Roles of Progranulin in Sexual Differentiation of the Developing Brain and Adult Neurogenesis

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Abstract. Progranulin (PGRN) is a growth modulating factor released by a variety of cells. This molecule has gained the attention of the neuroscience community with recent discoveries of multifunctional roles of PGRN in normal brain and neurodegenerative disorders. We focus on novel roles of PGRN as a sex steroid-responsible gene in the developing and adult rodent brain. While the developing brain is feminine by default, hormone exposure, including androgen and estrogen, induces masculinization during the critical period. We have shown that PGRN is a sex steroid-responsible gene that may be involved in masculinization of the perinatal rat brain. We also found that in adult rats PGRN gene expression was up-regulated by estrogen in the hippocampus, suggesting that PGRN may mediate the mitogenic effects of estrogen in the active area of neurogenesis. Since it has been recently reported that mutations in PGRN gene are responsible for a type of frontotemporal lobar degeneration in humans, PGRN appears to be also involved in modulating neurodegeneration. Together, PGRN gene expression is induced by estrogen in both developing and adult brains, and it may play multifunctional roles in the organization of functional masculinization in the developing brain and the maintenance of adult brain function.

Key words: Brain, Estrogen, Neurogenesis, Progranulin, Sexual differentiation

Progranulin (PGRN) is a trophic factor that has gained attention with recent discoveries of its multifunctional roles in normal brain development and neurodegenerative disorders. In the last decade, we have demonstrated novel biological aspects of PGRN as a sex steroid-responsible gene in the developing and adult rodent brain. This review summarizes the recent results obtained in our and other laboratories concerning the potential roles of PGRN as a mediator of sex steroids in sexual differentiation of the developing brain and adult neurogenesis.

Background: What is PGRN?

PGRN, also called as granulin/epithelin precursor (GEP), proepithelin (PEPI), PC cells-derived growth factor (PCDFG) and acrogranin, is a glycosylated protein released by a variety of cells and is potentially mitogenic in cell culture [1]. It consists of 7½ sequentially arranged granulin (GRN) motifs in tandem. PGRN can be cleaved by extracellular proteases into several GRN peptides, which probably have separate functions. GRN peptides, also called epithelins [2], were initially identified as peptides of approximately 6-kDa, some of which can modulate the growth of cells in tissue culture [1]. They are rich in cysteine and possess a unique structurally defined motif of six disulfide bonds. PGRN mRNA has been demonstrated in various tissues and organs including the reproductive organs, gastrointestinal tract, endocrinal organs, and neural tissues [3, 4]. It is particularly prominent in epithelial and hematopoietic cells, and tends to be more highly expressed in tissues with high turnover rates such as gastric mucosa, lymphoid tissue, and tumor cell lines [4, 5]. In contrast, most mitotically quiescent epithelia, such as those of the lung or renal tubules, express PGRN at relatively low levels [4]. PGRN is mitogenic for epithelial cells and several kinds of cancer cells and also promotes tumor cell invasiveness [6–12]. It has been suggested that PGRN is a steroid-regulated growth factor and mediates the mitogenic effect of estrogens in breast cancer tumorgenesis [13, 14]. PGRN is expressed in the acrosome of the sperm [15] and oocytes [16] and modulates the development of early embryos in vitro [17].

PGRN has been also implicated in wound healing and inflammation [18–20]. During the wound repair response, PGRN is upregulated and stimulates neutrophil and macrophage infiltration and neovascularization of wound tissue [19]. PGRN and GRN peptides also regulate inflammation with opposing effects, and their production is regulated by novel interactions between PGRN, secretory leukocyte protease inhibitor (SLPI), and a serine protease elastase [19]. SLPI is a protein with protease inhibitor domains that has been implicated in regulating proteolysis. In the periphery the relative balance between the activities of elastase and SLPI influences the levels of the anti-inflammatory PGRN and the pro-inflammatory GRN proteins [19]. PGRN is also upregulated in response to hypoxia and acidic stress in fibroblasts in culture [21], which indicates PGRN serves as a multiple-stress responsive factor.

