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Interleukin-1β impairs brain derived neurotrophic factor-induced signal transduction

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Abstract

The expression of IL-1 is elevated in the CNS in diverse neurodegenerative disorders, including Alzheimer's disease. The hypothesis was tested that IL-1β renders neurons vulnerable to degeneration by interfering with BDNF-induced neuroprotection. In trophic support-deprived neurons, IL-1β compromised the PI3-K/Akt pathway-mediated protection by BDNF and suppressed Akt activation. The effect was specific as in addition to Akt, the activation of MAPK/ERK, but not PLCγ, was decreased. Activation of CREB, a target of these signaling pathways, was severely depressed by IL-1β. As the cytokine did not influence TrkB receptor and PLCγ activation, IL-1β might have interfered with BDNF signaling at the docking step conveying activation to the PI3-K/Akt and Ras/MAPK pathways. Indeed, IL-1β suppressed the activation of the respective scaffolding proteins IRS-1 and Shc; this effect might involve ceramide generation. IL-1-induced interference with BDNF neuroprotection and signal transduction was corrected, in part, by ceramide production inhibitors and mimicked by the cell-permeable C2-ceramide. These results suggest that IL-1β places neurons at risk by interfering with BDNF signaling involving a ceramide-associated mechanism.

Keywords

IL-1β; BDNF; signal transduction; cortical neurons

Interleukin-1 (IL-1) is a pluripotent proinflammatory cytokine that is a potent activator of host defense responses to infection and injury both in the periphery and the CNS [59]. However, IL-1 can also exacerbate damage in the CNS resulting from acute insults, such as

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cerebral ischemia and trauma, and circumstantial evidence is consistent with a similar role in chronic neurological diseases, such as multiple sclerosis, Parkinson's disease and Alzheimer's disease (AD) [48]. Furthermore, recent genetic studies highlighted the relevance of IL-1 in AD pathogenesis, showing that specific polymorphisms in the IL-1 gene cluster are associated with greatly increased risk for AD, especially for the earlier onset of the disease [23, 50]. The view that inflammatory processes play an important role in pathogenesis has been supported by epidemiological studies, showing that certain anti-inflammatory drugs result in a slower progression of the disease [2, 8, 43, 77] and in transgenic AD models decrease the number of dystrophic neurites, activated microglia and IL-1 expression [35], although such drugs also modulate the production of the amyloidogenic $A\beta_{1-42}$ [18, 76]. The view that neuroinflammation contributes directly to AD pathogenesis is consistent with recent observations showing that selective inhibition of proinflammatory cytokine production suppresses in the hippocampus the chronic Aβ infusion-induced decrease in synaptic markers and attenuates deficit in a hippocampus-linked memory task [56].

In the healthy brain, the concentration of IL-1 is very low, but IL-1 is rapidly induced in response to injury. The increase in IL-1 after traumatic brain injury is followed by neuronal loss. IL-1 is linked to the loss because antibodies to IL-1 attenuate neuronal loss [36] and in IL-1 deficient mice neuronal loss and infarct volumes are reduced [6]. The early primary source of IL-1 is microglia, but later the cytokine is also produced by astrocytes and expression in oligodendrocytes and neurons has also been detected [59]. There is general consensus that after insults, IL-1 has adverse effects in the brain in vivo, but observations on cultured neurons are less clear. It seems that IL-1β is neurotoxic only at high concentrations and after relatively long exposure [3]. Several mechanism by which IL-1 may induce neuronal death have been proposed including the involvement of glia [59] and the regulation of NMDA receptor [39, 73].

Neurotrophins, such as BDNF, are important modulators for neuronal survival and functions. BDNF, a major trophic factor in the CNS, is critical for the development and survival of certain neuronal populations. In addition to protecting neurons from damage caused by insults of various kinds, BDNF also modulates synaptic transmission and plays a role in synaptic plasticity, including LTP and certain forms of learning and memory processes in animal models [7, 21, 28, 44, 52, 80]. Accordingly, conditions that may interfere with BDNF signaling may affect a variety of downstream neuronal functions and may contribute to neurodegenerative diseases, including Alzheimer's disease (AD) [13]. In AD, BDNF processing and levels are reduced even in pre-clinical stages [53]. Patients with Down syndrome (DS) almost invariably develop Alzheimer type of neuropathology and in an animal model of DS in trisomy 16 (Ts16) mouse BDNF-dependent survival of cultured hippocampal neurons is compromised, because of the dysregulation of the expression of BDNF receptor isoforms [17].

In the present study, we tested the hypothesis that IL-1β acting directly on neurons can compromise cell survival by interfering with the neuroprotective effect of BDNF. The observations have provided support for this proposal.

2. Materials and Methods

2.1. Cell culture

Primary cultures of dissociated cerebral cortical neurons were prepared from the brains of embryonic day 18 (E 18) rats as described previously [54]. Cell plated at 2.5×10^5 cells/cm^2 were cultured in poly-L-lysine coated six well plates (for western blot analysis) or on 24 well plates (for immunocytochemistry and assays for cell viability) and maintained in serum-free optimal Dulbecco's modified Eagles medium (DMEM) supplemented with B-27 components (Invitrogen, Carlsbad, CA). Cultures were maintained for 5-7 days before treatments. Neuronal survival was determined by trypan blue exclusion [54] or using the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [30]. The purity of the preparations was checked occasionally using double staining with MAP-2 (for neurons) and GFAP (for astrocytes). The cultures contained more than 95% of neurons (96.2±0.50, n=6).

2.2. Experimental treatment

Cells were treated with IL-1β for 2 h before the addition of BDNF (Peprotech, Rocky Hill, NJ). Rat recombinant IL-1β (Sigma, St. Louis, MO) was dissolved in DMEM and used after one freeze-thaw cycle. Cells were treated with tetrodoxin (TTX, 1 μM) and amino-5-phosphonovaleric acid (100 μM), respectively 2 h and 30 min before exposure to IL-1β to reduce endogenous synaptic activity and to block glutamate release induced by BDNF [34, 51] in order to reduce the basal level of activated signaling molecules [12]. The IL-1 receptor antagonist IL-1ra (R&D Systems, Minneapolis, MN) was used at 5 μg/ml, and when applied, cells were preincubated for 30 min before the addition of IL-1β. Exposure to BDNF or vehicle was usually for 10 min, unless otherwise mentioned, and then cells were processed for either biochemical or immunocytochemical analysis. Pilot experiments on the time course of the effect of IL-1β on BDNF signaling indicated that significant interference is detectable after 2 h exposure that was the preincubation time routinely used in these studies. The concentration of BDNF was 10ng/ml, unless mentioned otherwise.

2.3. Western blot analysis

Cells were lysed in a buffer containing 1% Triton X-100, 150 mM NaCl, 5 mM EDTA, 10 mM NaF, 2 mM Na_3VO_4, 1 mM phenylmethylsulponyl fluoride, 0.7 μg/ml pepstatin, 1 μg/ml leupeptin and 20 mM Tris-HCl, pH 7.5. Lysates were centrifuged at 12,000 g at 4°C for 30 min, and then the protein concentration was determined by the BCA protein assay (Pierce, Rockford, IL). Samples containing 20 μg of protein were electrophoresed on SDS-polyacrylamide gel (10% acrylamide gel). Proteins were then electrotransferred to PVDF membranes, blocked with 5% nonfat milk in Tris-buffered saline (TBS), and probed with various antibodies. The immunoreactivity was revealed using horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit IgG or goat anti-mouse IgG) (Vector Laboratories, Burlingame, CA) and enhanced chemiluminescence (ECL, Amersham Pharmacia, Piscataway, NJ) according to the recommended conditions. Immunoreactivity was quantified using densitometric analysis.

The following antibodies were used for the biochemical studies: from Upstate (Charlottesville, VA), phosphorylated CREB (P-CREB) (detects CREB phosphorylated at Ser-133, 1:2,000), total CREB (T-CREB, 1:2,000), Shc (the antibody recognizes all isoforms; 1:1000), total Trk (T-Trk, 1:1000), TrkB (1:1000); from Cell Signaling (Beverly, MA), phosphorylated MAPK/ERK (P-MAPK) (detects p44/42 MAPK phosphorylated at Thr-202 and Tyr-204, 1:2000), total-MAPK (T-MAPK, 1:2000), phosphorylated Akt (P-Akt) (detects Akt phosphorylated at Ser-473, 1:1000), total Akt (T-Akt, 1:1000), phosphorylated Trk (P-Trk) (detects Trk phosphorylated at Tyr490, 1:500).

Quantification of the data obtained from Western blots derived from cultures under the various experimental conditions was done relative to the estimates in the BDNF-treated cultures using Student's *t*-Test. In addition, we also analyzed data, when appropriate (e.g. the effect of IL-1 concentration on BDNF signaling), using a non-parametric, Friedman Analysis of Variance and obtained similar indication of significance.

2.4. Immunoprecipitation

Cells were lysed in 500 μl of immunoprecipitation buffer (1% Triton-X-100, 150 mM NaCl, 50 mM Tris pH 8.0, 0.2mM sodium ortho-vanadate, 0.2 mM phenylmethylsulfonyl fluoride, 1 mg/ml pepstatin, 1 mg/ml leupeptin, and 1 mg/ml antipain). Lysates were centrifuged at 10,000 g for 30 min, and protein concentration of the clarified lysates was determined using the Micro-BCA protein assay (Pierce, Rockford, IL). Proteins were immunoprecipitated with various antibodies at 4°C overnight. The immunoprecipitation was followed by addition of protein G-Sepharose and the samples were rotated at 4 °C for 1 h. The immune complexes were pelleted by centrifugation at 10,000 *g* at 4 °C for 1 min. The supernatant was decanted, and the pellet was washed with 1 ml immunoprecipitation buffer. The wash steps were repeated three times and finally the pellet was suspended in 60 μl SDS-sample buffer (62.5 mM Tris pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.01% bromphenol blue). Proteins of the suspended immunoprecipitate (30 μl) were separated on a 10% SDS-PAGE gel. The immunoprecipitates were analyzed by Western blotting with anti-phosphotyrosine 4G10 (Upstate, Charlottesville, VA), anti-p85 PI3-K, or PLCγ (Upstate, Charlottesville, VA) antibodies, as indicated.

2.5. Immunocytochemistry

For the detection of TrkB receptors, cultures at 5 DIV were fixed with 4% paraformaldehyde and permeabilized by incubation in PBS containing 0.3% Triton, blocked in blocking solution (5% goat serum and 0.3% Triton in PBS), and incubated with the anti-Trk antibody (Santa Cruz, CA; SC-12, 1:50) at 4°C overnight and then washed three times before incubation with biotinylated anti-rabbit antibodies The antibody recognizing Trk receptors was from Santa Cruz Biotech.. Cultures were incubated without shaking. After three washes, cultures were incubated in the presence of Streptavidin conjugated Alexa Fluor 568(1:200) (Molecular Probes) in PBS. Cells were then washed three times in PBS before being subjected to further processing. Similar to the procedure described for Trk receptor detection, cultures were incubated with monoclonal MAP-2 antibodies (1:500) (Sigma, St. Louis, MO) overnight, followed by exposure to anti-mouse antibodies conjugated with

Alexa Fluor 488. Finally, cells were washed three times in PBS and mounted in Fluoromount G.

