et al., 2000; Ness and Wood, 2002; Vemuri and McMorris, 1996).

Our data show that the effect of IGF-1 on autophosphorylation of the IGF-1R was much stronger than the phosphorylation of the IR (Figs. 8,9), suggesting that the protective action of IGF-1 is primarily mediated by the IGF-1R. Prevention of apoptosis by an activated IGF-1R plays a major role in the survival of many cell types, including neurons and hemopoietic cells after interleukin-3 withdrawal (Baserga et al., 1997; Mason et al, 2003). Moreover, it has been documented that IGF-1-stimulated IGF-1R autophosphorylation results in enhanced activation of its downstream targets Akt and Erk1/2 (Dupont and Le Roith, 2001; Shan et al., 2003). Conversely, inhibition of IGF-1R autophosphorylation leads to suppression of downstream substrates in cultured cells (Blum et al., 2000, 2003; Wang et al., 2004). Thus, the ability to protect against apoptosis is directly related to the ability of IGF-1 to bind to the IGF-1R.

In conclusion, our data indicate that psychosine induces the dephosphorylation of Akt and Erk1/2, IGF-1 suppresses psychosine-mediated dephosphorylation of Akt and Erk1/2, PI3K/Akt is required for IGF-1-promoted cell survival against the toxic effect of psychosine, and IGF-1 protection is mediated by activation of the IGF-1R. However, a small effect on the IR cannot be ruled out. The results presented here could have important implications for a role for IGF-1 in OL survival during stressful conditions, including genetic defects, in vivo. At this time, the only treatment for GLD is hematopoietic stem cell transplantation which slows down the course of the disease in human patients as well as GALC-deficient mice (Escolar et al., 2005; Krivit et al., 1995; Suzuki and Suzuki, 1995). In the future, it might be possible to develop more effective therapies, including the use of IGF-1 in conjunction with transplantation and gene therapy, to reduce the apoptotic events that take place early in the developing brain of humans and animal models with GLD.

Experimental methods

Materials

Cell culture medium (DMEM), trypsin and all other supplements were purchased from Invitrogen (Carlsbad, CA), Fischer Scientific (Pittsburgh, PA) or Sigma (St. Louis, CA). The oligodendrocyte progenitor cell line (OLP-II) was provided by Dr. Constantino-Ceccarini (Siena, Italy). Psychosine and insulin were purchased from Sigma. Recombinant human IGF-1 was generously provided by Cephalon, Inc. (West Chester, PA). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide thiazol blue (MTT) in vitro toxicity kit was purchased from Sigma. The in situ cell death detection kit-fluorescein was purchased from Roche Molecular Biochemicals (Indianapolis, IN). Antibodies to cleaved caspase-3, phospho-Akt, total Akt, phospho-Erk1/2, total Erk1/2, phospho-tyrosine, actin and conjugated secondary antibodies were purchased from Cell Signaling Technology (Beverly, MA). Antibodies to IGF-1Rβ and IRβ were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). LY-294002 and PD-98059 were purchased from Cell Signaling Technology. The enhanced chemiluminescence (ECL) detection kit was purchased from Amersham Pharmacia Biotech (Piscataway, NJ).

Cell culture and treatment conditions

The procedure for the isolation of OLP-II cells has been described in Luddi et al. (2001). Briefly, primary cultures of OL were prepared from pooled cerebra of normal C57BL6J mice. These cells undergo spontaneous transformation with time and were characterized as OL progenitors because they expressed A2B5 (a cell marker for OL progenitor). They underwent maximum cell death in the absence of B104-conditioned medium and other mitogens and could not be induced to differentiate into O4-positive OL (cell marker for mature OL) in vitro using several different growth conditions. However, these cells differentiated in vivo and expressed O4 (personal communication, Dr. Costantino-Ceccarini). OLP-II cells were cultured in polyornithine-coated plates with the complete nutrient media [DMEM medium supplemented with 0.29 g/l L-glutamine, 1.5% non-essential amino acids, 6 g/l glucose supplement, 50 μg/ml gentamicin, 30% B104-conditioned medium, 2% B-27 supplement, 5 μg/ml insulin, 15 nM thyroid hormone (T3), 10 ng/ml epidermal growth factor (EGF) and 10 ng/ml fibroblast growth factor (FGF)]. The media was replaced on alternate days. Cells were incubated in treatment media (complete media excluding insulin, EGF, FGF and T3) for 24 h prior to the addition of the various reagents. Psychosine was dissolved in ethanol at the concentration of 10 mM and was further diluted in treatment media to the indicated concentrations.

