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Gαz regulates BDNF-induction of axon growth in cortical neurons

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ABSTRACT

The disruption of neurotransmitter and neurotrophic factor signaling in the central nervous system (CNS) is implicated as the root cause of neuropsychiatric disorders, including schizophrenia, epilepsy, chronic pain, and depression. Therefore, identifying the underlying molecular mechanisms by which neurotransmitter and neurotrophic factor signaling regulates neuronal survival or growth may facilitate identification of more effective therapies for these disorders. Previously, our lab found that the heterotrimeric G protein, Gz, mediates crosstalk between G protein-coupled receptors and neurotrophin signaling in the neural cell line PC12. These data, combined with Gαz expression profiles – predominantly in neuronal cells with higher expression levels corresponding to developmental times of target tissue innervation – suggested that Gαz may play an important role in neurotrophin signaling and neuronal development. Here, we provide evidence in cortical neurons, both manipulated ex vivo and those cultured from Gz knockout mice, that Gαz is localized to axonal growth cones and plays a significant role in the development of axons of cortical neurons in the CNS. Our findings indicate that Gαz inhibits BDNF-stimulated axon growth in cortical neurons, establishing an endogenous role for Gαz in regulating neurotrophin signaling in the CNS.

Introduction

Understanding the mechanisms by which neurons develop polarity and extend axons and dendrites is critical for understanding nervous system development and disorders related to this development. While a number of growth factors have been shown to impact neuron development, much is yet to be learned regarding the regulation of intracellular signaling networks that govern this process. Several lines of evidence indicate G protein coupled receptors (GPCRs) that play important roles in synaptic communication may also play a significant role in neuron development (McCobb et al., 1988; Ponimaskin et al., 2007; Prokosch et al., 2010; Reinoso et al., 1996).

Neurotransmitter monoamines, including norepinephrine, serotonin, and dopamine have been shown to augment (Lieske et al., 1999; Reinoso et al., 1996; Song et al., 2004) or inhibit (Haydon et al., 1984; Reinoso et al., 1996; Spencer et al., 1996) neurite growth in a highly context-specific manner. Additionally, several disorders that have been traditionally characterized by disregulation of monoamines have in recent years also been identified as having a developmental and/or neurotrophic basis, some examples include schizophrenia, chronic pain, epilepsy, and depression (Hendry et al., 2000; Hinton et al., 1990; Hisata et al., 2007; Ho and Wong, 2001; Hsu et al., 1979; Huang et al., 1999; Hughes et al., 2001). Together, these findings are suggestive of an important role for G proteins and GPCRs in the regulation of growth pathways during neuron development.

Gαz is a member of the Gαi subfamily of heterotrimeric G proteins, and couples to GPCRs accordingly. Gαz has been shown to preferentially couple to several types of GPCRs in cells and in vivo (Ho and Wong, 2001; Kimple et al., 2009), including the u-opioid (Hendry et al., 2000; Sanchez-Blazquez et al., 2009), α_2-adrenergic (Kelleher et al., 2001; Meng and Casey, 2002; Yang et al., 2000), 5-HT_{1A} serotonin (Oleskevich et al., 2005; Serres et al., 2000; van den Buuse et al., 2007), and D2 dopamine (Leck et al., 2006; van den Buuse et al., 2005; Yang et al., 2000) receptors. Coupling to these receptors has been primarily demonstrated through altered behavioral responses to receptor-specific agonists in wild-type and Gαz-null mice. In general, Gαz-null mice exhibit increased anxiety and depressive-like behaviors (Oleskevich et al., 2005; van den Buuse et al., 2007). Evidence for Gz coupling to 5-HT_{1A} serotonin receptors comes from studies showing that Gαz-null mice are insensitive to induction of anxious behaviors by a 5-HT_{1A} agonist (van den Buuse et al., 2007), and show significantly increased amplitudes of 5-HT-mediated potassium current and conductance in CA1 pyramidal neurons (Oleskevich et al., 2005). Evidence that Gz couples to the α_{2A}-adrenergic receptor is supported by decreased platelet aggregation and impaired inhibition of cAMP formation in response to epinephrine in Gαz-null mice (Hsu et al., 1979; Kelleher et al., 2001; Yang et al., 2000, 2002). Gαz-null mice also exhibit a loss of the antidepressant effects of catecholamine