The specificity of Trk receptor staining was examined by repeating the procedure using antibodies to TrkB preabsorbed at room temperature for 2 h with the peptide used to raise the Trk antibody, which is specific to the caboxyl terminal cytoplasmic domains of TrkB (SC-12P) (ratio of antibodies to peptides 1:5 by weight).

For the detection of activated CREB, cultures were fixed, washed with PBS, and exposed to blocking solution similar to the procedure described for Trk receptor detection. Cultures were then incubated overnight with an antibody that selectively recognized CREB phosphorylated at Ser-133 (Upstate, 1:500), followed by exposure to anti-rabbit antibodies conjugated with Alexa Fluor 568. Finally, cells were washed three times in PBS and mounted in Fluoromount G.

2.6. Deprivation from trophic support

It has previously been shown that BDNF can protect neurons from cell death induced by serum deprivation [27]. Our cultures were maintained in the B-27-containing serum-free medium that contains a great number of trophic ingredients providing comparable support for neuron survival as serum does, but preventing the proliferation of glial cells. We observed that deprivation from B-27, like that from serum, compromised neuronal survival, thus permitting the testing of the effect of BDNF and the influence of IL-1β on the survival of the deprived cells. In these studies, the B-27-containing medium was removed from cultures at 5 DIV. Cells were washed twice with DMEM and then incubated in DMEM for 36 h in the absence or presence of BDNF (10 ng/ml) ± IL-1β (50 ng/ml). Control cells were treated the same way, but were incubated in B-27-containing DMEM.

3. Results

3.1. IL-1β interferes with the BDNF rescue of nerve cells deprived from trophic support

One of the important functions of BDNF is to protect neurons from the harmful effect of certain types of insults. We evaluated the hypothesis that IL-1β exposure may influence the capacity of BDNF to protect neurons under conditions that compromise their survival. Cultured neurons die when deprived of their trophic support, such as serum or in our case the B-27 supplement (Fig. 1). In the absence of trophic support neurons degenerate progressively so that by 36 h about 60% of the cells are lost.

BDNF rescues the trophic support-deprived cells, restoring neuronal survival to control levels (Fig. 1A). In the presence of IL-1β, however, the ability of BDNF to rescue neurons was compromised in a dose dependent manner by up to 50%. IL-1 β alone had no positive or negative effect on survival. In our cultures comprising primarily neurons (95%), no neurotoxic effect could be detected after 24 h exposure, using IL-1β over a wide concentration range (cell survival at 500 ng/ml was 95 ± 4% of control). IL-1β (50 ng/ml) did not interfere with cell survival over prolonged incubation times (as a percentage of the control the survival was 99±2.7% at 24 h, 100 ± 1.9% at 48 h and 103 ± 1.5% at 72 h). Neither did we observe neuroprotection by IL-1β against cell loss induced by deprivation

from trophic support. The removal of the B-27 trophic medium reduced cell survival in DMEM with or without supplementation with 50 ng/ml IL-1β to similar levels by 36 h (33 ± 3.0% or 39 ± 7.3% of the estimates in the B-27 control cultures). Thus IL-1β interferes with cell survival indirectly by compromising the trophic influence of BDNF.

The pathway involved in neuroprotection is dependent on the nature of the insult [27]. Under our experimental conditions, BDNF-protection of the trophic support-deprived cells involves the activation of the PI3-K/Akt rather than the Ras/MAPK pathway that is, however, known to be critical for protection from other types of insults, such as DNA fragmentation [27] (Fig. 2). Inhibition of PI3-K by LY294002 prevented BDNF from promoting survival, but the blockade of MAPK activation using PD98059 had no significant effect. LY294002 or PD98059 did not reduce survival of cells cultured in the B-27-containing medium (data not shown). The involvement of the PI3-K pathway on the effect of BDNF on B27-deprived neurons is in agreement with previous observations using serum deprivation protocol [27]. We examined, therefore the influence of IL-1β on the BDNF-induced activation of Akt the major effector of this pathway. Treatment with IL-1β for 2 h suppressed the BDNF-evoked phosphorylation of Akt (Fig. 3A and B), which was also suppressed when the cytokine exposure was prolonged for 24 h (Fig. 3D). Two hours exposure to IL-1β alone had no significant effect on the level of activated Akt (Fig. 3C).

3.2. The effect of the cytokine on BDNF signaling is specific

The IL-1 family comprises in addition to the agonists IL-1α and IL-1β, IL-1ra that is a selective competitive IL-1 antagonist [59]. IL-1ra prevented the IL-1β -induced suppression of the activation of Akt by BDNF, thus providing evidence that the effect of the cytokine on BDNF signal transduction is specific (Fig. 3E and F).

3.3. IL-1β interference with BDNF signal transduction is pathway specific

BDNF activates not only the PI3-K/Akt, but also other major signaling pathways [28]. To establish whether IL-1β affects BDNF signaling in general, we examined the influence of the cytokine on the activation of the Ras/MAPK and the PLCγ/PKC pathways.

The cytokine interfered with the activation of MAPK/ERK, monitored by measuring the level of p42/p44 MAPK phosphorylated at Thr202 and Tyr-204 (Fig. 4A and B). Exposure to the cytokine alone for 2 h had no significant effect on the level of activated MAPK/ERK (Fig. 4C). On the other hand, IL-1β did not interfere with another of the major signal transduction pathways the PLCγ/PKC cascade activated by BDNF (Fig. 4D). BDNF-induced Tyr phosphorylated PLCγ levels were not significantly affected by IL-1β pretreatment (93.1 ± 5.6% of control, $n = 3$). Thus the suppression of BDNF signaling by IL-1β is pathway specific.

3.4. IL-1β interferes with BDNF-induced activation of the transcription factor CREB

One of the targets of activated MAPK/ERK is via Rsk the transcription factor CREB that is also activated via the PI3-K/Akt pathway. Under certain conditions, CREB-induced gene transcription has survival-promoting effects [4] and also plays a critical role in learning and memory processes in different species, from *Drosophila* to mammals [1, 62]. Both ERK and

CREB have been implicated in processes of neuronal plasticity [1, 62, 66, 68]. We examined, therefore, the effect of IL-1β on the BDNF-induced activation of CREB (Fig. 4E and F). The transcription promoting activity of CREB involves phosphorylation at the Ser-133 residue [46], and BDNF exposure is known to lead to the phosphorylation of this residue [e.g. 67, 70]. Exposure of cortical neurons to BDNF induced a great increase in the Ser-133-phosphorylated CREB content. This was drastically suppressed by treatment with IL-1β that reduced the BDNF-elicited activation of CREB by 70% (Fig. 4 E and F). Exposure to IL-1β alone for 2 h had no significant effect on the level of activated CREB (Fig. 4E).

3.5. IL-1β does not affect the activation of TrkB receptors, but interferes with BDNF signaling at the level of the docking proteins

Virtually all neurons in our culture express TrkB receptors (Fig. 5A) and are activated by BDNF, as indicated by the immunocytochemical detection of phosphorylated CREB (Fig. 5B). As the activation of the Ras/MAPK and PI3-K/Akt pathways depends on BDNF-induced autophosphorylation of TrkB, inhibition of the TrkB phosphorylation would provide a simple mechanism for the interference by the cytokine with BDNF signaling. However, the BDNF-induced tyrosine phosphorylation of the TrkB receptor was not suppressed by IL-1β (Fig. 5C). In terms of the tyrosine phosphorylated Trk receptor level in the BDNF-exposed cultures, the estimate in the IL-1β -pretreated cells was 98 ± 4.5%.

IL-1β may, therefore, interfere with BDNF signaling downstream from the activated receptor. Phosphorylation of Tyr residues of the Trk receptor provides docking sites for proteins mediating the signaling functions of the receptor. The Tyr residues critical for the activation of the Ras/MAPK and PI3-K/Akt pathways (Tyr484) are different from the residue involved in the activation of PLCγ (Tyr785) [42]. Further, PLCγ -whose phosphorylation was not affected by IL-1β (Fig. 4D) - is activated by direct binding to the phosphorylated Tyr785 of TrkB. On the other hand, the stimulation of the Ras/MAPK and PI3-K/Akt pathways requires binding of the scaffolding proteins, Shc and IRS respectively. The ensuing Tyr phosphorylation of these docking proteins provides binding sites for the regulatory subunit of PI3-K and the Grb/SOS complex, the activation of which propagates the signaling cascade. Our observations are consistent with the view that IL-1β interference with BDNF signaling might involve the docking proteins mediating the activation of the Ras/MAPK and PI3-K pathways.

It has been observed previously that the docking step is critically involved in the inhibition by $A\beta_{1-42}$ of BDNF signaling [70] and in the downregulation by proinflammatory cytokines of insulin- and IGF-1-induced signaling in various cell types including neurons [71, 81]. We examined, therefore, the effect of IL-1β on Tyr phosphorylation of the adaptor proteins linking the activated receptor with the downstream lipid and protein kinase cascades. The cytokine treatment impaired the BDNF-induced activation of the key docking proteins IRS-1 and Shc (Fig. 6). IL-1β reduced BDNF-evoked Tyr phopshorylation of IRS-1 (Fig. 6A and B) and the association of IRS-1 with the regulatory subunit of PI3-K (p85) (Fig. 6C and D). The cytokine also reduced BDNF-induced Tyr phosphorylation of all the Shc isoforms (Fig. 6E and F).

3.6. The inhibitory effect of IL-1β on BDNF-mediated survival and Akt activation involves the sphingomyelin/ ceramide pathway

Next we addressed the question of the nature of the intracellular mechanisms that mediate the inhibition by IL-1β of TrkB receptor activation of the docking proteins. One early event initiated by IL-1β stimulation in many cell types is the activation of the sphingomyelin/ ceramide pathway [25, 29, 41, 57]. Ceramide is one of the factors which is implicated in the development of insulin resistance, as it can lead to interference with insulin signaling by affecting the activation of the docking proteins [65, 81]. Thus by analogy to insulin resistance, ceramide may participate in the impairment of BDNF signaling. It has been reported that ceramide levels increase in the brain with age and many chronic diseases, including Alzheimer's disease [15]. Ceramide generation may occur via either de-novo synthesis or the hydrolysis of sphingomylin by acidic or neutral sphingomyelinases. We examined, therefore, the effect on IL-1-induced suppression of BDNF signaling of inhibitors selective to these ceramide-producing routes, using fumonisin B1 (FB1) or myriocin (ISP-1) to block the *de novo* pathway, glutathione (GSH) or GW4869 to inhibit neutral sphingomyelinase, and desipramine to inhibit acidic sphingomyelinase. Inhibitors of the *de novo* pathway and neutral sphingomyelinase significantly attenuated the IL-1β-impairment of BDNF signaling (Fig. 7). Desipramine (10 μM) an inhibitor of acidic sphingomyelinase did not affect IL-1β-induced decrease of BDNF-induced Akt activation (data not shown). Ceramide inhibitors alone had no effect on P-Akt or P-CREB levels (data not shown). Further, ISP-1 and GSH, but not desipramine, attenuated the IL-1β-elicited reduction of the neuroprotective effect of BDNF in cultures deprived from the B-27 trophic support (Fig. 8).