Live/dead assay and in situ end labeling

The in situ DNA strand breaks were detected using the in situ death detection kit-fluorescein (Roche Molecular Biochemicals) as per manufacturer's instructions. OLP-II cells were treated as

follows: 50 μM psychosine in the absence or presence of 100 ng/ml IGF-1 for 0, 2, 5, 10 and 24 h, or 50 μM psychosine in the absence or presence of 100, 200 and 400 ng/ml, or 1 μg/ml IGF-1 for 16 h, or 50 μM psychosine in the absence or presence of 100, 400 ng/ml or 1 μg/ml IGF-1 for 24 h. Cells were then spun onto slides using a cytospin centrifuge, fixed with 4.0% paraformaldehyde in 0.1 M phosphate buffer saline (PBS) (pH 7.4) for 15 min at room temperature and washed three times with PBS for 5 min. Cells were then permeabilized with 0.1% Triton X-100, 0.1% sodium citrate, labeled with dUTP nick-end labeling (TUNEL) reaction mixture and incubated in a humidified chamber for 60 min at 37°C in the dark. After rinsing three times with PBS, the cells were mounted in Vectashield (Vector Laboratories, Burlingame, CA) with 4′,6-diamidino-2-phenylindole (DAPI) counterstain and visualized using an Olympus BX51 and Leica microscope with FITC and DAPI filters (Chroma Technology Corp., Brattleboro, VT). The digital images were captured with a Leica camera and compiled with IPLabs software (Scanalytics Inc., Fairfax, VA). The compiled images were processed with Adobe Photoshop 6.0 software (Adobe Systems Inc., San Jose, CA).

MTT assay of cell viability

OLP-II cells were either untreated, treated with 1 μg/ml IGF-1 or treated with 1 μg/ml IGF-1 and 50 μM psychosine in the presence or absence of 30 μM LY-294002 or 50 μM PD-98059 for 24 h, and the viability of cells was evaluated by the MTT assay using an in vitro toxicity kit (Sigma). Mitochondrial dehydrogenase activity assayed by cleavage of MTT was used to determine cell viability. The reaction detects only living cells and is based on cleavage of the tetrazolium ring by active mitochondria, producing a visible dark blue formazan product. After the treatments, the cells were incubated with the MTT reagent at 37°C for an additional 3 h to determine cell viability. After the incubation with the lysis buffer, the background absorbance measured at 690 nm was subtracted from the absorbance measured at 570 nm.

Western blotting

For Western blot analysis, protein from untreated and treated cultured cells was extracted using the RIPA lysis buffer (50 mM Tris–HCl, 50 mM NaCl, 0.25% sodium deoxycholate, 1 mM EGTA, 1% Igepal) (Sigma), containing protease inhibitors (20 μg/PMSF, 2.5 mM sodium-orthovanadate, 10 mM NaF, and 10 μg/μl Aprotinin) (Sigma) for 10–15 min on ice. The concentration of protein was determined according to the method of Lowry et al. (1951). The appropriate protein concentrations were denatured by boiling in Laemmli sample buffer for 5 min, cooled and loaded onto a 10% Tris-gradient gel (Bio-Rad, Hercules, CA). Proteins were then electrotransferred to either a PVDF (Millipore, Bedford, MA) or nitrocellulose (Millipore) transfer membranes. Membrane blocking and incubation with secondary antibodies were carried out in 5% milk TBS–0.5% Tween 20 (TTBS). Membrane incubation with primary antibodies was carried out in 5% milk or bovine albumin (BSA) (Sigma) in TTBS. Membrane blocking and reaction with secondary antibodies were performed for 1 h at room temperature, and primary incubations were done overnight at 4°C. Blots were washed three times for 5 min each time with TTBS after each incubation period. After reaction with horseradish-peroxidase (HRP)-conjugated secondary antibodies, the proteins were detected by enhanced chemiluminescence using the ECL detection kit. After detection of a particular protein, the membrane was stripped as per ECL manual instructions, and immunoblotting was followed with different antibodies. Data were analyzed using a Molecular Dynamics densitometer and quantified by ImageQuant 5.1 software. Phosphorylation data were represented as a ratio of the optical density (OD) of phosphorylated protein to the OD of total protein.

