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Presenilin mediates neuroprotective functions of ephrinB and brain-derived neurotrophic factor and regulates ligand-induced internalization and metabolism of EphB2 and TrkB receptors

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Abstract

Activation of EphB receptors by ephrinB (efnB) ligands on neuronal cell surface regulates important functions, including neurite outgrowth, axonal guidance, and synaptic plasticity. Here, we show that efnB rescues primary cortical neuronal cultures from necrotic cell death induced by glutamate excitotoxicity and that this function depends on EphB receptors. Importantly, the neuroprotective function of the efnB/EphB system depends on presenilin 1 (PS1), a protein that plays crucial roles in Alzheimer's disease (AD) neurodegeneration. Furthermore, absence of one PS1 allele results in significantly decreased neuroprotection, indicating that both PS1 alleles are necessary for full expression of the neuroprotective activity of the efnB/EphB system. We also show that the ability of brain-derived neurotrophic factor (BDNF) to protect neuronal cultures from glutamate-induced cell death depends on PS1. Neuroprotective functions of both efnB and BDNF, however, were independent of γ-secretase activity. Absence of PS1 decreases cell surface expression of neuronal TrkB and EphB2 without affecting total cellular levels of the receptors. Furthermore, PS1-knockout neurons show defective ligand-dependent internalization and decreased ligand-induced degradation of TrkB and Eph receptors. Our data show that PS1 mediates the neuroprotective activities of efnB and BDNF against excitotoxicity and regulates surface expression and ligand-induced metabolism of their cognate receptors. Together, our observations indicate that PS1 promotes neuronal survival by regulating neuroprotective functions of ligand-receptor systems.

Keywords: Presenilin; EphrinB; BDNF; EphB; TrkB; Neuroprotection; Neurodegeneration; Glutamate excitotoxicity

1. Introduction

Neuronal exposure to toxic insults, including glutamate excitotoxicity and oxidative stress, has been proposed to play crucial roles in neurodegenerative disorders of the CNS, including Alzheimer's disease (AD). Although glutamate receptors play key roles in neuronal functions, excessive activation of these receptors results in calcium overload and increased oxidative stress that may result in neuronal cell death (Choi, 1996; Greenamyre and Young, 1989; Mattson, 1997). Neurodegenerative disorders, including AD, are characterized by progressive loss of specific neuronal populations, but the mechanisms underlying this selective vulnerability are not fully understood. Aging, abnormal metabolism of the amyloid precursor protein (APP), and genetic mutations, however, may sen-

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sitize neuronal populations to excitotoxicity, thus promoting neurodegeneration and, ultimately, dementia (Mattson, 2003). Missense mutations of PS1, a protein important to the production of Aβ peptides and shown to have both γ-secretase-related and -unrelated functions, promote neurodegeneration and familial AD (FAD) in an autosomal dominant inheritance pattern, suggesting they may cause gain of neurotoxic functions or dominant loss of essential functions (Robakis, 2011). Additional reports indicate that PS1 regulates cell survival signaling (Baki et al., 2004), receptor trafficking (Leem et al., 2002), glutamate release, and calcium homeostasis (Tu et al., 2006; Zhang et al., 2009), and that PS1 mutations increase neuronal vulnerability to hypoxia and excitotoxicity (Mattson et al., 2000).