To examine further the potential role of ceramide on the IL-1β-induced suppression of neurotrophin signaling, cells were pretreated with an exogenous, cell-permeable ceramide analogue, C2-ceramide, for 2 h before they were challenged with BDNF (Fig. 9A and B). C2-ceramide mimicked the effect of IL-1β by reducing the neurotrophin-induced activation of Akt. The effect of C2-ceramide on BDNF-mediated neuroprotection was further investigated. C2-ceramide treatment significantly compromised the ability of BDNF to rescue neurons from trophic deprivation-induced cell death (Fig. 9C).

4. Discussion

Accumulating evidence suggests that proinflammatory cytokines play important roles in CNS injury and neurodegenerative diseases, including Alzheimer's disease (AD)[22, 37]. It is generally accepted that insults of various kinds can induce activation of astrocytes and microglial, which leads to increased production of proinflammatory cytokines, such as IL-1β and TNFα. In this study, we explored the possibility that IL-1β renders neurons vulnerable by interfering with neurotrophin signaling. We demonstrate that IL-1β reduces the neuroprotective capacity of BDNF in trophic support-deprived cells by impairing BDNF activation of the survival promoting PI3-K pathway. Our observations show that (1) IL-1β alone does not affect the viability of neurons in agreement with previous reports [69], (2) IL-1β interferes with neurotrophin signal transduction through effects on the PI3-K/Akt and Ras/MAPK pathways, (3) the cytokine suppression of signaling is localized to the scaffolding proteins IRS and Shc that convey activation from the Trk receptors to the PI3-

K/Akt and Ras/MAPK pathways, (4) IL-1β interference involves ceramide-mediated processes, and (5) the cytokine suppresses drastically the activation of downstream targets, such as the transcription factor CREB that is a point of convergence of signaling through the Ras/MAPK and PI3-K/Akt pathways.

Our results complement and extend previous work on possible mechanism for the interference by IL-1β with neuronal function and viability. While IL-1β can cause neuronal toxicity in vivo after CNS damage, the cytokine is not directly neurotoxic to neurons in culture, but exacerbates neuronal damage by other agents [58, 69]. For example, IL-1β increased excitotoxicity in vitro [39, 73] and in vivo [26]. IL-1β induced neurotoxicity in vitro has been recently shown to be mediated by glia and requires free radical release and caspase activation [69]. In an AD animal model, elevated IL-1 levels in the IL-1 receptor antagonist knock-out mice increased the susceptibility of neurons to Aβ-induced injury [14]. Our observations that IL-1β alone did not affect the viability of neurons, but compromised their BDNF-induced survival suggest, therefore, a subtle regulatory mechanism that may contribute to increased neuronal vulnerability resulting from inflammation in the brain.

We observed that IL-1 caused an approximately 50% decrease in the BDNF rescue of trophic support-deprived cells, while the suppression of the activation of certain key components of BDNF signaling cascades was less (20-40%). We speculate that this decrease is sufficient to reduce BDNF signaling below a neuroprotective threshold. The suggestion has some precedents. Thus Poser et al [55] observed that cAMP elevation decreases in cortical neurons BDNF-induced PI3-K/Akt signaling by 40-50%, while BDNF protection of serum-deprived cells is almost completely abolished. In Ts16 an approximately 30% reduction in TrkB phosphorylation leads to about a 50% increase in the death of trophic support-deprived neurons in the presence of BDNF, the suppression being comparable with that elicited by TrkB antibodies that completely blocked BDNF signaling [17].

The present observation that BDNF protection of B27-deprived cells is mediated by the PI3-K/Akt pathway is consistent with previous findings on serum-deprived cells [27]. IL-1 interfered with BDNF neuroprotection by affecting PI3-K/Akt signaling. However, IL-1 also suppressed signaling through the Ras/ERK pathway that is critical for neuroprotection under different experimental conditions, including exposure to toxic substances that cause DNA fragmentation [27] or consequent to ischemic insults [63].

The BDNF-induced activation of the transcription factor CREB was very severely depressed by IL-1. This may be related to the summation of the IL-1-induced decrease of BDNF signaling through various pathways, including PI3-K/Akt and Ras/ERK (e.g. [79]), which converge at this transcription factor. Under our experimental conditions, IL-1 alone had no effect on the basal level of P-CREB. This is consistent with the finding of Srinivasan et al. [64], who observed that IL-1β activation by CREB is transient, declining towards the basal level by 30 min. In our studies the IL-1 preincubation time was 2 h. Under specific conditions CREB can play a critical role in promoting the survival of various types of cells, including neurons [27, 31, 45, 74, 75]. Recently, Willaime-Morawek et al. have shown that CREB is not involved in the IGF-1-mediated attenuation of neuron loss induced by ceramide treatment [79]. However, in addition to their role in promoting neuron survival under

specific conditions, CREB and the Ras/MAPK pathway play essential roles in synaptic plasticity, including learning and memory processes [1, 62, 66]. Taken together, these findings are consistent with the view that IL-1β can compromise a broad range of BDNF-mediated functions, ranging from synaptic plasticity to neuronal survival through the regulation of multiple pathways.

The antiapoptotic effect of Akt may involve a great number of downstream effectors (e.g. [10]), including the inhibition of the function of FOXO transcription factors that upregulate key death genes, the activation of T-cell factor (TCF) via inactivation of GSK-3β, the activation of NF-kappa B, inactivation of p53, and the prevention of the release of cytochrome *c* from the mitochondria via Bag1-dependent phosphorylation of BAD [19]. One or more of these pathways may participate in the effect of IL-1β in compromising the Akt-mediated BDNF survival signal and it will be interesting to elucidate their relative contribution in future studies.

Our data demonstrated that IL-1β targets the scaffolding proteins that convey activation from the Trk receptors to the PI3-K/Akt and Ras/ERK pathways. We also observed that IL-1β interferes by the same mechanism with signaling of another neurotrophic factor NT-3 that under the experimental conditions exerted its action primarily through TrkC activation (manuscript in preparation). Scaffolding proteins are also critically involved in the regulation of insulin and IGF-1 signaling in both neurons and non-neural, peripheral tissues [20, 33, 71, 81]. In neurons the wide-ranging nature of regulation at the level of the docking proteins, such as IRS-1, is indicated by the observation that interference at the docking site is not restricted to the effect of IL-1β on neurotrophin signaling in cerebral cortical cells. In an earlier study Venters et al. [72] have shown that another proinflammatory cytokine, TNFα interferes with signaling of a member of another trophic factor IGF-1 at the level of IRS-2 in cerebellar granule cells.

Several potential intracellular signaling mechanisms may mediate the inhibition by IL-1β of TrkB receptor activation of the docking proteins. Previously, it has been reported that in peripheral tissues the second messenger ceramide, can trigger protein kinase cascades that suppress insulin-IGF-1 signaling at the docking step [65, 81]. IL-1β -induced ceramide signaling has also been demonstrated in neurons [16]. Warm-sensitive hypothalamic neurons are rapidly hyperpolarized by IL-1β. This is mediated by the activation of Src family protein tyrosine kinases, which is effected by neutral sphingomyelinase-generated ceramides. The activation of neutral sphingomyelinase involves the recruitment of the scaffolding protein MyD88 to the agonist-charged IL-1 receptor complex [60]. In the present study, we observed the involvement of ceramide in IL-1β-mediated suppression of BDNF signaling. The effect of the cytokine was blocked by inhibition of ceramide generation by both the de novo pathway and neutral sphingomyelinase, but not acidic sphingomyelinase. It is important to note that the level of ceramide increases in brain with age, injury and in certain chronic neurological diseases and, thus may be a candidate to contribute to the suppression of BDNF signaling after injury and chronic diseases. Further studies should address the question of the mechanism of ceramide-induced attenuation of IRS and Shc activation by neurotrophins. It is known that ceramides activate stress activated protein kinases through different routes including pathways, which involve either PKCζ [5] or Rac-1 [5, 9] and

TRAF-6 [11] depending on the tissue. These kinases impair signaling through the docking step (e.g. [81]) and are candidates for the IL-1 effect.

Previously we demonstrated that BDNF signal transduction and neuronal survival are also suppressed in the presence of the β-amyloid peptide ($A\beta_{1-42}$) [70]. Like IL-1β, Aβ impaired BDNF activation of the PI3-K/Akt and Ras/MAPK pathways and suppressed the activation of transcription factors, such as CREB and Elk-1, and transcription mediated by these factors. In addition, the regulatory mechanism elicited by Aβ involved the docking proteins IRS-1 and Shc. Exposure of cultured neurons to Aβ evokes an increase in ceramide levels [15]. Further, aggregated Aβ can directly activate neutral sphingomyelinase [24] and soluble Aβ oligomers activate both the neutral and the acidic sphingomyelinase [40]. Thus ceramide may be a common intracellular signal mediating both the action of IL-1β and Aβ on BDNF signaling.

The suppression of BDNF/neurotrophin signaling has a parallel in the concept of insulin resistance. It is well know that proinflammatory cytokines, such as IL-1β can compromise insulin/IGF-1 signaling in a variety of peripheral tissues [65, 78, 81], a condition referred to as “insulin resistance”. The mechanism involves a suppression of the activation of the docking proteins [81]. It has also been shown that the IGF-1-promoted survival of cerebellar granule cells is compromised by the proinflammmatory cytokine TNFα by suppressing the activation of the docking protein IGF-2 [72]. Thus like insulin resistance in peripheral tissues and a similar resistance to IGF-1 in cerebellar neurons there may be a mechanism of resistance to neurotrophins in the brain, predisposing neurons to dysfunction and placing them at increased risk for functional defects and degeneration. Insulin resistance progressing to type 2 diabetes is an age-related disorder. In age-related diseases of the nervous system, including AD, neuronal dysfunction may precede the onset of degeneration and development of pathology. For example, AD is characterized by a progressive decline in cognitive functions that occurs prior to the formation of plaques and neurodegenerative changes [32, 38, 47, 49]. Furthermore, synaptic dysfunction in both the AD brain and in animal models of AD are impacted before the accumulation of hallmark pathological lesions [for review 61], suggesting that non-degenerative mechanisms may contribute to cognitive decline. It is possible that cognitive decline is linked in part to mechanisms involving interference with neurotrophin/insulin/IGF-1 signaling via early development of inflammation and the accumulation of select assembly states of Aβ (e.g., oligomeric Aβ). Thus while insulin resistance is associated with inflammation and increased risk for type II diabetes, “neurotrophic factor resistance” may be associated with elevation of proinflammatory cytokines and Aβ that compromise neurotrophin, IGF-1 and insulin functions and give rise to increased risk for cognitive decline and neuronal degeneration in AD.

