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Research Report

Progranulin promotes activation of microglia/macrophage after pilocarpine-induced status epilepticus

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ABSTRACT

Progranulin (PGRN) haploinsufficiency accounts for up to 10% of frontotemporal lobe dementia. PGRN has also been implicated in neuroinflammation in acute and chronic neurological disorders. Here we report that both protein and mRNA levels of cortical and hippocampal PGRN are significantly enhanced following pilocarpine-induced status epilepticus. We also identify intense PGRN immunoreactivity that colocalizes with CD11b in seizure-induced animals, suggesting that PGRN elevation occurs primarily in activated microglia and macrophages. To test the role of PGRN in activation of microglia/macrophages, we apply recombinant PGRN protein directly into the hippocampal formation, and observe no change in the number of $CD11b^+$ microglia/macrophages in the dentate gyrus. However, with pilocarpine-induced status epilepticus, PGRN application significantly increases the number of $CD11b^+$ microglia/macrophages in the dentate gyrus, without affecting the extent of hilar cell death. In addition, the number of $CD11b^+$ microglia/macrophages induced by status epilepticus is not significantly different between PGRN knockout mice and wildtype. Our findings suggest that status epilepticus induces PGRN expression, and that PGRN potentiates but is not required for seizure-induced microglia/macrophage activation.

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; BSA, bovine serum albumin; DIV, days in vitro; FJB, fluoro-Jade B; FTLD, frontotemporal lobe dementia; GFAP, glial fibrillary acidic protein; HPLC, high performance liquid chromatography; IL-6, interleukin 6; KO, knockout; PGRN, progranulin; LPS, lipopolysaccharide; SE, status epilepticus; SLPI, secretory leukocyte protease inhibitor; WT, wildtype

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1. Introduction

More than 60 different null mutations of the progranulin gene (PGRN) have been identified in patients with frontotemporal lobe dementia (FTLD) (Sun and Eriksen, 2011) and PGRN haploinsufficiency appears to be a major cause of FTLD. The PGRN gene encodes a 68 KDa secretable protein that is widely expressed by neurons and microglia. Recent findings suggest critical roles for PGRN in the central nervous system. First, recombinant PGRN protein promotes neuronal survival and neurite growth in cortical and motor neuron cultures (Van Damme et al., 2008). Loss-of-function studies also support the neurotrophic role of PGRN. Knocking down PGRN with siRNA increases the vulnerability of cultured neuron to NMDA excitotoxicity (Guo et al., 2010). Cultured slices from PGRN knockout mice are more sensitive to lipopolysaccharide (LPS)-induced cell loss (Yin et al., 2010). In fact, PGRN knockout mice have a significantly shorter lifespan (Ghoshal et al., 2012; Wils et al., 2012). Secondly, upregulation of PGRN has been detected in chronic diseases such as Alzheimer's disease (AD) (Baker et al., 2006) and amyotrophic lateral sclerosis (ALS) (Philips et al., 2010), and has also been reported following acute lesions such as sciatic nerve axotomy (Moisse et al., 2009), spinal cord contusion (Naphade et al., 2010), and the quinolinic acid-induced striatal lesion (Petkau et al., 2010). These findings suggest that PGRN may regulate pathological responses in a variety of chronic and acute neurological disorders.

The first aim of this study is to broaden our knowledge of PGRN pathology in the acute response to status epilepticus (SE). SE causes acute injury of the hippocampus, accompanied by microglial activation and infiltration of macrophages (Fabene et al., 2010). Using a well-characterized pilocarpine SE model, we have established the kinetics of PGRN induction. Secondly, in view of evidence showing FTLD-associated symptoms in PGRN-deficient mice (Ghoshal et al., 2012; Petkau et al., 2012; Wils et al., 2012; Yin et al., 2010), supplementing PGRN in vivo may become a valuable therapeutic strategy. Few studies have addressed the side effect of such a strategy. Thus we aim to investigate how recombinant PGRN might affect neuronal survival and activation of microglia/macrophages both in the normal brain and in the pilocarpine SE model.

