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INTERLEUKIN-1β ENHANCES NEURONAL VULNERABILITY TO PRONGF-MEDIATED APOPTOSIS BY INCREASING SURFACE EXPRESSION OF P75^{NTR} AND SORTILLIN

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Abstract—Many types of injury such as seizure, ischemia, and oxidative stress cause upregulation of the p75 neurotrophin receptor (p75^{NTR}) in brain neurons, where it promotes apoptosis, however the mechanism by which p75^{NTR} is regulated under these conditions is not well understood. Proinflammatory cytokines such as interleukin-1β (IL-1β) are highly produced under these injury conditions and, in particular, are expressed rapidly in the rat hippocampus after seizure. IL-1β is known to increase neuronal vulnerability under many conditions, although it does not directly induce neuronal death. Recently, we have shown that these cytokines regulate p75^{NTR} induction both in neurons and astrocytes *in vitro*. Here, we show that IL-1β infusion into the brain induces p75^{NTR} in neurons of the CA1 area of the hippocampus. While IL-1β induction of p75^{NTR} is not sufficient to induce cell death, we demonstrate that IL-1β primes the neurons by recruiting p75^{NTR} and its coreceptor sortilin to the cell surface, making the neurons more vulnerable to subsequent challenge by proNGF. These results suggest a mechanism by which IL-1β exacerbates neuronal death following injury.

Key words: interleukin-1, neurotrophin, brain injury, apoptosis, NGF, p75.

INTRODUCTION

Interleukin-1β (IL-1β) is a major proinflammatory cytokine released under conditions of injury, infection, or disease, and is known to be involved in diverse actions in the central nervous system. Although many studies have shown that IL-1β alone does not induce neuronal death either *in vitro* (Thornton et al., 2006) or *in vivo* (Lawrence et al., 1998), it has synergistic effects on neuronal damage when provided with other cytokines (Chao et al., 1995; Hu et al., 1997). Moreover, release of IL-1β exacerbates traumatic, ischemic or excitotoxic stimulated neurotoxicity (Yamasaki et al., 1995; Patel et al., 2003), and blocking IL-1β with the receptor antagonist (IL-1ra) attenuates neuronal loss (Allan et al., 2005), suggesting that IL-1β indirectly contributes to neuronal injury.

Our lab has recently reported that proinflammatory cytokines such as IL-1β and TNFα regulate expression of the p75 neurotrophin receptor (p75^{NTR}) both in neurons and astrocytes *in vitro* (Choi and Friedman, 2009). The p75^{NTR} has diverse roles in regulating neuronal survival, death and axonal growth (Greene and Rukenstein, 1981; Rabizadeh et al., 1993; Frade et al., 1996; Maggirwar et al., 1998; Friedman, 2000). This multifunctional receptor is abundantly expressed in the brain during development, however its expression is limited in the adult brain (Yan and Johnson, 1988). p75^{NTR} is upregulated following many types of brain injury such as traumatic brain injury, seizure, ischemia, oxidative stress and axonal injury (Kokaia et al., 1998; Roux et al., 1999; Casha et al., 2001; Ramos et al., 2007) as well as in CNS neurodegenerative diseases such as Alzheimer's disease (Hu et al., 2002). The upregulated p75^{NTR} in these pathological conditions has been suggested to be directly involved in neurodegeneration. p75^{NTR} is highly expressed in the hippocampus after pilocarpine-induced seizure (Roux et al., 1999) and induces neuronal cell death by activating the intrinsic caspase cascade (Troy et al., 2002). Furthermore, the unprocessed NGF precursor, proNGF, which is a potent ligand for p75^{NTR}, is also released after injury and induces neuronal apoptosis (Beattie et al., 2002; Volosin et al., 2008).

IL-1β is also known to regulate NGF mRNA expression (Spranger et al., 1990; Friedman et al., 1991), although the form of the NGF protein that is produced has not been identified. Therefore, IL-1β may be involved in neurodegeneration by regulating the p75^{NTR} as well as ligands that promote neuronal death.

In this study, IL-1β infusion into the brain increased p75^{NTR} expression but did not induce cell death *in vivo*. We therefore investigated the functional role of p75^{NTR} upregulation after IL-1β treatment and show that IL-1β specifically exacerbated proNGF-induced hippocampal neuronal death by recruiting the p75^{NTR} and sortilin receptors to the cell surface. This study, therefore, identifies a mechanism by which IL-1β may enhance neuronal vulnerability following brain injury.

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Abbreviations: CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; IL-1β, interleukin-1β; p75^{NTR}, p75 neurotrophin receptor; TdT, terminal deoxynucleotidyl transferase.

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EXPERIMENTAL PROCEDURES

Materials

Human recombinant IL-1β was a gift from Dr. Ron Hart, (Rutgers University, Piscataway, NJ, USA) and human NGF was provided by Genentech, Inc. (South San Francisco). Furin-resistant proNGF was generously provided by Dr. Barbara Hempstead. Eagle's MEM, Ham's F12, and penicillin-streptomycin were purchased from Invitrogen (Carlsbad, CA, USA). All other materials were obtained from Sigma (St. Louis, MO, USA).