Immunoprecipitation of IGF-1R and IR

Confluent cells in 100-mm plates were serum/trophic factor-starved overnight and then incubated either without or with 100 ng/ml or 1 μg/ml IGF-1 at 37°C for 24 h. Cleared cell lysates were prepared as described above. For immunoprecipitation, 500 μg of protein was incubated with either 2 μg anti-IGF-1Rβ antibody or 2 μg anti-IRβ antibody at 4°C for 2 h. Then, 20 μl protein A agarose beads (Santa Cruz Biotechnology) was added, and cells were incubated at 4°C overnight on a rocking platform. After immunocomplexes were collected by centrifugation and washed four times with the RIPA buffer, the final products were boiled in Laemmli sample buffer for 5 min and separated on a 10% Tris-gradient gel (Bio-Rad). The proteins were electrophoretically transferred to a nitrocellulose membrane. After blocking with 5% non-fat milk in TTBS, membranes were probed with an antibody against phospho-tyrosine for phosphorylation studies of the IGF-1R and IR. Blots were then stripped and incubated with either anti-IGF-1Rβ or anti-IRβ antibodies to detect total levels of the receptors. Anti-phospho-tyrosine antibody was detected by a horseradish-peroxidase-conjugated anti-mouse antibody. Anti-IGF-1Rβ and anti-IRβ antibodies were detected by a horseradish-peroxidase-conjugated anti-rabbit antibody. Detection was carried out with the ECL detection kit.

Statistics

The data were expressed as mean ± SD based on data derived from three independent experiments. The intensity of bands from Western blots was scanned with densitometry and digitally analyzed. The statistical significance was tested by F test two-sample for variance followed by Student's t test. A P value below 0.05 was considered statistically significant.

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References

Barres, B.A., Hart, I.K., Coles, H.S., Burne, J.F., Voyvodic, J.T., Richardson, W.D., Raff, M.C., 1992. Cell death and control of cell survival in the oligodendrocyte lineage. Cell 70, 31–46.

Barres, B.A., Schmid, R., Sendnter, M., Raff, M.C., 1993. Multiple extracellular signals are required for long-term oligodendrocyte survival. Development 188, 283–295.

Baserga, R., 2000. The contradictions of the insulin-like growth factor 1 receptor. Oncogene 19, 5574–5581.

Baserga, R., Hongo, A., Rubini, M., Prisco, M., Valentinis, B., 1997. The IGF-1 receptor in cell growth, transformation and apoptosis. Biochim. Biophys. Acta 1332, 105–126.

Blum, G., Gazit, A., Levitzki, A., 2000. Substrate competitive inhibitors of IGF-1 receptor kinase. Biochemistry 39, 15705–15712.

Blum, G., Gazit, A., Levitzki, A., 2003. Development of new insulin-like growth factor-1 receptor kinase inhibitors using catechol mimics. J. Biol. Chem. 278, 40442–40454.

Campana, W.M., Darin, S.J., O'Brien, J.S., 1999. Phosphatidyl 3-kinase and Akt protein kinase mediate IGF-I- and prosaptide-induced survival in Schwann cells. J. Neurosci. Res. 57, 332–341.

Cho, K.H., Kim, M.W., Kim, S.U., 1997. Tissue culture model of Krabbe's disease: psychosine cytotoxicity in rat oligodendrocyte cultures. Dev. Neurosci. 19, 321–327.

Cui, Q.L., Zheng, W.H., Quirion, R., Almazan, G., 2005. Inhibition of Src-like kinases reveals Akt-dependent and -independent pathways in insulin-like growth factor-1-mediated oligodendrocyte progenitor survival. J. Biol. Chem. 280, 8918–8928.

Datta, S.R., Brunet, A., Greenberg, M.E., 1999. Cellular survival: a play in three Akts. Genes Dev. 13, 2905–2927.

Dubois-Dalcq, M., Murray, K., 2000. Why are growth factors important in oligodendrocyte physiology. Pathol. Biol. 48, 80–86.

Dudek, H., Datta, S.R., Franke, T.F., Birnbaum, M.J., Yao, R., Cooper, G.M., Segal, R.A., Kaplan, D.R., Greenber, M.E., 1997. Regulation of neuronal survival by the serine–threonine protein kinase Akt. Science 275, 661–665.

Dupont, J., Le Roith, D., 2001. Insulin-like growth factor 1 and oestradiol promote cell proliferation of MCF-7 breast cancer cells: new insights into their synergistic effects. J. Clin. Pathol. Mol. Pathol. 54, 149–154.

Ebner, S., Dunbar, M., McKinnon, R.D., 2000. Distinct roles for PI3K in proliferation and survival of oligodendrocyte progenitor cells. J. Neurosci. Res. 62, 336–345.