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References

1. Abel T, Kandel E. Positive and negative regulatory mechanisms that mediate long-term memory storage. Brain Res Brain Res Rev. 1998; 26:360–78. [PubMed: 9651552]
2. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL, Finch CE, Frautschy S, Griffin WS, Hampel H, Hull M, Landreth G, Lue L, Mrak R, Mackenzie IR, McGeer PL, O'Banion MK, Pachter J, Pasinetti G, Plata-Salaman C, Rogers J, Rydel R, Shen Y, Streit W, Strohmeyer R, Tooyoma I, Van Muiswinkel FL, Veerhuis R, Walker D, Webster S, Wegrzyniak B, Wenk G, Wyss-Coray T. Inflammation and Alzheimer's disease. Neurobiol Aging. 2000; 21:383–421. [PubMed: 10858586]
3. Araujo DM, Cotman CW. Differential effects of interleukin-1 beta and interleukin-2 on glia and hippocampal neurons in culture. Int J Dev Neurosci. 1995; 13:201–12. [PubMed: 7572276]
4. Bonni A, Brunet A, West AE, Datta SR, Takasu MA, Greenberg ME. Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. Science. 1999; 286:1358–62. see comments. [PubMed: 10558990]
5. Bourbon NA, Sandirasegarane L, Kester M. Ceramide-induced inhibition of Akt is mediated through protein kinase Czeta: implications for growth arrest. J Biol Chem. 2002; 277:3286–92. [PubMed: 11723139]
6. Boutin H, LeFeuvre RA, Horai R, Asano M, Iwakura Y, Rothwell NJ. Role of IL-1alpha and IL-1beta in ischemic brain damage. J Neurosci. 2001; 21:5528–34. [PubMed: 11466424]
7. Bramham CR, Messaoudi E. BDNF function in adult synaptic plasticity: The synaptic consolidation hypothesis. Prog Neurobiol. 2005; 76:99–125. [PubMed: 16099088]
8. Breitner JC, Gau BA, Welsh KA, Plassman BL, McDonald WM, Helms MJ, Anthony JC. Inverse association of anti-inflammatory treatments and Alzheimer's disease: initial results of a co-twin control study. Neurology. 1994; 44:227–32. [PubMed: 8309563]
9. Brenner B, Koppenhoefer U, Weinstock C, Linderkamp O, Lang F, Gulbins E. Fas- or ceramide-induced apoptosis is mediated by a Rac1-regulated activation of Jun N-terminal kinase/p38 kinases and GADD153. J Biol Chem. 1997; 272:22173–81. [PubMed: 9268362]
10. Brunet A, Datta SR, Greenberg ME. Transcription-dependent and - independent control of neuronal survival by the PI3K-Akt signaling pathway. Curr Opin Neurobiol. 2001; 11:297–305. [PubMed: 11399427]
11. Cao Z, Xiong J, Takeuchi M, Kurama T, Goeddel DV. TRAF6 is a signal transducer for interleukin-1. Nature. 1996; 383:443–6. [PubMed: 8837778]
12. Chandler LJ, Sutton G, Dorairaj NR, Norwood D. N-methyl D-aspartate receptor-mediated bidirectional control of extracellular signal-regulated kinase activity in cortical neuronal cultures. J Biol Chem. 2001; 276:2627–36. [PubMed: 11062237]
13. Chao MV, Rajagopal R, Lee FS. Neurotrophin signalling in health and disease. Clin Sci (Lond). 2006; 110:167–73. [PubMed: 16411893]
14. Craft JM, Watterson DM, Hirsch E, Van Eldik LJ. Interleukin 1 receptor antagonist knockout mice show enhanced microglial activation and neuronal damage induced by intracerebroventricular infusion of human beta-amyloid. J Neuroinflammation. 2005; 2:15. [PubMed: 15967035]
15. Cutler RG, Kelly J, Storie K, Pedersen WA, Tammara A, Hatanpaa K, Troncoso JC, Mattson MP. Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. Proc Natl Acad Sci U S A. 2004; 101:2070–5. [PubMed: 14970312]
16. Davis CN, Tabarean I, Gaidarova S, Behrens MM, Bartfai T. IL-1beta induces a MyD88-dependent and ceramide-mediated activation of Src in anterior hypothalamic neurons. J Neurochem. 2006; 98:1379–89. [PubMed: 16771830]
17. Dorsey SG, Bambrick LL, Balice-Gordon RJ, Krueger BK. Failure of brain-derived neurotrophic factor-dependent neuron survival in mouse trisomy 16. J Neurosci. 2002; 22:2571–8. [PubMed: 11923422]
18. Eriksen JL, Sagi SA, Smith TE, Weggen S, Das P, McLendon DC, Ozols VV, Jessing KW, Zavitz KH, Koo EH, Golde TE. NSAIDs and enantiomers of flurbiprofen target gamma-secretase and lower Abeta 42 in vivo. J Clin Invest. 2003; 112:440–9. [PubMed: 12897211]

19. Frebel K, Wiese S. Signalling molecules essential for neuronal survival and differentiation. Biochem Soc Trans. 2006; 34:1287–90. [PubMed: 17073803]
20. Garcia-Galloway E, Arango C, Pons S, Torres-Aleman I. Glutamate excitotoxicity attenuates insulin-like growth factor-I prosurvival signaling. Mol Cell Neurosci. 2003; 24:1027–37. [PubMed: 14697666]
21. Gooney M, Lynch MA. Long-term potentiation in the dentate gyrus of the rat hippocampus is accompanied by brain-derived neurotrophic factor-induced activation of TrkB. J Neurochem. 2001; 77:1198–207. [PubMed: 11389170]
22. Griffin WS. Inflammation and neurodegenerative diseases. Am J Clin Nutr. 2006; 83:470S–474S. [PubMed: 16470015]
23. Grimaldi LM, Casadei VM, Ferri C, Veglia F, Licastro F, Annoni G, Biunno I, De Bellis G, Sorbi S, Mariani C, Canal N, Griffin WS, Franceschi M. Association of early-onset Alzheimer's disease with an interleukin- 1alpha gene polymorphism. Ann Neurol. 2000; 47:361–5. [PubMed: 10716256]
24. Grimm MO, Grimm HS, Patzold AJ, Zinser EG, Halonen R, Duering M, Tschape JA, De Strooper B, Muller U, Shen J, Hartmann T. Regulation of cholesterol and sphingomyelin metabolism by amyloid-beta and presenilin. Nat Cell Biol. 2005; 7:1118–23. [PubMed: 16227967]
25. Hannun YA, Obeid LM. The Ceramide-centric universe of lipid-mediated cell regulation: stress encounters of the lipid kind. J Biol Chem. 2002; 277:25847–50. [PubMed: 12011103]
26. Hara H, Friedlander RM, Gagliardini V, Ayata C, Fink K, Huang Z, Shimizu-Sasamata M, Yuan J, Moskowitz MA. Inhibition of interleukin 1beta converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. Proc Natl Acad Sci U S A. 1997; 94:2007–12. [PubMed: 9050895]
27. Hetman M, Kanning K, Cavanaugh JE, Xia Z. Neuroprotection by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol 3-kinase. J Biol Chem. 1999; 274:22569–80. [PubMed: 10428835]
28. Huang EJ, Reichardt LF. Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem. 2003; 72:609–42. [PubMed: 12676795]
29. Huwiler A, Pfeilschifter J. Interleukin-1 stimulates de novo synthesis of mitogen-activated protein kinase in glomerular mesangial cells. FEBS Lett. 1994; 350:135–8. [PubMed: 8062912]
30. Ivins KJ, Ivins JK, Sharp JP, Cotman CW. Multiple pathways of apoptosis in PC12 cells. CrmA inhibits apoptosis induced by beta-amyloid. J Biol Chem. 1999; 274:2107–12. [PubMed: 9890971]
31. Lee B, Butcher GQ, Hoyt KR, Impey S, Obrietan K. Activity-dependent neuroprotection and cAMP response element-binding protein (CREB): kinase coupling, stimulus intensity, and temporal regulation of CREB phosphorylation at serine 133. J Neurosci. 2005; 25:1137–48. [PubMed: 15689550]
32. Lesne S, Koh MT, Kotilinek L, Kayed R, Glabe CG, Yang A, Gallagher M, Ashe KH. A specific amyloid-beta protein assembly in the brain impairs memory. Nature. 2006; 440:352–7. [PubMed: 16541076]
33. Li Y, Eitan S, Wu J, Evans CJ, Kieffer B, Sun X, Polakiewicz RD. Morphine induces desensitization of insulin receptor signaling. Mol Cell Biol. 2003; 23:6255–66. [PubMed: 12917346]
34. Li YX, Zhang Y, Lester HA, Schuman EM, Davidson N. Enhancement of neurotransmitter release induced by brain-derived neurotrophic factor in cultured hippocampal neurons. J Neurosci. 1998; 18:10231–40. [PubMed: 9852560]
35. Lim GP, Yang F, Chu T, Chen P, Beech W, Teter B, Tran T, Ubeda O, Ashe KH, Frautschy SA, Cole GM. Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. J Neurosci. 2000; 20:5709–14. [PubMed: 10908610]
36. Lu KT, Wang YW, Yang JT, Yang YL, Chen HI. Effect of interleukin-1 on traumatic brain injury-induced damage to hippocampal neurons. J Neurotrauma. 2005; 22:885–95. [PubMed: 16083355]
37. Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. Br J Pharmacol. 2006; 147(Suppl 1):S232–40. [PubMed: 16402109]

38. Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE, Rogers J. Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am J Pathol. 1999; 155:853–62. [PubMed: 10487842]

39. Ma XC, Gottschall PE, Chen LT, Wiranowska M, Phelps CP. Role and mechanisms of interleukin-1 in the modulation of neurotoxicity. Neuroimmunomodulation. 2002; 10:199–207. [PubMed: 12584407]

40. Malaplate-Armand C, Florent-Bechard S, Youssef I, Koziel V, Sponne I, Kriem B, Leininger-Muller B, Olivier JL, Oster T, Pillot T. Soluble oligomers of amyloid-beta peptide induce neuronal apoptosis by activating a cPLA2-dependent sphingomyelinase-ceramide pathway. Neurobiol Dis. 2006; 23:178–89. [PubMed: 16626961]

41. Mathias S, Pena LA, Kolesnick RN. Signal transduction of stress via ceramide. Biochem J. 1998; 335:465–80. [PubMed: 9794783]

42. McCarty JH, Feinstein SC. The TrkB receptor tyrosine kinase regulates cellular proliferation via signal transduction pathways involving SHC, PLCgamma, and CBL. J Recept Signal Transduct Res. 1999; 19:953–74. [PubMed: 10533983]

43. McGeer PL, Rogers J. Anti-inflammatory agents as a therapeutic approach to Alzheimer's disease. Neurology. 1992; 42:447–9. [PubMed: 1736183]

44. Minichiello L, Korte M, Wolfer D, Kuhn R, Unsicker K, Cestari V, Rossi-Arnaud C, Lipp HP, Bonhoeffer T, Klein R. Essential role of trkB receptors in hippocampus-mediated learning. Neuron. 1999; 24:401–414. [PubMed: 10571233]

45. Monti B, Marri L, Contestabile A. NMDA receptor-dependent CREB activation in survival of cerebellar granule cells during in vivo and in vitro development. Eur J Neurosci. 2002; 16:1490–8. [PubMed: 12405962]

