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Systemic or Forebrain Neuron-Specific Deficiency of Geranylgeranyltransferase-1 Impairs Synaptic Plasticity and Reduces Dendritic Spine Density

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Abstract—Isoprenoids and prenylated proteins regulate a variety of cellular functions, including neurite growth and synaptic plasticity. Importantly, they are implicated in the pathogenesis of several diseases, including Alzheimer's disease (AD). Recently, we have shown that two protein prenyltransferases, farnesyltransferase (FT) and geranylgeranyltransferase-1 (GGT), have differential effects in a mouse model of AD. Haplodeficiency of either FT or GGT attenuates amyloid-β deposition and neuroinflammation but only reduction in FT rescues cognitive function. The current study aimed to elucidate the potential mechanisms that may account for the lack of cognitive benefit in GGT-haplodeficient mice, despite attenuated neuropathology. The results showed that the magnitude of long-term potentiation (LTP) was markedly suppressed in hippocampal slices from GGT-haplodeficient mice. Consistent with the synaptic dysfunction, there was a significant decrease in cortical spine density and cognitive function in GGT-haplodeficient mice. To further study the neuron-specific effects of GGT deficiency, we generated conditional forebrain neuron-specific GGT-knockout ($GGT^{f/f}Cre+$) mice using a Cre/LoxP system under the CAMKIIα promoter. We found that both the magnitude of hippocampal LTP and the dendritic spine density of cortical neurons were decreased in $GGT^{f/f}Cre+$ mice compared with $GGT^{f/f}Cre-$ mice. Immunoblot analyses of cerebral lysate showed a significant reduction in cell membrane-associated (geranylgeranylated) Rac1 and RhoA but not (farnesylated) H-Ras, in $GGT^{f/f}Cre+$ mice, suggesting that insufficient geranylgeranylation of the Rho family of small GTPases may underlie the detrimental effects of GGT deficiency. These findings reinforce the critical role of GGT in maintaining spine structure and synaptic/cognitive function in development and in the mature brain.

Key words: protein prenylation, geranylgeranyltransferase, knockout mouse models, small GTPases, synaptic plasticity, dendritic spine density.

INTRODUCTION

Proteins can undergo different types of posttranslational modifications which result in proper tertiary structure, function, and subcellular location (Krishna and Wold, 1993). One important posttranslational modification is prenylation (Lane and Beese, 2006), which is the process of adding short-chain lipid molecules (isoprenoids) to target proteins via an irreversible covalent bond. Isoprenoids are intermediates in the mevalonate/cholesterol biosynthesis pathway (Goldstein and Brown, 1990) (Fig. 1). The two major isoprenoids are the 15-carbon farnesyl pyrophosphate (FPP) and the 20-carbon geranylgeranyl pyrophosphate (GGPP). FPP and GGPP are substrates of farnesyltransferase (FT)

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Abbreviations: ALS, amyotrophic lateral sclerosis; aCSF, artificial cerebrospinal fluid; AD, Alzheimer's disease; ANOVA, analysis of variance; AO, apical oblique; BS, basal shaft; CaMKII α, Ca2+/calmodulin-dependent protein kinase; CNS, central nervous system; Cre+, Cre recombinase transgene positive; fEPSPs, field excitatory post-synaptic potentials; FPP, farnesyl pyrophosphate; FT, farnesyltransferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GGPP, geranylgeranyl pyrophosphate; GGT, geranylgeranyltransferase-1; GGT+/−, GGT-haplodeficient; $GGT^{f/f}$, GGT gene floxed; $GGT^{f/f}Cre+$, conditional forebrain neuron-specific GGT knockout; GTP, guanosine triphosphate; I/O, input/output; LTP, long-term potentiation; PPF, paired-pulse facilitation; WT, wild type.

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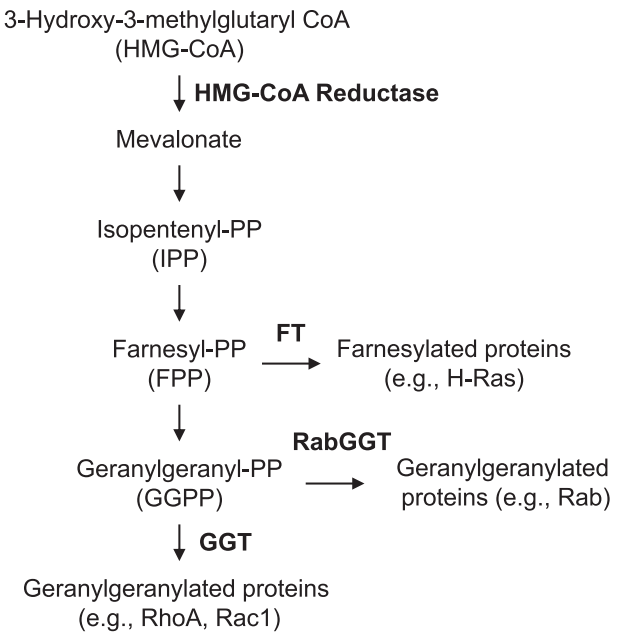