Stereotaxic cannulation of the hippocampus

Male Sprague Dawley rats (250–275 g) were anaesthetized with ketamine (50 mg/kg)/xylazine (10 mg/kg) and placed in a stereotaxic frame for bilateral implantation of cannula into the dorsal hippocampus. The following coordinates were used: anterior–posterior = −3.1 mm from bregma, lateral = ± 2 mm from midline, dorsoventral = −3 mm from skull (Paxinos et al., 1985). Skull holes were made with a dental drill and the guide cannula and support screw were fixed with dental cement. After 7 days, 10 ng (in 0.5 μl) of IL-1β was infused unilaterally into the hippocampus via the guide cannula at a rate of 0.5 μl/min. Animals found to have an incorrectly placed cannula were excluded.

All animal studies were conducted using the NIH (National Institutes of Health) guidelines for the ethical treatment of animals with approval of the Rutgers Institutional Animal Care and Facilities Committee.

Immunocytochemistry

Two days after IL-1β infusion, rats were anesthetized with ketamine/xylazine and perfused transcardially with saline followed by 4% paraformaldehyde. The brains were removed and postfixed in 4% paraformaldehyde before being cryoprotected in 30% sucrose for 2 d. Brains were then sectioned on a cryostat (Leica), and mounted onto charged slides for immunostaining. Frozen brain sections (12 μm) were warmed at 37 °C for 1 min, washed with PBS, blocked in 10% goat serum with PBS plus 0.3% Triton X-100, and then incubated with anti-$p75^{NTR}$ (Upstate; 1:500) and NeuN (Chemicon; 1:500) overnight at 4 °C. The sections were then washed three times with PBS for 15 min, followed by incubation with the secondary antibodies, Fluor 488-conjugated donkey anti-rabbit and Texas Red-conjugated goat anti-mouse (Jackson; 1:500) at room temp in the dark for 1 h, washed three times with PBS for 15 min. Hoechst 33342 dye (1 μg/ml; Sigma) was added into PBS during the last wash to label nuclei. Cell death was examined by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) staining following the manufacturer's manual (Roche, Mannheim, Germany). Sections were mounted with anti-fading medium (ProLong Gold; Invitrogen) and were examined by fluorescence microscopy (Nikon). Images were obtained using MetaMorph and figures were assembled in Adobe Photoshop.

Analysis of cerebrospinal fluid (CSF)

Rats were anesthetized with ketamine/xylazine and placed in a stereotaxic frame for collecting CSF from cisterna magna using a 25-gauge needle. Only CSF samples that did not contain blood contamination were mixed with protease inhibitors, flash frozen, and stored at −80 °C until analysis.

Western blot analysis

Cells were lysed in RIPA buffer (50 mM Tris–HCl, pH7.5 150 mM NaCl, 5 mM EDTA, 1% Nonidet P-40, 0.5% deoxycholic acid, 0.5% SDS) supplemented with a protease inhibitor mixture (Roche Products, Welwyn Garden City, UK), 1 mM sodium vanadate, and 5 mM sodium fluoride. Proteins were quantified by Bradford assay (Bio-Rad, Hercules, CA), equal amounts of proteins were run on 10% polyacrylamide gel, and transferred to a nitrocellulose membrane. Membranes were blocked in 5% non-fat milk in TBST and then probed with antibodies to $p75^{NTR}$ (Upstate Biotechnology, Inc., Lake Placid, NY, USA), and actin (Sigma, St. Louis, MO, USA), NGF (Sigma). Bands were visualized by enhanced chemical luminescence (Pierce, Rockford, IL, USA).

Quantitative real-time reverse transcription PCR

Dorsal hippocampus was freshly homogenized, mRNA and proteins were isolated using TRI**zol** reagent (Invitrogen, Carlsbad, CA, USA). cDNA was generated using SuperScript II Reverse Transcriptase with random hexamers (Invitrogen), and SYBR green-based quantitative real-time PCR was performed using primers specific for $p75^{NTR}$ (rat, forward: 5′-CTGATGCT GAATGCGAAGAG-3′, reverse: 5′-TCACCATATCCGCC ACTGTA-3′), NGF (rat, forward: 5′-CAAGGACGCAGC TTTCTATCCTG-3′, reverse: 5′-CTTCAGGGACAGAG TCTCCCTCT-3′), or actin (forward:5′-TCATGAAGTGTG ACGTTGACATCCGT-3′, reverse:5′-CTTAGAAGCATT TGCGGTG CACGATG-3′) with the comparative C_T method ($\Delta\Delta C_T$)(ABI).

Neuronal cultures

Hippocampal neuronal cultures were prepared as described previously (Farinelli et al., 1998; Friedman, 2000). Rat hippocampi were dissected from embryonic day 18, dissociated, plated on poly-D-lysine (0.1 mg/ml)-coated dishes, and maintained in a serum-free environment. The medium consisted of a 1:1 mixture of Eagle's MEM and Ham's F12 supplemented with glucose (6 mg/ml), insulin (25 μg/ml), putrescine (60 μM), progesterone (20 nM), transferrin (100 μg/ml), selenium (30 nM), penicillin (0.5 U/ml), and streptomycin (0.5 μg/ml). Cultures were maintained in 5% CO_2 at 37 °C for 5 days and subjected to IL-1β treatment for the times indicated.