46. Montminy M. Transcriptional regulation by cyclic AMP. Annu Rev Biochem. 1997; 66:807–22. [PubMed: 9242925]

47. Morris JC, Storandt M, McKeel DW Jr, Rubin EH, Price JL, Grant EA, Berg L. Cerebral amyloid deposition and diffuse plaques in “normal” aging: Evidence for presymptomatic and very mild Alzheimer's disease. Neurology. 1996; 46:707–19. [PubMed: 8618671]

48. Mrak RE, Griffin WS. Interleukin-1, neuroinflammation, and Alzheimer's disease. Neurobiol Aging. 2001; 22:903–8. [PubMed: 11754997]

49. Naslund, j, et al. Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. JAMA. 2000; 283:1571–77. [PubMed: 10735393]

50. Nicoll JA, Mrak RE, Graham DI, Stewart J, Wilcock G, MacGowan S, Esiri MM, Murray LS, Dewar D, Love S, Moss T, Griffin WS. Association of interleukin-1 gene polymorphisms with Alzheimer's disease. Ann Neurol. 2000; 47:365–8. [PubMed: 10716257]

51. Numakawa T, Matsumoto T, Adachi N, Yokomaku D, Kojima M, Takei N, Hatanaka H. Brain-derived neurotrophic factor triggers a rapid glutamate release through increase of intracellular Ca(2+) and Na(+) in cultured cerebellar neurons. J Neurosci Res. 2001; 66:96–108. [PubMed: 11599006]

52. Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K, Zhen S, Teng KK, Yung WH, Hempstead BL, Lu B. Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science. 2004; 306:487–91. [PubMed: 15486301]

53. Peng S, Wuu J, Mufson EJ, Fahnestock M. Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. J Neurochem. 2005; 93:1412–21. [PubMed: 15935057]

54. Pike CJ, Burdick D, Walencewicz AJ, Glabe CG, Cotman CW. Neurodegeneration induced by beta-amyloid peptides in vitro: the role of peptide assembly state. J Neurosci. 1993; 13:1676–87. [PubMed: 8463843]

55. Poser S, Impey S, Xia Z, Storm DR. Brain-derived neurotrophic factor protection of cortical neurons from serum withdrawal-induced apoptosis is inhibited by cAMP. J Neurosci. 2003; 23:4420–7. [PubMed: 12805282]

56. Ralay Ranaivo H, Craft JM, Hu W, Guo L, Wing LK, Van Eldik LJ, Watterson DM. Glia as a therapeutic target: selective suppression of human amyloid-beta-induced upregulation of brain

proinflammatory cytokine production attenuates neurodegeneration. J Neurosci. 2006; 26:662–70. [PubMed: 16407564]

57. Rolz W, Xin C, Ren S, Pfeilschifter J, Huwiler A. Interleukin-1beta inhibits ATP-induced protein kinase B activation in renal mesangial cells by two different mechanisms: the involvement of nitric oxide and ceramide. Br J Pharmacol. 2003; 138:461–8. [PubMed: 12569071]
58. Rothwell N. Interleukin-1 and neuronal injury: mechanisms, modification, and therapeutic potential. Brain Behav Immun. 2003; 17:152–7. [PubMed: 12706413]
59. Rothwell NJ, Luheshi GN. Interleukin 1 in the brain: biology, pathology and therapeutic target. Trends Neurosci. 2000; 23:618–25. [PubMed: 11137152]
60. Sanchez-Alavez M, Tabarean IV, Behrens MM, Bartfai T. Ceramide mediates the rapid phase of febrile response to IL-1beta. Proc Natl Acad Sci U S A. 2006; 103:2904–8. [PubMed: 16477014]
61. Selkoe DJ. Alzheimer's disease is a synaptic failure. Science. 2002; 298:789–91. [PubMed: 12399581]
62. Silva AJ, Kogan JH, Frankland PW, Kida S. CREB and memory. Annu Rev Neurosci. 1998; 21:127–48. [PubMed: 9530494]
63. Skaper SD, Floreani M, Negro A, Facci L, Giusti P. Neurotrophins rescue cerebellar granule neurons from oxidative stress-mediated apoptotic death: selective involvement of phosphatidylinositol 3-kinase and the mitogen-activated protein kinase pathway. J Neurochem. 1998; 70:1859–68. [PubMed: 9572269]
64. Srinivasan D, Yen JH, Joseph DJ, Friedman W. Cell type-specific interleukin-1beta signaling in the CNS. J Neurosci. 2004; 24:6482–8. [PubMed: 15269258]
65. Strle K, Broussard SR, McCusker RH, Shen WH, Johnson RW, Freund GG, Dantzer R, Kelley KW. Proinflammatory cytokine impairment of insulin-like growth factor I-induced protein synthesis in skeletal muscle myoblasts requires ceramide. Endocrinology. 2004; 145:4592–602. [PubMed: 15256490]
66. Sweatt JD. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. J Neurochem. 2001; 76:1–10. [PubMed: 11145972]
67. Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ, Greenberg ME. Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. Neuron. 1998; 20:709–26. published erratum appears in Neuron 1998 Jun;20(6):1297. [PubMed: 9581763]
68. Thomas GM, Huganir RL. MAPK cascade signalling and synaptic plasticity. Nat Rev Neurosci. 2004; 5:173–83. [PubMed: 14976517]
69. Thornton P, Pinteaux E, Gibson RM, Allan SM, Rothwell NJ. Interleukin-1-induced neurotoxicity is mediated by glia and requires caspase activation and free radical release. J Neurochem. 2006; 98:258–66. [PubMed: 16805812]
70. Tong L, Balazs R, Thornton PL, Cotman CW. Beta-amyloid peptide at sublethal concentrations downregulates brain-derived neurotrophic factor functions in cultured cortical neurons. J Neurosci. 2004; 24:6799–809. [PubMed: 15282285]
71. Venters HD, Dantzer R, Kelley KW. A new concept in neurodegeneration: TNFalpha is a silencer of survival signals. Trends Neurosci. 2000; 23:175–80. [PubMed: 10717677]
72. Venters HD, Tang Q, Liu Q, VanHoy RW, Dantzer R, Kelley KW. A new mechanism of neurodegeneration: a proinflammatory cytokine inhibits receptor signaling by a survival peptide. Proc Natl Acad Sci U S A. 1999; 96:9879–84. [PubMed: 10449788]
73. Viviani B, Bartesaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, Binaglia M, Corsini E, Di Luca M, Galli CL, Marinovich M. Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. J Neurosci. 2003; 23:8692–700. [PubMed: 14507968]
74. Walton M, Woodgate AM, Muravlev A, Xu R, During MJ, Dragunow M. CREB phosphorylation promotes nerve cell survival. J Neurochem. 1999; 73:1836–42. [PubMed: 10537041]
75. Watt WC, Sakano H, Lee ZY, Reusch JE, Trinh K, Storm DR. Odorant stimulation enhances survival of olfactory sensory neurons via MAPK and CREB. Neuron. 2004; 41:955–67. [PubMed: 15046727]
76. Weggen S, Eriksen JL, Das P, Sagi SA, Wang R, Pietrzik CU, Findlay KA, Smith TE, Murphy MP, Bulter T, Kang DE, Marquez-Sterling N, Golde TE, Koo EH. A subset of NSAIDs lower

amyloidogenic Abeta42 independently of cyclooxygenase activity. Nature. 2001; 414:212–6. [PubMed: 11700559]

77. Weninger SC, Yankner BA. Inflammation and Alzheimer disease: the good, the bad, and the ugly. Nat Med. 2001; 7:527–8. [PubMed: 11329045]
78. White MF. Insulin signaling in health and disease. Science. 2003; 302:1710–1. [PubMed: 14657487]
79. Willaime-Morawek S, Arbez N, Mariani J, Brugg B. IGF-I protects cortical neurons against ceramide-induced apoptosis via activation of the PI-3K/Akt and ERK pathways; is this protection independent of CREB and Bcl-2? Brain Res Mol Brain Res. 2005; 142:97–106. [PubMed: 16290312]
80. Yin KJ, Lee JM, Chen SD, Xu J, Hsu CY. Amyloid-beta induces Smac release via AP-1/Bim activation in cerebral endothelial cells. J Neurosci. 2002; 22:9764–70. [PubMed: 12427831]
81. Zick Y. Insulin resistance: a phosphorylation-based uncoupling of insulin signaling. Trends Cell Biol. 2001; 11:437–41. [PubMed: 11684411]

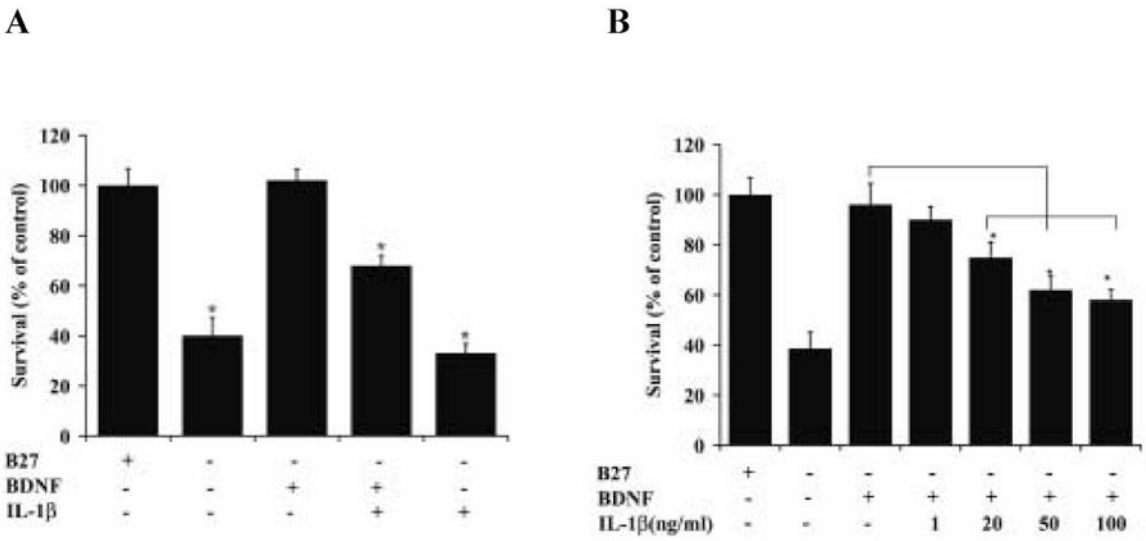

Fig. 1. A, IL-1β abrogates BDNF protection of cortical neurons from trophic support deprivation-induced cell death

The serum-free "trophic" medium, containing B-27, was removed from cultures and after a DMEM wash, cultures were incubated for 36 h in DMEM in the presence or absence of BDNF (10 ng/ml) ± IL-1β (50 ng/ml). When IL-1β was added, there was a 1 h preincubation period in the presence of the cytokine before the removal of the B-27-containing medium. In controls, the B-27-containing medium was removed, but replaced, after a DMEM wash, with B-27-containing medium. Cell survival was assayed by MTT assay; data are mean ± S.E. (n = 3). B, The effect of IL-1 β on neuroprotection by BDNF is concentration-dependent. In both A. and B., significance (p<0.05) was determined by ANOVA (with Fisher's PLSD as the post hoc test) in comparison with either the control cells or neurons treated with BDNF alone (*).