Fig. 1. Isoprenoid synthesis and protein prenylation pathways. PP pyrophosphate; FT, farnesyltransferase; GGT, geranylgeranyltransferease-1; RabGGT, geranylgeranyltransferase-2.

and geranylgeranyltranfersase-1 (GGT), which respectively catalyze the attachment of farnesyl or geranylgeranyl group to target proteins with the CAAX motif (McTaggart, 2006). A third prenyltransferase, geranylgeranyltranfersase-2 (RabGGT), also uses GGPP as its substrate to prenylate target proteins. Over 100 proteins are known to undergo prenylation, including heterotrimeric G-protein subunits and nuclear lamins (McTaggart, 2006; Berndt et al., 2011). Notably, the largest and most well-studied group of prenylated proteins is the Ras superfamily of small GTPases such as Ras, Rho, and Rab proteins. These small GTPases serve as molecular switches and regulate a plethora of cellular processes and functions, including dendritic spine morphogenesis and synaptic plasticity (Hottman and Li, 2014). The importance of protein prenylation is further underscored by the findings that germline deletion of FT or GGT is embryonically lethal (Mijimolle et al., 2005; Sjogren et al., 2007), and dysregulation of prenylated proteins causes cancers and a number of other diseases including cardiovascular and cerebrovascular diseases, bone diseases, progeria, and potentially, neurodegenerative diseases such as Alzheimer's disease (AD) (McTaggart, 2006; Li et al., 2012; Wang and Casey, 2016).

We previously demonstrated that haplodeficiency of either GGT or FT reduced amyloid-β accumulation in a transgenic mouse model of AD (Cheng et al., 2013). However, only FT haplodeficiency rescued cognitive function in these animals. GGT haplodeficiency similarly reduced amyloid plaques and neuroinflammation, but was not sufficient to rescue memory function of the animals. As geranylgeranylated Rho family proteins are crucial in synapse/spine formation and remodeling (Newey et al., 2005; Tolias et al., 2011), we hypothesized that GGT deficiency might have detrimental effects that could neutralize the benefits of attenuated AD-related neuropathology. The current study was undertaken to address the impact of GGT deficiency on dendritic spine density and synaptic plasticity, the cellular basis of learning and memory formation (Bliss and Collingridge, 1993; McGaugh, 2000). Our results showed that either germline/systemic GGT haplodeficiency or forebrain neuron-specific GGT deficiency reduced the magnitude of hippocampal long-term potentiation (LTP) and decreased the dendritic spine density of cortical neurons in mice. These findings corroborate the pivotal role of GGT in the development and maintenance of neurophysiological function of the brain.

EXPERIMENTAL PROCEDURES

Animals

Germline/systemic GGT-haplodeficient (GGT+/−) mice have been described previously (Liu et al., 2010; Cheng et al., 2013). The forebrain neuron-specific GGT-deficient mice were generated by breeding the GGT-floxed ($GGT^{f/f}$) mice (Sjogren et al., 2007) with a CaMKIIα promoter-driven Cre recombinase (Cre+) mice (Tsien et al., 1996). Further interbreeding of resulting siblings with genotypes of $GGT^{f/+}$Cre+ and $GGT^{f/+}$Cre− produced $GGT^{f/f}$Cre+ and $GGT^{f/f}$Cre− (wild-type, WT) mice, which were used in this study. All genotypes were determined using DNA extracted from tail biopsies and amplified via PCR using gene-specific primers. The average mouse age was 8–12 months and both male and female were used. Littermate controls were used whenever possible. Investigators were blinded to genotypes of the mice during the experiments. All animal procedures in this study were prospectively reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Minnesota.

Electrophysiology

Mice were anesthetized using isoflurane and the depth of anesthesia was confirmed with a foot pinch, followed by decapitation as previously described (Parent et al., 2014). Briefly, brains were collected and cooled in a "cutting solution" containing (mM): 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 0.5 $CaCl_2$, 7 $MgCl_2$, 7 Dextrose, and 240 sucrose (Sigma). Transverse hippocampal slices (400-μm) were prepared using a vibratome (Leica) while immersed in the ice-cold cutting solution. The slices were allowed to recover for at least 1 h (up to 4 h) in an artificial cerebrospinal fluid (aCSF) containing (mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, and 25 dextrose (pH 7.4) with constant bubbling of 95% O_2/5% CO_2. After recovery, slices were placed into the recording chamber (Automate Scientific, Berkeley, CA) with aCSF flowing at approximately 1.5 ml/min at 28–30 °C. Using a bipolar tungsten electrode (FHC) driven by a constant-current stimulus isolator (World Precision Instruments, Sarasota, FL), electrical stimulations were delivered to the CA3/CA1 boundary of the hippocampus