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In the central nervous system (CNS), PGRN is widely expressed during early neural development but later on its expression becomes restricted to specific neuronal populations including cortical neurons in several layers, pyramidal cell layer and dentate gyrus of the hippocampus, ventromedial and arcuate nuclei of the hypothalamus, amygdale, and Purkinje cell layer in the cerebellum [4, 22, 23]. At the cellular level, PGRN immunoreactivity is found in neuronal perikarya, dendrites and axons. PGRN is also expressed in many non-neuronal cell types in CNS and particularly prominent in microglia [24], but also present in endothelial and smooth muscle cells and in the choroid plex and ependyma. There is no or little PGRN immunoreactivity in astrocytes or oligodendrocytes under normal conditions, but PGRN expression is observed in reactive astrocytes. While the exact function of PGRN in normal CNS is still unclear, recent results suggest that this molecule is involved in neurotrophic activity and neuroinflammation [23, 25]. An in vitro study shows that PGRN enhances neuronal survival and neurite length in cultured cortical and motor neurons [26]. Interestingly, these effects were abolished by coadministration of SLPI, suggesting that PGRN/GRN conversion plays a crucial role in their actions. In the CNS, SLPI has been known to be expressed in reactive astrocytes and upregulated with neuroprotective properties in ischemic stroke [27]. Furthermore, cultured microglial cells are known to produce elastase. Consequently, there may be interaction between PGRN, SLPI, elastase in the CNS neuronal repair process as seen in wound healing in peripheral tissues [25].

Recently, much attention has been paid to the functional role of PGRN in the CNS, because PGRN plays a key role for disease progression in neurodegenerative diseases [23, 24, 28–33]. Mutations in PGRN gene were recently identified as the cause of some forms of autosomal dominant tau-negative frontotemporal lobar degeneration (FTLD) [24, 28], which is represented by severe atrophy in the frontal lobe and temporal lobe of the brain and recognized as the common cause of dementia after Alzheimer’s disease (AD). The majority of FTLD-causing mutations in PGRN are predicted to cause functional null alleles by nonsense-mediated decay of the mutant mRNA [24, 28]. Therefore, haploinsufficiency with reduced PGRN-induced neuronal survival is thought to cause neurodegeneration in FTLD. Apart from null-function mutations in FTLD, PGRN gene expression is increased in a number of neurodegenerative diseases in which microglial activation occurs, including lysosomal storage disorders, viral encephalitis, prion-related disease (Creutzfeldt-Jakob disease), AD, and Amyotrophic Lateral Sclerosis (ALS) [23]. It is still unclear how PGRN contributes in the pathological progression of these inflammatory neurodegenerative disorders. In contrast to FTLD, mechanisms other than the lack of neurotrophic effects may play a role in PGRN-associated neurodegeneration as well. In a recent study, PGRN knockdown was found to induce caspase-dependent cleavage of TAR DNA binding protein-43 (TDP-43) with accumulation of its fragments, which is thought to work as the pathologic substrate of neuronal glial inclusions in FTLD and ALS [34].

**PGRN as a Steroid-inducible Gene in the Developing and Adult Rat Brain**

While the roles of PGRN in the normal brain and neurodegeneration remain to be determined, our recent observations propose novel biological aspects of this molecule. We have shown that PGRN is a sex steroid-responsive gene that may be involved in masculinization of the perinatal rat brain [22, 35–38]. We also showed that PGRN may be involved in the mitogenic effects in the active area of neural generation (neurogenesis) of the adult rat brain [39].

**PGRN in sexual differentiation of the developing brain**

Some physiological and behavioral brain functions are gender-specific [40–42]. This is particularly evident, but not limited, in reproductive physiology such as patterning secretion of reproductive hormones and sexual behaviors. A variety of hormones and neurotransmitters show different degrees of variety between males and females, but sex differences have also been reported in non-sexual behaviors like spatial orientation and verbal fluency, or adaptive mechanisms of the adrenal axis to stress [43].