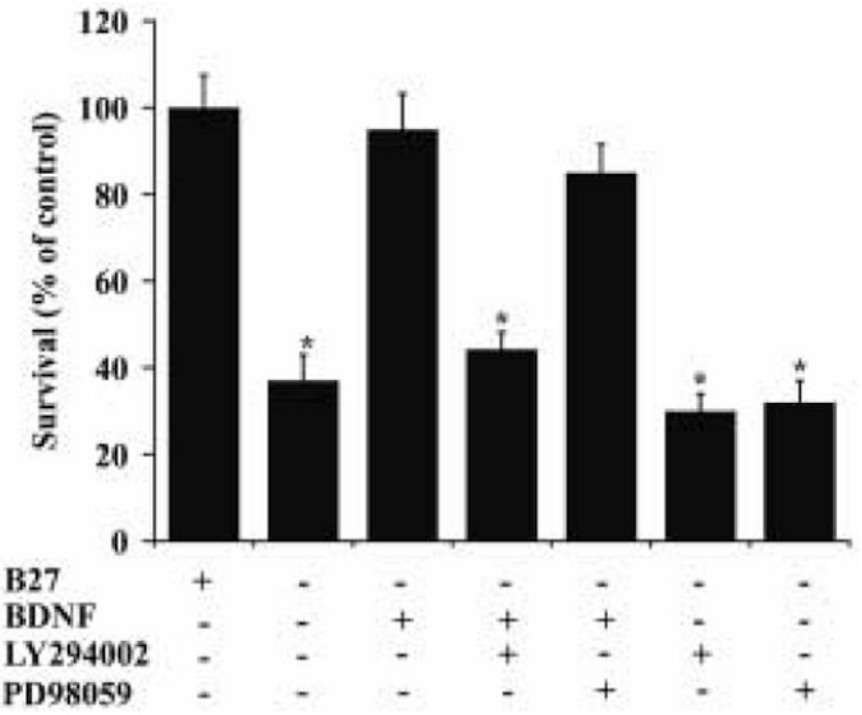

Fig. 2. Activation of the PI3K pathway by BDNF is required for BDNF-mediated neuroprotection

Experimental conditions of deprivation from trophic support were done as described in the legend of Fig. 1. Inhibitors of the PI3-K/Akt and Ras/MAPK pathways, LY294002 (30 μM) and PD98059 (40 μM) respectively, were added to the cultures 30 min prior to the BDNF treatment. Cell survival was assayed by MTT assay; data are mean ± S.E. (n = 3). Significance (p<0.05) determined by ANOVA (with Fisher's PLSD as the post hoc test) in comparison with either control cells or cultures treated with BDNF only is indicated (*).

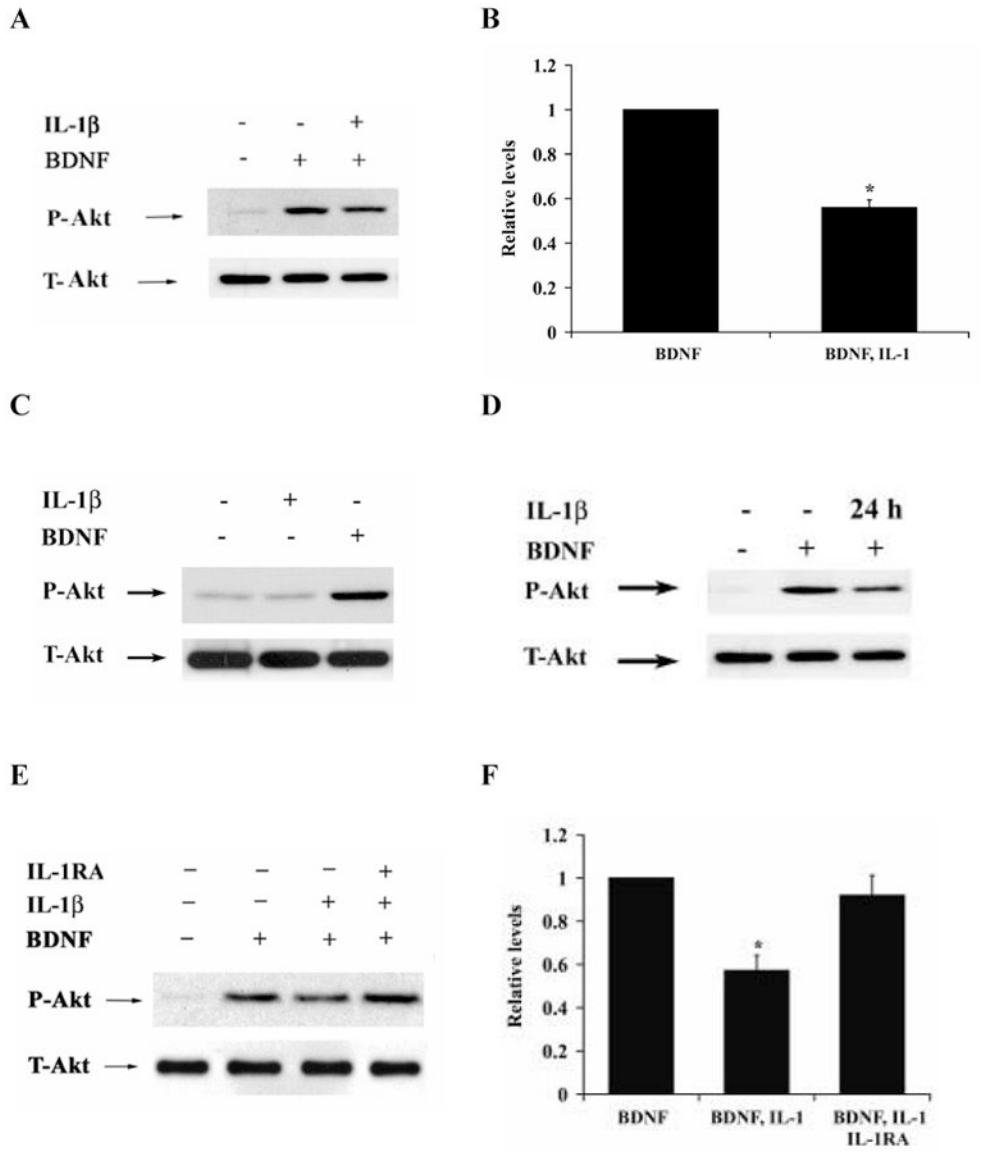

Fig. 3. IL-1β pretreatment decreased BDNF-induced Akt activation

A, Exposure to BDNF (10 ng/ml, 10 min) increased the amount of P-Akt. Pretreatment with IL-1β (50 ng/ml) suppressed the effect of BDNF on P-Akt, without influencing total Akt (T-Akt) content. *B*, Quantification of the blots corresponding to that shown in *A*. Unless otherwise indicated, here and in the other legends, the quantification data for Western blots are expressed relative to the estimates in the BDNF-treated cultures (n =3) and significance ($p<0.05$) is indicated by *. *C*, Exposure to IL-1β for 2 h had no significant effect on the level of activated Akt. The competence of the cells is indicated by the elevation of P-Akt content induced by BDNF (10 ng/ml, 10 min). *D*. The effect of 24 h pretreatment with IL-1β was similar to that of 2 h exposure. *E*, IL-1ra blocks the suppression by IL-1β of BDNF-induced activation of Akt. *F*, Quantification of the blots corresponding to *E*.

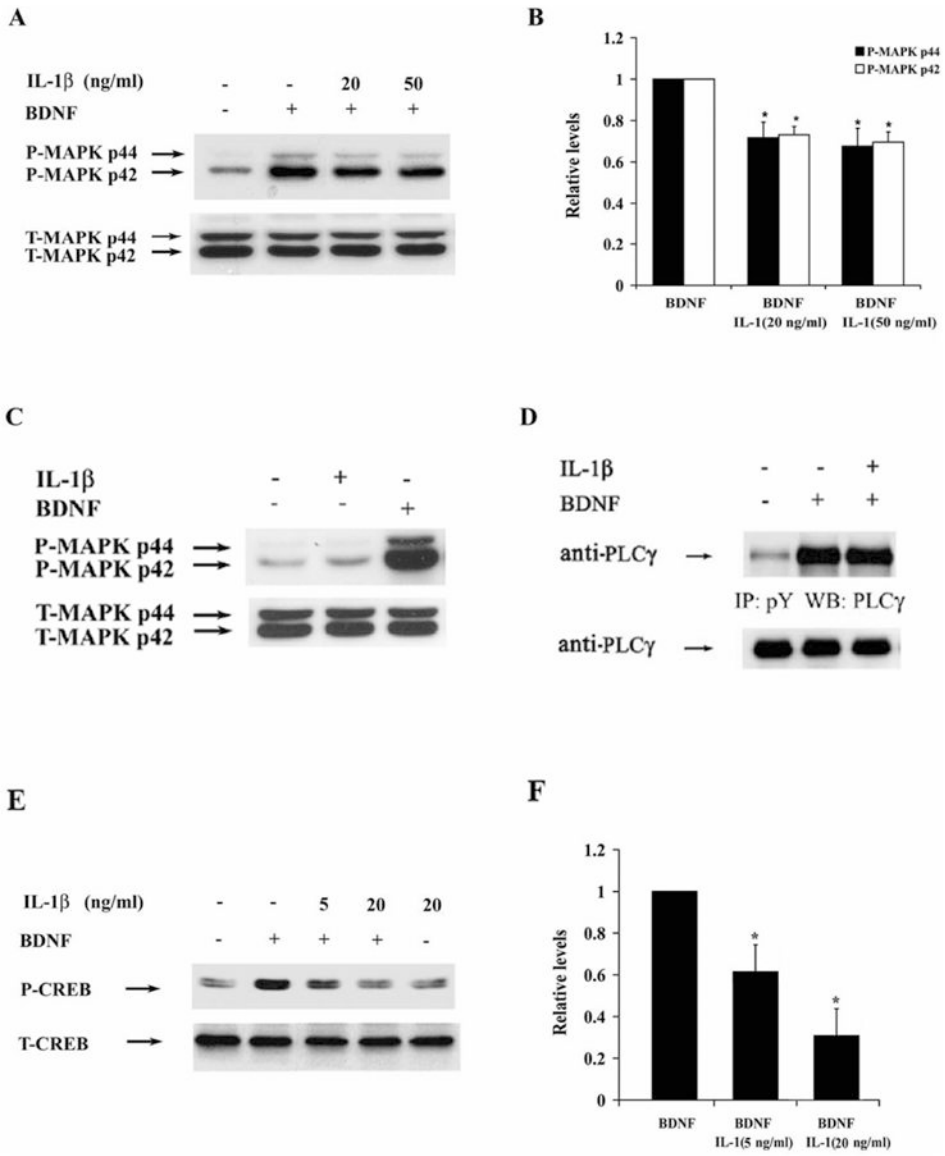

Fig. 4. Treatment with IL-1β decreases the BDNF-induced activation of MAPK/ERK, CREB, but not PLCγ