Recent data show that PS1 functionally interacts with the EphB2 tyrosine kinase receptor (Litterst et al., 2007), a member of the EphB family of receptors shown to play pivotal roles in development and cell function (Martínez and Soriano, 2005; Pasquale, 2008). Neuronal EphB receptors are activated by binding to their cognate ephrinB (efnB) ligands, including efnB1 and efnB2, at the surface of adjacent neurons, a process followed by internalization and degradation of EphB receptors (Litterst et al., 2007; Pitulescu and Adams, 2010). Ligand-activated EphB2 interacts with extracellular sequence of the *N*-methyl-D-aspartate (NMDA) receptor (NMDAR), and recent reports indicate this interaction may be involved in neurodegeneration caused by Aβ oligomers (Cissé et al., 2011). Furthermore, the efnB-EphB binding regulates neuronal physiology and memory-related functions, including neurite outgrowth and synaptic plasticity (Dalva et al., 2000; Pasquale, 2008). At the molecular level, the efnB-EphB interaction stimulates autophosphorylation of the tyrosine kinase domain of EphB2 (Egea and Klein, 2007; Pasquale, 2008), factor binding to the cytosolic sequence of this receptor, and endocytosis and degradation of the efnB-EphB complexes (Pitulescu and Adams, 2010). In addition, we showed that the PS1/γ-secretase system participates in the downstream processing of endocytosed EphB2 receptor after ligand stimulation (Litterst et al., 2007). Here, we report that the efnB-EphB system protects cortical neurons from glutamate excitotoxicity and necrotic death and that this function requires the presence of PS1. We also show that PS1 is required for the neuroprotective activity of other factors, including brain-derived neurotrophic factor (BDNF), and that PS1 regulates the cell surface expression and ligand-induced degradation of EphB2 and TrkB receptors. Surprisingly, however, the neuroprotective function of PS1 is independent of γ-secretase activity. Our data suggest that PS1 promotes neuronal survival by mediating ligand-dependent neuroprotection.

2. Methods

2.1. Materials and antibodies

Polyclonal anti-EphB2 antibody and monoclonal anti-EphB2 antibodies were obtained from Zymed (San Francisco, CA, USA). Anti-TrkB (C-14) was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA); anti-phospho-TrkB (Tyr516) rabbit monoclonal antibody (mAb) was from Cell Signaling (Danvers, MA, USA); and anti-phospho-tyrosine (clone 4G10) was from Upstate Biotechnology (Millipore, Billerica, MA, USA). Antibodies against spectrin antibodies were from Millipore, and anti-cleaved caspase-3 antibody was from Cell Signaling. Necrostatin-1 was from EMD Chemicals (Gibbstown, NJ, USA); EfnB1-Fc and BDNF were obtained from R & D Systems (Minneapolis, MN, USA) and Sigma St. Louis, MO, respectively; Progranulin (PGRN) was prepared as described (Xu et al., 2011); ZVLL (N-benzyloxycarbonyl-Val-Leu-leucinal) and γ-secretase inhibitors (GSIs) were from Calbiochem; and z-VAD (N-benzyloxyxarbonyl-VAL-ALA-ASP fluoromethyl ketone) was from R & D Systems. Clustering of recombinant mouse ephrin-B1/Fc ligands and anti-Fc was performed as described and used at 2 μg/mL for stimulation (Litterst et al., 2007).

2.2. Primary neuronal cultures

All animal experiments were carried out in accordance with the rules and regulations of the Mount Sinai School of Medicine (New York, NY, USA). Rat brain cortical neuronal cultures were prepared as described recently (Xu et al., 2011). Briefly, neurons were plated at a density of 1×10^5 neurons per cm^2 on poly-D-lysine-coated plates in Neurobasal medium supplemented with 2% B27 (Invitrogen, Eugene, OR, USA), L-glutamine (0.5 mM), and penicillin/streptomycin (1% vol/vol), and kept for at least 8 days *in vitro* (DIV) before use as indicated in the figure legends. Under these conditions, postmitotic neurons represent more than 98% of cultured cells (Xu et al., 2011). Neurons from mouse brains of embryonic day 15.5 (E15.5) from wild-type (WT) and transgenic PS1-knockout mice (KOs), heterozygous (PS1+/−) or homozygous (PS1−/−) for PS1, were prepared and genotyped for PS1 as described (Baki et al., 2008). Transgenic KO mice heterozygous for EphB2 (EphB2+/−) and homozygous KOs for EphB1 and EphB3 (EphB1−/−; EphB3−/−) as well as triple KOs for EphB1, EphB2, and EphB3 receptors (EphB2−/−; EphB1−/−; EphB3−/−) were obtained from Dr Henkemeyer (Henkemeyer et al., 2003). Dissociated brain cells were plated onto poly-D-lysine-coated plates at a density of approximately 60,000 cells/cm^2. Cells were maintained in Neurobasal medium supplemented as described previously and used at 8 DIV. Under these conditions, postmitotic neurons represent more than 98% of cultured cells (Baki et al., 2008).