The developing brain is feminine by default, unless some specific stimuli drive it to a male phenotype. The mechanisms of sexual differentiation of the brain by sex steroids seem to be conserved throughout the mammalian species, although there may be some species differences. In rats, the critical period for sexual differentiation of the brain has been considered from embryonic 18 until around postnatal day 10 [41]. The organization of the brain during the critical period is followed by the activational effects of hormones on sexual behavior in adulthood, leading to the organizational/activational hypothesis of the brain and behavior [44]. While testicular androgen is a primary molecule to induce sexual differentiation in the male rat brain, administering large doses of estrogen can also induce masculinization of females and appears to fully mimic the effects of endogenous androgen. The developing fetus has high levels of circulating α-fetoprotein, a protein that potently binds estrogen. Furthermore, brain nuclei that are sexually dimorphic express high levels of aromatase, the enzyme that converts testosterone to estrogen [45, 46]. In the developing male rat, testosterone secreted from the testes, which is not bound to α-feto-protein, can enter the brain, and is locally converted to estrogen by aromatase in specific nuclei. This hypothesis, named the aromatization hypothesis, has been supported by additional data that blocking of neuronal aromatase or absence of this key enzyme during development prevents normal sexual differentiation of the male rodent brain.

A major unveiled question is the underlying mechanism by which estrogen mediates sexual differentiation of the rat brain. Sex steroids exert profound and selective influences on brain development through the regulation of neuronal and glial proliferation and differentiation [40, 45, 47]. Most of these effects by estrogen occur through interactions with estrogen receptors, which serve as transcription factors for a wide variety of cellular target genes. Therefore, analysis of the specific gene expression or protein synthesis induced by sex steroids would provide an approach for understanding the underlying mechanism of sexual differentiation of the rat brain [48–50].
Under this hypothesis, we investigated genes differentially expressed between sexes or induced expression by steroid treatment in neonatal rat hypothalamus using cDNA subtraction [22], and the PGRN gene was identified as one of the sex steroid-inducible genes [22]. The cDNA subtraction method is a simple and efficient technique for isolating clones that are unequally distributed between two samples of cells or tissues. PGRN mRNA was strongly enriched in the hypothalamus of male and androgenized (testosterone-treated) female 5-day-old pups. Transcription of PGRN was also up-regulated by exogenous estrogen in the neonatal hypothalamus [35], indicating that in male rats androgen may induce PGRN gene expression after being converted to estrogen. The expression of PGRN in the hypothalamus of males is maintained at high levels throughout the critical period, while in females it gradually decreased and abruptly declines after birth [22]. In the brain of a 5-day-old male rat, PGRN mRNA was strongly enriched in the ventromedial hypothalamic nucleus (VMH) and the arcuate nucleus (ARC) of the hypothalamus [22]. Interestingly, a dense assembly of estrogen receptors has been shown in VMH and ARC, and sex steroids have been shown to affect to the synaptic structures in these area. Furthermore, VMH is known to play essential roles in the dimorphism of sexual behavior in rats.

Different patterns of PGRN expression between the sexes led us to hypothesize that higher PGRN expression in the neonatal hypothalamus during the critical period is requisite for the process of masculinization of the brain. To test this possibility, we adapted the antisense oligo-deoxynucleotide (ODN) method [36]. This method has been applied to cell culture and animals to block translation of the selective mRNA into protein. We designed antisense ODN complementary to PGRN mRNA sequence and infused them into the third ventricle of male rats at 2 days of age. After maturation, the subject animals that were treated with the antisense ODN had compromised male sexual behaviors as adults [36].