A, Pretreatment with IL-1β (50 ng/ml) for 2 h decreased phosphorylated MAPK/ERK (P-MAPK) levels in cultures stimulated with BDNF(10 ng/ml, 10 min), without influencing total MAPK/ERK (T-MAPK) content. The cytokine on its own did not increase P-MAPK levels (not shown). *B*, Quantification of blots corresponding to *A* (n=3). *C*, Western blots show that exposure to IL-1β (50 ng/ml, 2 h) had no significant effect on the level of activated MAPK/ERK. Cells treated with BDNF (10 ng/ml, 10 min) served as positive control. *D*, BDNF (10 ng/ml, 10min) -induced tyrosine phosphorylation of PLCγ is unaffected by IL-1β. Tyrosine phosphorylation of PLCγ was examined by immunoprecipitation (IP), followed by Western blotting (WB), as described in Methods. BDNF-induced PLCγ phosphorylation was not influenced significantly by IL-1β pretreatment; in terms of phosphorylated PLCγ levels obtained in the BDNF-exposed cultures, the estimate in the IL-1β-treated cultures was 93.1 ± 5.6% (n = 3). Total PLCγ

estimated from cell lysates was not influenced by IL-1β. *E*, pretreatment with IL-1β at the concentrations used at ≥5 ng/ml for 2 h compromised the increase in the BDNF-induced level of phosphorylated CREB (P-CREB), but had no effect on total CREB (T-CREB) levels. Note that IL-1β on its own had no significant effect on the basal level of P-CREB. *F*, Quantification of the effect of IL-1β on CREB activation.

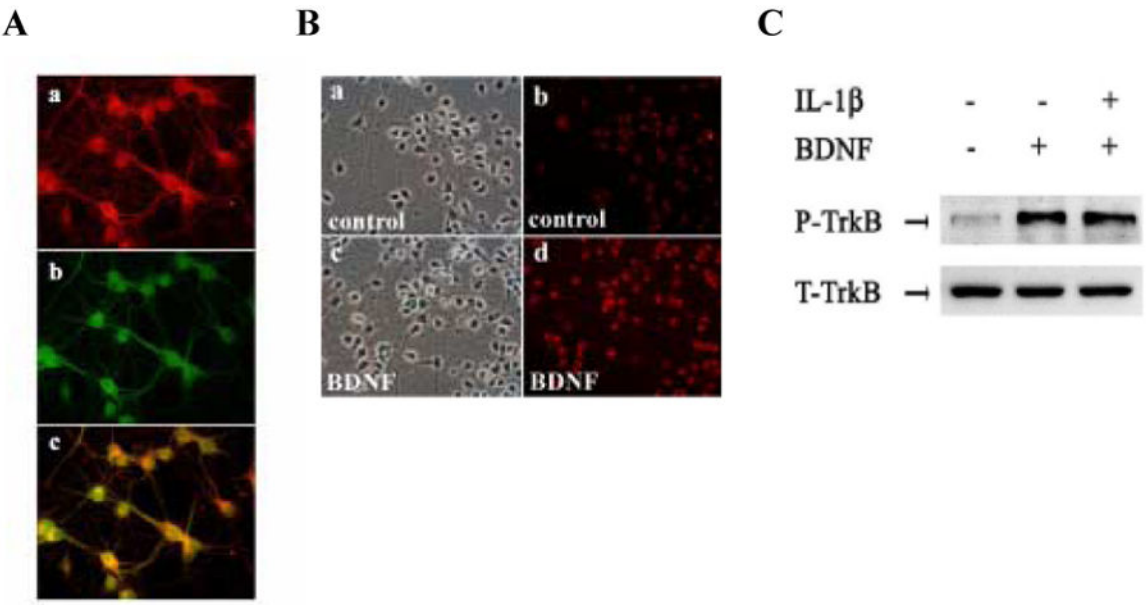

Fig. 5. IL-1β does not interfere with BDNF-induced activation of TrkB

A, Immunocytochemical detection of TrkB receptors using specific antibodies (red) (a). Neurons were identified using MAP-2 antibodies (b) (green). The merged images (orange) (c) show that almost all neurons express TrkB receptor. In independent experiments (not shown), an additional staining with Hoechst 33342 dye showed that virtually all the live nerve cells expressed TrkB receptors. *B* TrkB receptors on the neurons are functionally active. Under basal conditions, P-CREB staining using an antibody recognizing Ser-133-phosphorylated CREB was very weak (b), and BDNF (50 ng/ml, 10 min) caused a great increase in immunostaining in virtually all cells (d) (BDNF at 10 ng/ml also induced a similar marked increase in P-CREB labeling in almost all the cells; not shown). Phase contrast images of the corresponding fields are shown in (a) and (c). Magnification: X200. *C*, Phosphorylation of TrkB was examined using Western blotting with an antibody against Trk phosphorylated at Tyr-490 (P-TrkB). Pretreatment with 50 ng/ml IL-1β had no significant influence on the BDNF-induced P-TrkB content: in terms of P-TrkB levels obtained in the BDNF-exposed culture, the estimate in the IL-1β-pretreated cells was 98 ± 4.5% (n = 3).

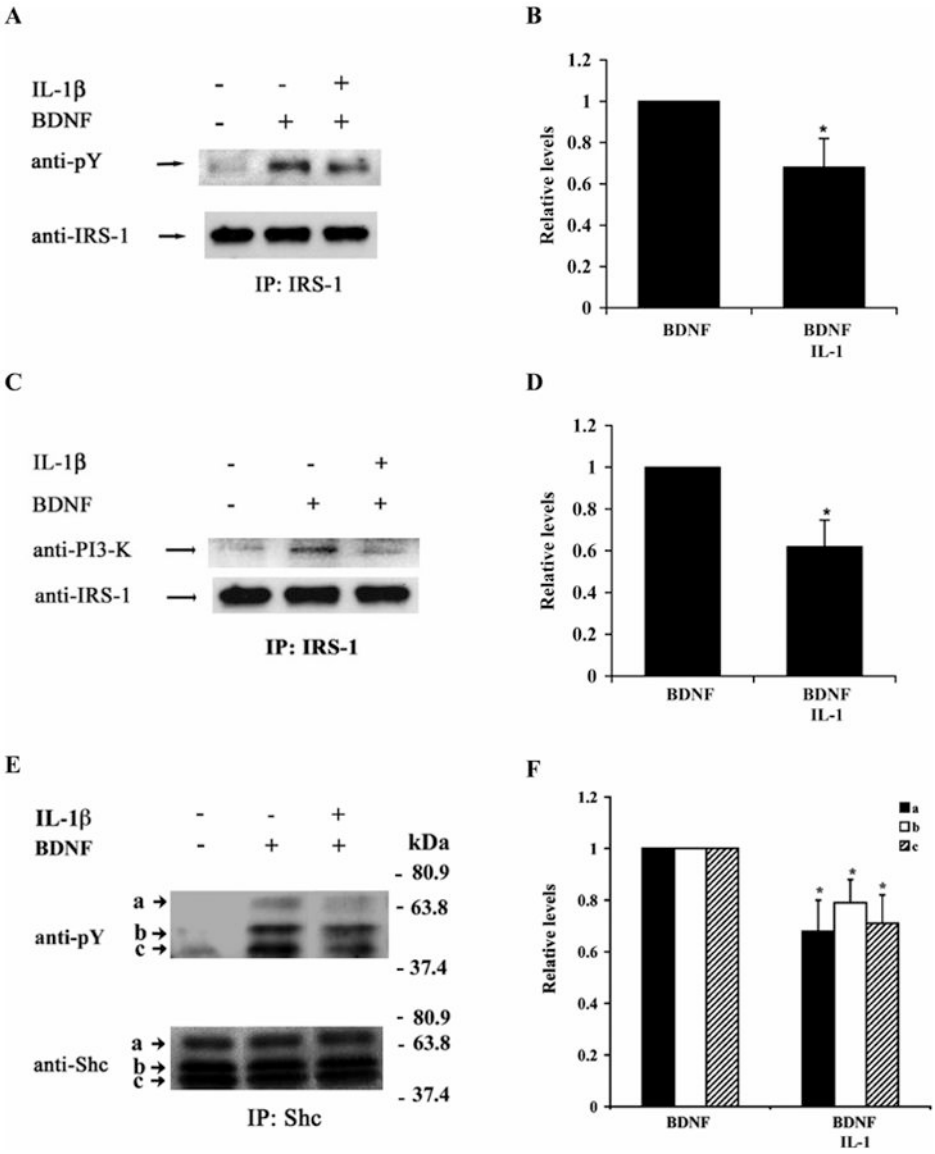

Fig. 6. IL-1β interferes with BDNF-induced phosphorylation of the docking proteins IRS-1 and Shc

A, IL-1β pretreatment (50 ng/ml, 2 h) decreased BDNF-induced level of Tyr-phosphorylated IRS-1 analyzed by immunoprecipitation with an IRS-1 antibody, followed by Western blotting with anti-phosphotyrosine antibody (top panel) and with anti-IRS-1 antibody (bottom panel). *B*, Quantification of blots corresponding to *A* (n=3). *C*, IL-1β pretreatment (50 ng/ml, 2 h) decreased BDNF-induced increase in the association between PI3-K and IRS-1 analyzed by immunoprecipitation with an IRS-1 antibody followed by Western blotting with anti-PI3-K antibody (top panel) and with anti-IRS-1 antibody (bottom panel). *D*, Quantification of blots corresponding to *C* (n=3). *E*, IL-1β pretreatment (50 ng/ml, 2 h) decreased BDNF-induced levels of Tyr-phosphorylated Shc isoforms analyzed by immunoprecipitation with an Shc antibody, followed by Western blotting with anti-phosphotyrosine antibody (top panel) and with anti-Shc antibody (bottom panel). The isoforms are indicated as a, b and c (approximate molecular weights, kDa: 66, 52 and 46,

respectively). *F*, Quantification of the effects of pretreatment with IL-1β on Shc activation (n=3).