Escolar, M.L., Poe, M.D., Provenzale, J.M., Richards, K.C., Allison, J., Wood, S., Wenger, D.A., Pietryga, D., Wall, D., Champagne, M., Morse, R., Krivit, W., Kurtzberg, J., 2005. Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease. N. Engl. J. Med. 352, 2069–2081.

Flores, A., Mallon, B.S., Matsui, T., Mallon, B.S., Matsui, T., Ogawa, W., Rosenzweig, A., Okamoto, T., Macklin, W.B., 2000. Akt-mediated survival of oligodendrocytes induced by neuregulin. J. Neurosci. 20, 7622–7630.

Hannun, Y.A., Bell, R.M., 1987. Lysosphingolipids inhibit protein kinase C: implication for the sphingolipidoses. Science 235, 670–674.

Haq, E., Giri, S., Singh, I., Singh, A.K., 2003. Molecular mechanism of psychosine-induced cell death in human oligodendrocyte cell line. J. Neurochem. 86, 1428–1440.

Hideki, I., Suzuki, K., 1984. Progressive accumulation of toxic metabolite in a genetic leukodystrophy. Science 224, 753–755.

Hsieh, J., Aimone, J.B., Kasper, B.K., Kuwabara, T., Nakashima, K., Gage, F.H., 2004. IGF-1 instructs multipotent adult neural progenitor cells to become oligodendrocytes. J. Cell Biol. 164, 111–122.

Jatana, M., Giri, S., Singh, A.K., 2002. Apoptotic positive cells in Krabbe brain and induction of apoptosis in rat C6 glial cells by psychosine. Neurosci. Lett. 330, 183–187.

Komoly, S., Hudson, L.D., Webster, H.D., Bondy, C.A., 1992. Insulin-like growth factor 1 gene expression is induced in astrocytes during experimental demyelination. Proc. Natl. Acad. Sci. U. S. A. 89, 1894–1898.

Kulik, G., Weber, M.J., 1998. Akt-dependent and -independent survival signaling pathways utilized by insulin-like growth factor I. Mol. Cell. Biol. 18, 6711–6718.

Kulik, G., Klippel, A., Weber, M.J., 1997. Antiapoptotic signaling by the insulin-like growth factor 1 receptor, phosphatidylinositol 3-kinase, and Akt. Mol. Cell. Biol. 17, 1595–1606.

Krivit, W., Sung, J.H., Shapiro, E.G., Lockman, L.A., 1995. Microglia: the effector cell for reconstitution of the central nervous system following bone marrow transplantation for lysosomal and peroxisomal storage diseases. Cell Transplant 4, 385–392.

LeVine, S., Brown, M.A., 1997. IL-6 and TNF-α expression of twitcher, quaking and normal mice. J. Neuroimmunol. 73, 47–56.

Liu, Q., Ning, W., Dantzer, R., Freund, G.G., Kelley, K.W., 1998. Activation of protein kinase C-zeta and phosphatidylinositol 3′-kinase and promotion of macrophage differentiation by insulin-like growth factor-I. J. Immunol. 160, 1393–1401.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Luddi, A., Volterrani, M., Strazza, M., Smorlesi, A., Rafi, M.A., Datto, J., Wenger, D.A., Constantino-Ceccarini, E., 2001. Retrovirus-mediated gene transfer and galactocerebrosidase uptake into twitcher glial cells results in appropriate localization and phenotype correction. Neurobiology 8, 600–610.

Maira, S.M., Galetic, I., Brazil, D.P., Kaech, S., Ingley, E., Thelen, M., Hemmings, B., 2001. Carboxyl-terminal modulator protein (CTMP), a negative regulator of PKB/Akt and v-Akt at the plasma membrane. Science 294, 374–380.

Mason, J.L., Jones, J., Taniike, M., Morell, M., Suzuki, K., Matsushima, G., 2000a. Mature oligodendrocyte apoptosis precedes IGF-1 production and oligodendrocyte progenitor accumulation and differentiation during demyelination/remyelination. J. Neurosci. Res. 61, 251–262.

Mason, J.L., Ye, P., Suzuki, K., D'Ercole, A.J., Matsushima, G., 2000b. Insulin-like growth factor-1 inhibits mature oligodendrocyte apoptosis during primary demyelination. J. Neurosci. 20, 5703–5709.