As an alternative approach to block PGRN expression in the brain and to elucidate the physiological roles of PGRN in vivo, we recently generated a line of mice with targeted disruption of the PGRN gene, and investigated male sexual behavior, aggression and anxiety [38]. PGRN-deficient mice exhibited a decrease in ejaculation incidence, while the latency and frequency of both mount and intromission were unchanged. For the aggressive behavior test, the resident-intruder paradigm was used, and PGRN-deficient mice exhibited enhanced aggressiveness. In wild-type mice, males exhibited lower levels of anxiety than females by the open field test, while male PGRN-deficient mice exhibited an elevated level of anxiety and sex difference in anxiety was not observed. Interestingly, mRNA expression of the serotonergic receptor 5-HT1A, which could be related to the inhibition of aggression and anxiety, was significantly reduced in the hippocampus of PGRN-deficient mice after aggressive encounters. On the other hand, deficiency of the PGRN gene did not affect serum testosterone concentrations. These results suggest that PGRN gene plays a role in establishing sexual dimorphic behaviors at least partially by modulating the brain serotonergic system. We have further demonstrated that PGRN-deficient mice have a larger volume of the locus ceruleus (LC), which is known to participate in inducing anxiety-like behavior [51]. This suggests that PGRN plays a role in the organization of the LC, which eventually modulates anxiety in novel environments.

**PGRN and endocrine disruptors**

Endocrine disruptors (or environmental endocrine disrupting chemicals, EDCs) are xenobiotic substances that act like hormones in the endocrine system and disrupt the physiologic function of endogenous hormones [52]. Studies have linked endocrine disruptors to adverse biological effects in animals, giving rise to concerns that low-level exposure might cause similar effects in human beings. Due to the plasticity and the high responsiveness of the fetal brain, its exposure to compounds able to interfere with these mechanisms during the critical period of brain sexual differentiation might cause detrimental effects on reproductive physiology [53, 54]. More precise methods are necessary to define the impact of EDCs on the sexual differentiation of the brain. According to our observations of the steroid-dependent induction and sexually different expressing patterns, PGRN gene may be a good parameter for assessing sex steroid properties of EDCs in the neonatal brain. We recently assessed the effects of perinatal exposure of some phthalate/adipate esters, which are suspected to interfere with the endocrine system as EDCs, on PGRN gene expression in the neonatal hypothalamus and sexual behaviors after maturation [55]. PGRN expression was affected in the brains of male and female neonatal rats by perinatal exposure to these chemicals, while these treatments decreased sexual behaviors after maturation in other cohorts of rats. Perinatal exposure to these compounds may lead inappropriate expression of PGRN gene in the hypothalami of neonatal rats, followed by permanent effects on the hypothalamus to alter the exhibition of sexual behaviors after maturation.

**PGRN in adult neurogenesis**

Recent studies have shown the presence of active neurogenesis even in specific areas of the adult brain, and it has been suggested that estrogen and various growth factors influence the processes of adult neurogenesis. In the hippocampus, neural precursor cells are located in the subgranular zone, which is the border region between the granule cell layer and the hilus in the dentate gyrus, and these precursor cells proliferate and produce daughter cells that are capable of differentiation into mature granule neurons [56]. Adult neurogenesis in the dentate gyrus has been recognized to be involved in learning and also the behavioral effects of antidepressants. Among the factors regulating neurogenesis, more attention has been paid to estrogen since it is known to enhance cell proliferation and increase the number of immature neurons in the adult dentate gyrus [57–60]. Enhanced neurogenesis is thought to be one of the routes through which estrogen exerts its effect on cognitive functions [61].

Given increasing evidence that PGRN is potentially mitogenic in culture and its expression is up-regulated by sex steroids in the developing CNS, we hypothesized that PGRN is also involved in the mitogenic effects of sex steroids in the active areas of neurogenesis in the adult brain. To answer this possibility, we recently assessed cell proliferation in the dentate gyrus and the mRNA expression levels of PGRN in the hippocampus 4 hours after treatment with estrogen in young adult (3-month old) and aged (12-month old) ovariectomized rats [39]. In young adult rats, PGRN
gene expression and cell proliferation were increased by estrogen. However, neither PGRN gene expression nor cell proliferation in the dentate gyrus was affected by estrogen in aged females. Additionally, estrogen enhanced the proliferation of neural progenitor cells derived from hippocampal tissue of 3-month-old female rats in vitro; this was inhibited by neutralization of PGRN with specific antibodies. Together, these results suggest that PGRN may be involved in the mitogenic effects of estrogen in active areas of neurogenesis in the adult brain and that the product of this gene is involved in the mitotic effects of estrogen in the dentate gyrus, although the responses to estrogen decline with age.