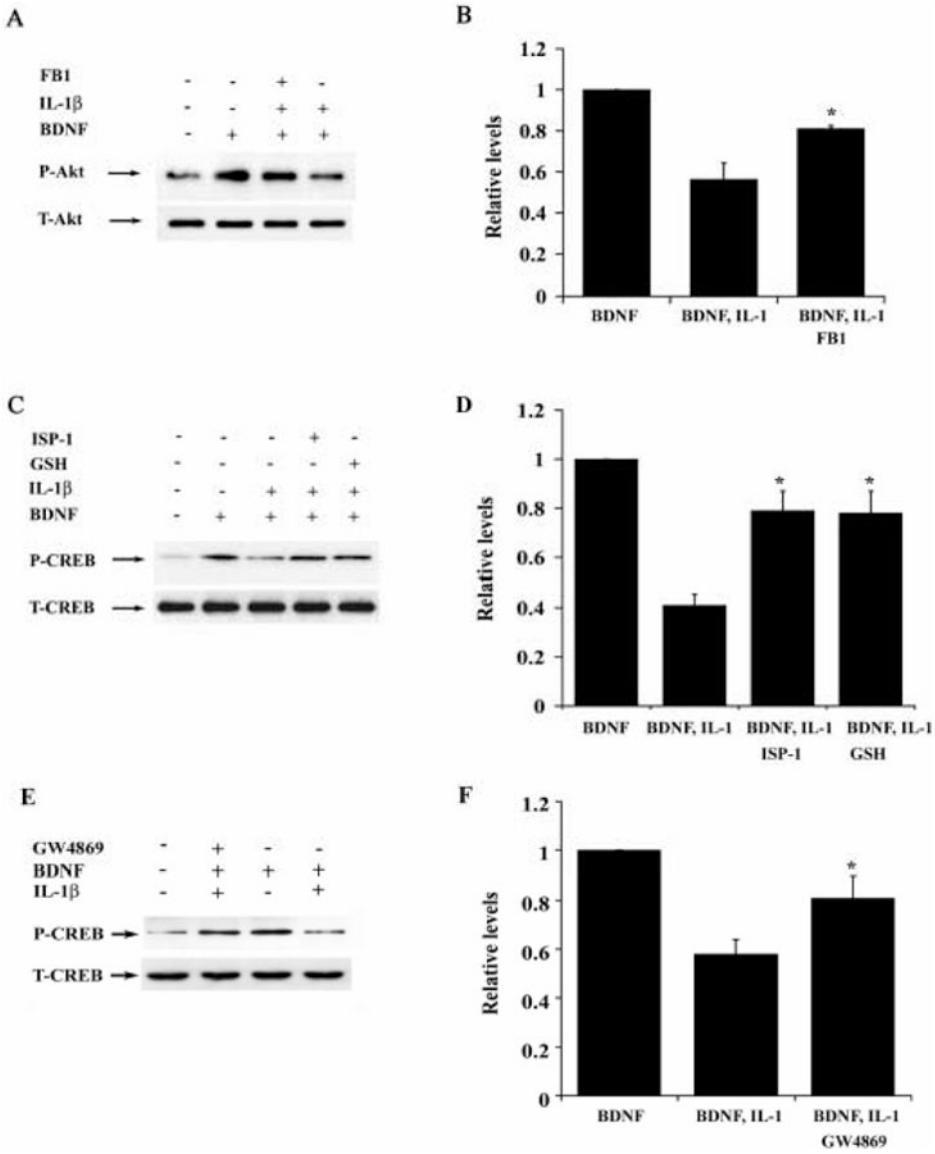

Fig. 7. Inhibitors of ceramide synthesis attenuate the inhibition by IL-1β of BDNF signaling

A, The *de novo* pathway inhibitor FB1 (5 μM) attenuated the suppression by IL-1β (50 ng/ml) of Akt activation induced by BDNF (10 ng/ml). *B*, Quantification of the effect of FB1. Significance ($p<0.05$) is indicated here and in Fig. 7D and F by * in comparison with IL-1β-pretreated cultures stimulated by BDNF (BDNF+IL-1β. *C*, Another inhibitor of the *de novo* pathway myriocin (ISP-1; 50 nM) or the neutral sphingomyelinase inhibitor glutathione (GSH; 2 mM) attenuated IL-1β-mediated suppression of BDNF-induced CREB phosphorylation. *D*, Quantification of blots corresponding to that shown in *C*. *E*, the neutral sphingomyelinase inhibitor GW4869 (10 μM) attenuated IL-1β-mediated suppression of BDNF-induced CREB phosphorylation. *F*, Quantification of the blots corresponding to that shown in *E*.

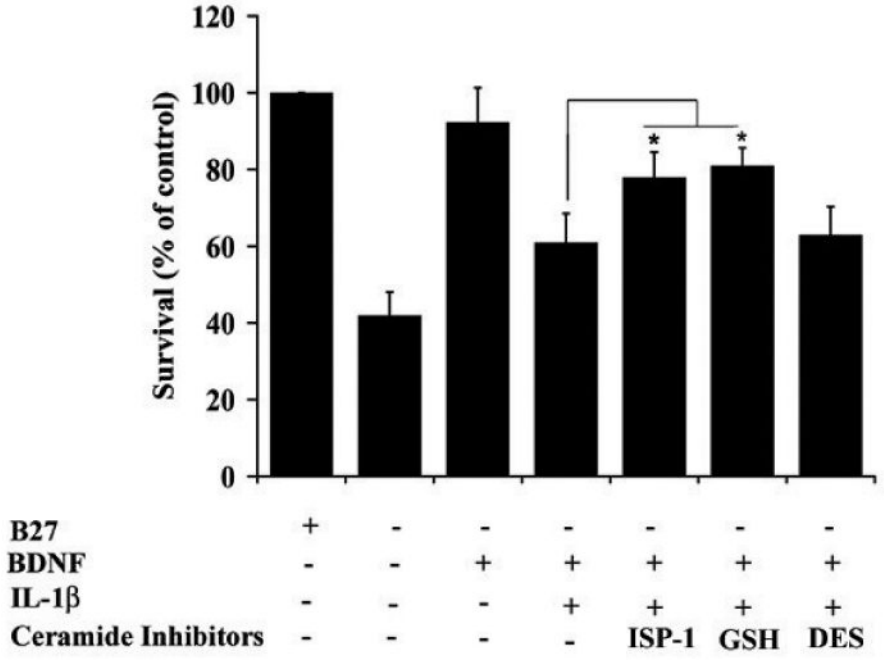

Fig. 8. Inhibitors of ceramide synthesis attenuate the inhibition by IL-1β of BDNF-mediated neuroprotection

The *de novo* pathway inhibitor ISP-1 (ISP, 100 nM) and the neutral sphingomyelinase inhibitor glutathione (GSH, 2 mM) attenuated IL-1β-mediated suppression of BDNF (10 ng/ml)-mediated protection of cortical neurons from trophic support deprivation-induced cell death. The acidic sphingomyelinase inhibitor desipramine (DES, 10 μM) had no effect on the action of IL-1β. Experimental conditions were as described in Fig. 1. When inhibitors of ceramide synthesis were employed, they were added to the cultures 30 min before exposure to IL-1β (50 ng/ml). Cell survival was assayed using the MTT assay; data are mean ± S.E. ($n = 3$). Significance ($p<0.05$) determined by ANOVA (with Fisher's PLSD as the post hoc test) in comparison with cultures treated with BDNF in the presence of IL-1β (*).

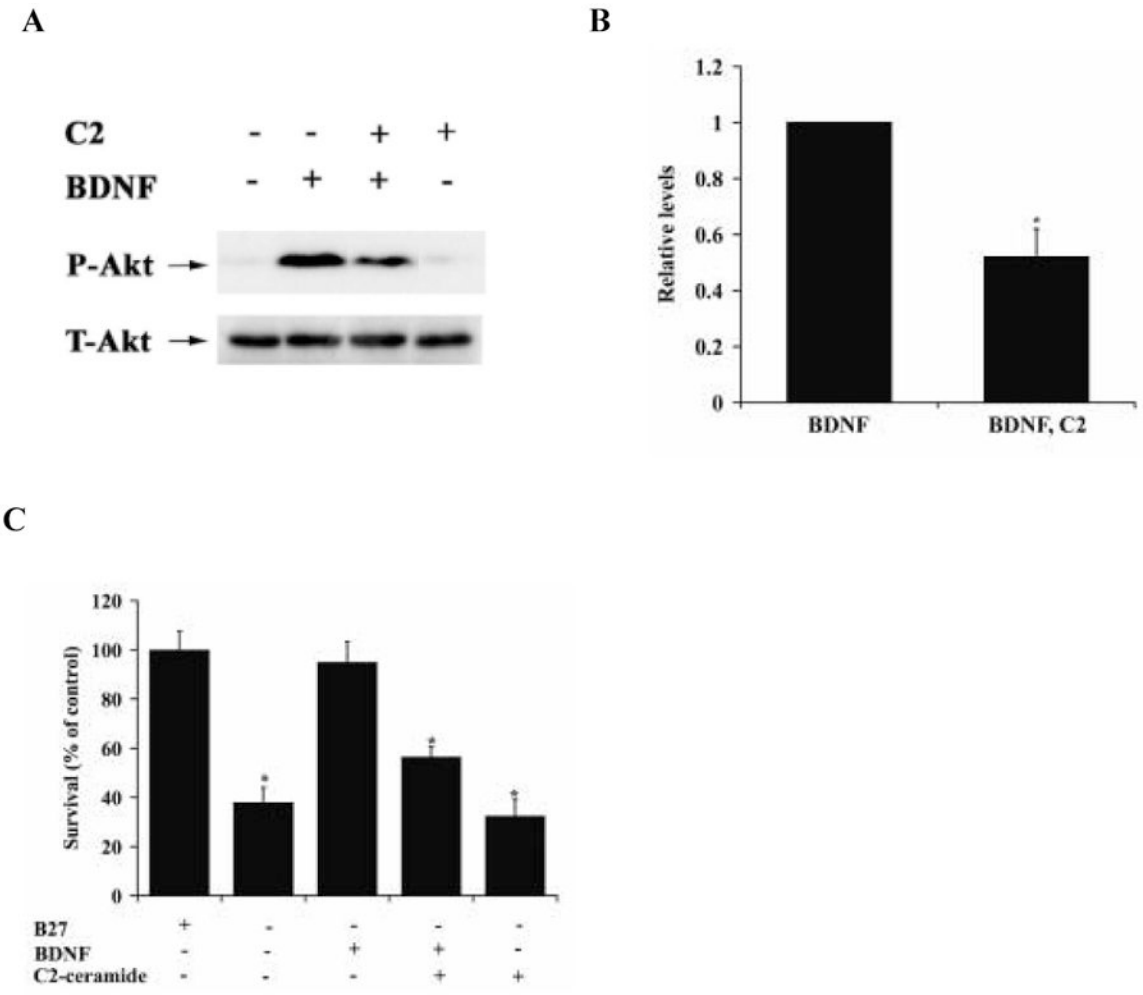

Fig. 9. Treatment with the ceramide analogue C2-ceramide decreased BDNF-induced Akt activation and neuroprotection

A, Phosphorylated Akt (P-Akt) levels were determined with an antibody specific to Akt phosphorylated at Ser-473 (P-Akt). Exposure to BDNF (10 ng/ml, 10 min) increased the amount of P-Akt. Pretreatment with the cell-permeable C2-ceramide at 10 μM for 2 h suppressed the effect of BDNF on P-Akt, but had no effect on total Akt (T-Akt) levels. C2-ceramide on its own had no significant effect on P-Akt content. *B*, Quantification of the C2-ceramide effect. *C*, C2-ceramide at 10 μM abrogates BDNF protection of cortical neurons from trophic support deprivation-induced cell death. The experiment conditions were as described in Fig. 1. Cell survival was assayed using the MTT assay; data are mean ± S.E. ($n = 3$). Significance ($p<0.05$) determined by ANOVA (with Fisher's PLSD as the post hoc test) in comparison with control (B-27) or BDNF-treated cultures (*).