Mason, J.L., Xuan, S., Dragatsis, J., Efstratiadis, A., Goldman, J.E., 2003. Insulin-like growth factor (IGF) signaling through type 1 IGF receptor plays an important role in remyelination. J. Neurosci. 23, 7710–7718.

Mitsuo, K., Kobayashi, T., Shinnoh, N., Goto, I., 1989. Biosynthesis of galactosylsphingosine (psychosine) in the twitcher mouse. J. Chem. Res. 14, 899–903.

Miyatake, T., Suzuki, K., 1972. Globoid cell leukodystrophy: additional deficiency of psychosine galactosidase. Biochem. Biophys. Res. Commun. 48, 538–543.

Mozell, R.L., McMorris, F.A., 1988. Insulin-like growth factor I stimulates regeneration of oligodendrocytes in vitro. Ann. N. Y. Acad. Sci. 540, 430–432.

Myers Jr., M.G., Grammer, T.C., Wang, L.M., Sun, X.J., Pierce, J.H., Blenis, J., White, M.F., 1993. Insulin receptor subtrate-1 mediates phophatidylinositol 3′-kinase and $p70^{S6K}$ signaling during insulin, insulin-like growth factor-1, and interleukin-4 stimulation. J. Biol. Chem. 269, 28783–28789.

Ness, J.K., Wood, T.L., 2002. Insulin-like growth factor I, but not neurotrophin-3, sustains Akt activation and provides long-term protection of immature oligodendrocytes from glutamate-mediated apoptosis. Mol. Cell. Neurosci. 20, 476–488.

Parrizas, M., Saltiel, A.R., LeRoith, D., 1997. Insulin-like growth factor I inhibits apoptosis using the phosphatidylinositol 3′-kinase and mitogen-activated protein kinase pathways. J. Biol. Chem. 272, 154–161.

Peruzzi, F., Prisco, M., Dews, M., Salomoni, P., Grassilli, E., Romano, G., Calabretta, B., Baserga, R., 1999. Multiple signaling pathways of the insulin-like growth factor-1 receptor in protection from apoptosis. Mol. Cell. Biol. 19, 7203–7215.

Prisco, M., Romano, G., Peruzzi, F., Baserga, R., 1999. Insulin and IGF-1 receptors signaling in protection from apoptosis. Horm. Metab. Res. 31, 80–89.

Rodgers, E.E., Theibert, A.B., 2002. Functions of PI3-kinase in development of the nervous system. Int. J. Dev. Neurosci. 20, 187–197.

Sakakura, C., Igarashi, Y., Anand, J.K., 1996. Plasmalopsychosine of human brain mimics the effects of nerve growth factor by activating its receptor kinase and mitogen-activated protein kinase in PC12 cells. J. Biol. Chem. 271, 946–952.

Seger, R., Krebs, E.G., 1995. The MAPK signaling cascade. FASEB J. 9, 726–735.

Sell, C., Baserga, R., Rubin, R., 1995. Insulin-like growth factor-I (IGF-I) and the IGF-I receptor prevent etoposide-induced apoptosis. Cancer Res. 55, 303–306.

Shan, Y., Yang, T.-L., Mestril, R., Wang, P.H., 2003. Hsp10 and Hsp60 suppress ubiquitination of insulin-like growth factor-1 receptor and augment insulin-like growth factor-1 receptor signaling in cardiac muscle. J. Biol. Chem. 278, 45492–45498.

Singleton, R.J., Dixit, V., Feldman, E.L., 1996. Type I insulin-like growth factor receptor activation regulates apoptotic proteins. J. Biol. Chem. 271, 31791–31794.

Stambolic, V., Suzuki, A., de la Pompa, J.L., Brothers, G.M., Mirtsos, C., Sasaki, T., Ruland, J., Penninger, J.M., Siderovski, D.P., Mak, T.W., 1998. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell 95, 29–39.

Sueyoshi, N., Maehara, T., Ito, M., 2001. Apoptosis of Neuro2a cells induced by lysosphingolipids with naturally occurring stereochemical configuration. J. Lipid Res. 42, 1197–1202.

Sugama, S., Kim, S.U., Ida, H., Eto, Y., 1990. Psychosine cytotoxicity in rat neural cell cultures and protection by phorbol ester and dimethyl sulfoxide. Pediat. Res. 28, 473–476.

Suzuki, K., 1998. Twenty five years of the "Psychosine Hypothesis": a personal perspective of its history and present status. Neurochem. Res. 23, 251–259.