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reuptake inhibitors reboxitine and desipramine (Hendry et al., 2000; Yang et al., 2000). A role for Gz in dopaminergic signaling was first demonstrated with the finding that Gαz-null mice exhibited a highly exaggerated response to cocaine (Yang et al., 2000), and these mice are less sensitive to the impact a D2-specific receptor agonist in a number of behavioral and physiologic responses (Leck et al., 2006). Gαz-null mice also exhibited altered responses to amphetamine with regard to locomotor activity and prepulse inhibition response (Ralph et al., 1999; van den Buuse et al., 2005).

In almost every case of receptors that couple to Gz, there is evidence suggesting a developmental role for the pathways they control (Haydon et al., 1987; Koert et al., 2001; Prokosch et al., 2010; Reinoso et al., 1996). In this regard, we previously identified a regulatory role for Gαz in neurotrophin signaling and cellular differentiation. Stimulation of α_{2A}-adrenergic receptors in PC12 cells expressing Gαz resulted in impairment of nerve growth factor (NGF)-mediated differentiation (Meng and Casey, 2002). This impact of Gαz on neurotrophin signaling was shown to be mediated through the monomeric GTPase Rap1; activated Gαz blunted both the activation of Rap1 and subsequent phosphorylation of Erk by NGF in PC12 cells (Meng and Casey, 2002).

Gαz tissue distribution is quite restricted, primarily to neuronal and neuroendocrine cells, and the protein has been shown to be highly expressed in cortex, hippocampus, cerebellum, and as well as in ganglion cells of the retina (Hinton et al., 1990; Kimple et al., 2009). In further support of a role for Gαz in neuronal development, Gαz mRNA is highest in sensory neurons near birth (E16 to P14), corresponding to the time of target tissue innervation (Kelleher et al., 1998). Together, these findings suggest an important role for Gz signaling in neuronal development. Here, we explored the impact of Gαz on cortical neuron development by examining morphological aspects of neurons at early developmental stages in wild-type and Gz-null neurons, as well as in several systems designed to modulate the activity of Gz. We found that Gαz plays a regulatory role in axon growth, particularly with regard to that stimulated by the neurotrophin BDNF.

Results

Cortical neurons from Gz-null mice exhibit enhanced axon growth

The localization Gαz specifically to neurons of the cortical, hippocampal and cerebellar regions of the CNS (Hinton et al., 1990), its developmental regulation (Kelleher et al., 2001; Schuller et al., 2001), and see Supplemental Fig. 1, combined with previous findings regarding its impact on cell growth and morphology (Meng and Casey, 2002), have indicated that Gαz expression increases at early postnatal stages, particularly with regard to cortical brain regions. Such expression suggests that Gαz might provide an important developmental context for regulation of neurotrophin-sensitive neuron development and led us to examine the impact of Gz on cortical neuron morphology and development.

In order to probe the functional impact of Gαz on neuron development, we first examined differences in morphology of cortical neurons cultured from wild-type and Gz-null mice. At early developmental stages (48 to 72 h in culture), cortical neurons cultured from Gαz-null mice demonstrated significantly enhanced axon growth compared to those cultured from wild-type mice (Fig. 1a,b,f,g). Neuron development has been well-characterized as occurring in a series of specific stages of development, with different modes of growth and polarization occurring at each stage (Craig and Banker, 1994; Polleux and Snider, 2010). In this model, initially described by Banker and colleagues, in developmental stage 1, a few hours after plating in culture, a neuron starts to sprout lamellipodia. By stage 2, a growth period of 12–24 h, neurites grow at equal rates and axons and dendrites are indistinguishable. By stage 3, one of the neurites has begun rapid growth and quickly becomes much longer than the other neurites, and cell polarity is apparent (Dotti et al., 1988; Goslin and Banker, 1989). At developmental stage 4, the slower-growing dendrites resume growth. Finally, by stage 5 neurons become mature and fully differentiated with dendritic spine and synapse formation. Thus, the differences in axon length between wild-type and Gz-null cortical neurons at early growth stages could be due to acceleration of the entire developmental process, or of growth during one particular developmental stage.