Our findings demonstrate the induction of PGRN expression in microglia following pilocarpine-induced SE. Using cannula-guided administration of PGRN protein, we found that PGRN promoted SE-induced microglial activation, but did not affect SE-induced cell death in the dentate gyrus. Our findings indicate the upregulation of PGRN protein by epileptic insults, and reveal potential side effects for any PGRN-supplementing strategy.

2. Results

2.1. *Induction of PGRN protein and mRNA after pilocarpine-induced SE*

Seizures have been shown to increase expression of numerous growth factors such as nerve growth factor (Holtzman and Lowenstein, 1995), brain-derived neurotrophic factor (Rudge et al., 1998), vascular endothelial growth factor (Nicoletti et al., 2008), and basic fibroblast growth factor (Riva et al., 1994; Van Der Wal et al., 1994). As a growth factor that plays a key role in the central nervous system, PGRN may be regulated by seizure activity. We tested this hypothesis in the rat pilocarpine model of SE. In the cortex, the PGRN protein levels first remained unchanged at 3 h or 12 h, elevated at 24 h, reached a maximal level at 48 h, and persisted until 96 h after SE (2.0 ± 0.1 fold at 24 h, 4.6 ± 0.7 fold at 48 h, and 3.2 ± 0.2 fold at 96 h post-SE compared with non-SE controls; Fig. 1B). Using real time PCR, we observed a similar induction pattern of PGRN mRNA levels (unaffected at 3 h or 12 h, 1.3 ± 0.1 fold at 24 h, 2.4 ± 0.2 fold at 48 h and 2.3 ± 0.2 fold at 96 h post-SE compared with non-SE controls; Fig. 1C). In the hippocampus, the mRNA and protein levels of PGRN were also enhanced by SE in the following manner: first insignificant at 3 h or 12 h, evident at 24 h (1.9 ± 0.2 fold for protein and 1.5 ± 0.1 fold for mRNA, compared with respective non-SE controls; Fig. 1B and C), further elevated at 48 h (3.1 ± 0.2 fold for protein and 2.3 ± 0.1 fold for mRNA, compared with non-SE controls; Fig. 1B and C), and reaching the maximal levels at 96 h (4.5 ± 0.3 fold for protein and 4.6 ± 0.2 fold for mRNA compared with non-SE controls; Fig. 1B and C). Our data indicated that PGRN expression was markedly increased at 24–96 h following SE at both the transcriptional and translational level.

2.2. *Localization of PGRN in microglia/macrophages after SE*

When PGRN was highly expressed in the brain at 48 h post-SE, reactive gliosis was also observed. We found increased levels of glial fibrillary acidic protein (GFAP), a marker for hypertrophic astrocytes, in the cortical and hippocampal brain lysates at 48 h and 96 h after SE in rats (Supplementary Fig. 2A–2D). We also found greater numbers of $CD11b^+$ microglia/macrophages at 48 h post-SE than in non-SE controls (Supplementary Fig. 2E). The Ox42 antibody recognizes CD11b expressed on the surface of microglia and numerous leukocytes including monocytes and macrophages (Kettenmann et al., 2011). Previous study has shown that leukocytes infiltrate the brain in the pilocarpine model of SE (Fabene et al., 2010). Thus our study does not differentiate between activated microglia and infiltrating macrophages.

We next asked whether PGRN upregulation could be associated with reactive gliosis using double immunohistochemistry. We found PGRN-positive cells that co-stained with CD11b antibody at 48 h post-SE in rats (Fig. 2A). $PGRN^+$/$CD11b^+$ cells significantly outnumbered those in non-SE controls by 4.9 fold in the hippocampus, 30.5 fold in the thalamus, and 31.5 fold in the cortex (Fig. 2D). However, the $PGRN^+$ cells did not colocalize with $GFAP^+$ cells at 48 h post-SE (Fig. 2C). Our results thus indicate that activated microglia but not astrocytes strongly express PGRN after pilocarpine-induced SE.

2.3. *PGRN protein increased $CD11b^+$ microglia/macrophages after seizure induction*

Another approach to study the association between PGRN and microglial activation is to apply PGRN protein in the