Suzuki, K., Suzuki, Y., 1970. Globoid cell leukodystrophy (Krabbe's disease): deficiency of galactocerebroside β-galactosidase. Proc. Natl. Acad. Sci. U. S. A. 66, 302–309.

Suzuki, K., Suzuki, K., 1995. The twitcher mouse: a model for Krabbe disease and for experimental therapies. Brain Pathol. 5, 249–258.

Svennerholm, L., Vanier, M.-T., Mansson, J.E., 1980. Krabbe disease: a galactosylsphingosine (psychosine) lipidosis. J. Lipid Res. 21, 53–58.

Tanaka, K., Webster, H.D., 1993. Effects of psychosine (galactosylsphingosine) on the survival and the fine structure of cultured Schwann cells. J. Neuropathol. Exp. Neurol. 52, 490–498.

Taniike, M., Mohri, I., Eguchi, N., Irikura, D., Urade, Y., Okada, S., Suzuki, K., 1999. An apoptotic depletion of oligodendrocytes in the twitcher, a murine model of globoid cell leukodystrophy. J. Neuropathol. Exp. Neurol. 58, 644–653.

Tohyama, J., Matsuda, J., Suzuki, K., 2001. Psychosine as potent an inducer of cell death as C6-Ceramide in cultured fibroblasts and in MOCH-1 cells. Neurochem. Res. 26, 667–671.

Vanier, M., Svennerholm, L., 1976. Chemical pathway of Krabbe disease: the occurrence of psychosine and other neural sphingoglycolipids. Adv. Exp. Med. Biol. 68, 115–126.

Vemuri, G.S., McMorris, A.F., 1996. Oligodendrocytes and their precursors require phosphatidylinositol 3-kinase signaling for survival. Dev. 122, 2529–2537.

Wang, Y., Malkowski, M., Hailey, J., Turek-Etienne, T., Tripodi, T., Pachter, J.A., 2004. Screening for small molecule inhibitors of insulin-like growth factor-1 receptor (IGF-1R) kinase: comparison of homogeneous time-resolved fluorescence and 33P-ATP plate assay formats. J. Exp. Ther. Oncol. 4, 111–119.

Wenger, D.A., Suzuki, K., Suzuki, Y., Suzuki, K., 2001. Galactosylceramide lipidosis. Globoid cell leukodystrophy (Krabbe disease). In: Scriver, C.R., Beaudet, A.L., Valle, D., Sly, W.S. (Eds.), The Metabolic and Molecular Bases of Inherited Disease, 8th ed. McGraw-Hill, New York, pp. 3669–3694.

Woodgett, J.R., Avruch, J., Kyriakis, J., 1996. The stress activated protein kinase pathway. Cancer Surv. 27, 127–138.

Xia, Z., Dickens, M., Raingeaud, J., Davis, R.J., Davis, R.J., Greenberg, M.E., 1995. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 270, 1326–1331.

Yao, D.L., Liu, W., Hudson, L.D., Webster, H.D., 1995. Insulin-like growth factor treatment reduces demyelination and up-regulates gene expression of myelin-related proteins in experimental autoimmune encephalomyelitis. Proc. Natl. Acad. Sci. U. S. A. 13, 6190–6194.

Ye, P., Lee, K.H., D'Ercole, A.J., 2000. Insulin-like growth factor-I (IGF-I) protects myelination from undernutritional insult: studies of transgenic mice overexpressing IGF-I in brain. J. Neurosci. Res. 62, 700–708.

Ye, P., Li, L., Richards, R.G., DiAugustine, R.P., D'Ercole, A.J., 2002. Myelination is altered in insulin-like growth factor-1 null mutant mice. J. Neurosci. 22, 6041–6051.

Yu, C., Takeda, M., Soliven, B., 2000. Regulation of cell cycle proteins by TNF-alpha and TGF-beta in cells of oligodendroglial lineage. J. Neuroimmunol. 108, 2–10.

Zaka, M., Wenger, D.A., 2004. Psychosine-induced apoptosis in a mouse oligodendrocyte progenitor cell line is mediated by caspase activation. Neurosci. Lett. 358, 205–209.

Zheng, W.H., Kar, S., Dore, S., Quirion, R., 2000. Insulin-like growth factor-I (IGF-I): a neuroprotective trophic factor acting via the Akt kinase pathway. J. Transm., Suppl. 60, 261–272.

Zumkeller, W., 1997. The effect of insulin-like growth factors on brain myelination and their potential therapeutic application in myelination disorders. Eur. J. Paediatr. Neurol. 4, 91–101.