Initial characterization of the number of neurons populating each of the above-noted developmental stages did not reveal any significant differences between the two genotypes (Supplemental Table 1), suggesting that the difference observed in axon growth in the neurons from the Gαz-null mice was specifically due to an impact at the stage of axon development. Hence, we focused our attention on the axon growth of neurons specifically at stage 3 in their development; i.e. just as they begin to extend their specified axons. Fixed neurons were stained with Tau-1, an axonal marker, and neurons fitting into the stage 3 category, defined by the presence of Tau-1 staining and a clearly defined axon that was $\geq 2\times$ the length of the longest dendrite, was measured. As shown in Fig. 1b, f and g, cortical neurons from Gz-null mice exhibited significantly enhanced axon growth and branching at stage 3 as compared to the wild-type counterparts. No significant differences in dendrite growth were observed between wild-type and Gz-null neurons at either 48 or 72 h in culture (Fig. 1c,d). These data indicate that loss of Gαz impacts the early stages of axon growth.

Localization of Gαz to axon growth cones

In order to better characterize the localization of Gz in cortical neurons, we constructed a YFP-Gαz fusion protein. Several groups have demonstrated that internal fluorescent protein fusions of Gα could be constructed without disrupting their functionality (Hughes et al., 2001; Hynes et al., 2004; Yu and Rasenick, 2002). For the expression of the FP-tagged Gαz, we used an internal fusion of Gαz modeled after a CFP-Gαs created by Berlot and colleagues by inserting YFP inside the constitutively-active variant of Gαz, GαzQ205L (Hynes et al., 2004). This YFP-Gαz internal fusion was characterized for its localization in HEK cells and it ability to inhibit cAMP (Supplemental Fig. 2). The YFP-Gαz Q205L displayed the same functionality as the untagged GzQ205L in each of these respects.

Expression of YFP-Gαz Q205L in cortical neurons revealed that the protein was localized at growth cones. YFP-GαzQL co-localized well with standard markers of axon growth cones, phospho-JNK (Fig. 2a) and GAP-43 (Fig. 2b), but also appears to a lesser extent to be present throughout the axon, including in filopodial protrusions (Fig. 2c). In further support of a specific targeting of Gαz to growth cone isolations, we performed differential centrifugation followed by a Ficoll gradient and found that endogenous Gαz was enriched with other growth cone proteins (Fig. 2d).

Activated Gαz inhibits BDNF-stimulated axon growth

The finding that cortical neurons lacking Gαz have enhanced axon growth raised the question as to the underlying cellular mechanism of this impact. We could envision three possible places where Gz could impact on axon development: i) on the inherent axon growth properties, ii) on the sensitivity of neurons to a growth factor in the media, or iii) on growth factor release, i.e. in an autocrine function. In order to further probe the underlying mechanism, we employed a single-cell based assay where axon growth of a few cells expressing a dominant-active variant of Gαz could be monitored amidst a neuronal culture predominantly expressing the endogenous levels of Gαz found in wild-type cortical neurons. To do this, we implemented a low-efficiency transfection method to express the fluorescent protein fusion of the dominant-active Gαz Q205L in ~10% of the neuronal population.

In cortical neurons isolated from wild-type mice cultured under basal conditions, no significant difference was observed in axon growth between those expressing the YFP control protein and those expressing