**Conclusion**

We have shown that sex-steroid exposure during the perinatal period increased PGRN expression in the rat hypothalamus. The reduction or depletion of PGRN expression influenced the exhibition of sexual dimorphic behaviors in the antisense ODN-treated rats or PGRN-deficient mice. Furthermore, PGRN works as a good parameter for assessing the estrogenic or anti-estrogenic actions of EDCs on the neonatal brain. These observations support our hypothesis that PGRN is involved in the organization of the male brain during the critical period. PGRN during the critical period might play a role in modulating the proliferation and differentiation of neurons and/or glial cells in an autocrine or paracrine manner, and consequently may masculinize the neuronal circuit necessary for exhibiting sexual dimorphic behaviors [37]. PGRN is a complex protein that has distinct functional properties as a precursor protein and its cleaved peptides. Although secretory and processing mechanisms of PGRN in the CNS still need to be resolved, SLPI may contribute to PGRN processing in the CNS since this protein is known to be generated in astrocytes. Additionally, the steroid-dependent induction of PGRN gene was also observed in the dentate gyrus of adult rat hippocampus, suggesting PGRN may play a key role to modulate the mitotic effects of estrogen in active areas of neurogenesis. Our findings indicate that PGRN may be involved in both sexual differentiation of the brain during perinatal period and neurogenesis in the hippocampus in adulthood in a sex-steroid dependent manner. This suggests that there is a common molecular mechanism between the developmental and neurotrophic effects of sex-steroids in the brain. Since haptolinsufficiency of PGRN has been shown to cause FTLD, PGRN appears to play multifunctional roles in the organization of functional masculinization in developing brain and the maintenance of adult brain functions by modulating neurogenesis and neurodegeneration (Fig. 1).

It is important to understand how estrogen modulates PGRN expression and its trophic effects in the developing and adult CNS. Additional studies using in vitro systems and PGRN-deficient mice might be helpful to answer these questions. Further investigations will lead us to understand the biological roles of PGRN in the normal CNS and neurodegenerative diseases.

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**References**

Protein Kinase C Isoforms: Unique Mediators of Membrane Dysfunction

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Abstract

Protein kinase C (PKC) isozymes have long been implicated in the regulation of membrane function. Recent findings have revealed unique roles for PKC isozymes in mediating stress-induced cell death and in regulating the expression of membrane receptors and ion channels. This review focuses on the mechanisms by which PKC isozymes regulate membrane function and presents evidence that PKC isozymes may act as mediators of cell death. The review also highlights recent findings that suggest PKC isozymes may provide a link between membrane function and stress-induced cell death.

Keywords: protein kinase C, membrane function, stress-induced cell death, membrane receptors, ion channels

Introduction

PKC isozymes have been implicated in the regulation of membrane function, including the regulation of ion channels and membrane receptors. Recent findings have revealed unique roles for PKC isozymes in mediating stress-induced cell death and in regulating the expression of membrane receptors and ion channels. This review focuses on the mechanisms by which PKC isozymes regulate membrane function and presents evidence that PKC isozymes may act as mediators of cell death. The review also highlights recent findings that suggest PKC isozymes may provide a link between membrane function and stress-induced cell death.

Mechanisms of PKC Regulation of Membrane Function

PKC isozymes have been shown to regulate the activity of membrane receptors and ion channels. For example, PKC activation has been shown to increase the activity of the adrenergic receptor

Possible Mechanisms of PKC Regulation of Membrane Function

One possible mechanism by which PKC isozymes regulate membrane function is through the regulation of membrane receptor activity. PKC activation has been shown to increase the activity of the adrenergic receptor

Conclusion

In conclusion, PKC isozymes have long been implicated in the regulation of membrane function. Recent findings have revealed unique roles for PKC isozymes in mediating stress-induced cell death and in regulating the expression of membrane receptors and ion channels. This review highlights the unique roles of PKC isozymes in regulating membrane function and presents evidence that PKC isozymes may act as mediators of cell death. Future studies are needed to fully understand the mechanisms by which PKC isozymes regulate membrane function and to determine the role of PKC isozymes in stress-induced cell death.

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References


