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Receptor-stimulated transamidation induces activation of Rac1 and Cdc42 and the regulation of dendritic spines

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ABSTRACT

Regulation of dendritic spines is an important component of synaptic function and plasticity whereas dendritic spine dysregulation is related to several psychiatric and neurological diseases. In the present study, we tested the hypothesis that serotonin (5-HT)2A/2C receptor-induced Rho family transamidation and activation regulates dendritic spine morphology and that activation of multiple types of receptors can induce transglutaminase (TGase)-catalyzed transamidation of small G proteins. We previously reported a novel 5-HT2A receptor downstream effector, TGase-catalyzed serotonylation of the small G protein Rac1 in A1A1v cells, a rat embryonic cortical cell line. We now extend these findings to rat primary cortical cultures which develop dendritic spines; stimulation of 5-HT2A/2C receptors increased transamidation of Rac1 and Cdc42, but not RhoA. Inhibition of TGases significantly decreased transamidation and activation of Rac1 and Cdc42, suggesting that transamidation led to their activation. In primary cortical cultures, stimulation of 5-HT2A/2C receptors by 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) caused a transient dendritic spine enlargement, which was blocked by TGase inhibition. Stimulation of both 5-HT2A and 5-HT2C receptors contributed to DOI-induced Rac1 transamidation in primary cortical cultures as demonstrated by selective antagonists. Furthermore, stimulation of muscarinic acetylcholine receptors and NMDA receptors also increased TGase-catalyzed Rac1 activation in SH-SY5Y cells and N2a cells, respectively. Receptor-stimulated TGase-catalyzed transamidation of Rac1 occurs at Q61, a site previously reported to be important in the inactivation of Rac1. These studies demonstrate that TGase-catalyzed transamidation and activation of small G proteins results from stimulation of multiple types of receptors and this novel signaling pathway can regulate dendritic spine morphology and plasticity.

1. Introduction

In the central nervous system, the majority of the excitatory synapses are composed of postsynaptic terminals located on dendritic spines (Phillips and Pozzo-Miller, 2015). Changes in size, number and morphology of dendritic spines are tightly coordinated with synaptic function and plasticity, underlying the establishment and remodeling of neuronal circuits, learning and memory, and behavior (Penzes et al., 2011; Kennedy, 2016). Notably, malfunction of dendritic spines accompanies a large number of brain disorders, including bipolar disorder, autism spectrum disorder, schizophrenia and Alzheimer's disease, suggesting that dendritic spines can serve as a common target for those complex diseases (Penzes et al., 2011, 2013; Konopaske et al., 2014; Phillips and Pozzo-Miller, 2015). Understanding the molecular underpinnings of dendritic spine regulation may provide essential insights into the etiologies of those disorders and may reveal new drug targets.

Morphological changes of dendritic spines are driven by actin dynamics, which can be regulated by small G proteins of the Rho-family. At the synapse, Rac1, Cdc42 and RhoA play a pivotal role in spine formation and morphogenesis, and synaptic plasticity (Martino et al., 2013). Activation of Rac1 and Cdc42 promotes spine formation, growth and stabilization; conversely, RhoA activation leads to spine pruning. Perturbations in Rho family signaling are

Abbreviations: DIV, days in vitro; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; 5-HT, serotonin; TGase, transglutaminase.

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implicated in various brain disorders, particularly those associated with cognitive deficits, such as mental retardation, schizophrenia and Alzheimer's diseases (Ba et al., 2013; Bolognin et al., 2014; Datta et al., 2015).

Numerous studies have demonstrated that the activity of small G proteins including those of the Rho family is regulated by monoaminylation (Muma and Mi, 2015). Monoaminylation is a post-translational modification of proteins in which transglutaminases (TGases) catalyze the transamidation of a primary amine molecule such as serotonin (5-HT) or dopamine to a protein-bound glutamine residue (Muma and Mi, 2015). Serotonylation is a term for the specific transamidation of 5-HT to a protein (Walther et al., 2003). Stimulation of serotonin 2A (5-HT2A) receptors induces serotonylation of Rac1, resulting in Rac1 activation (Dai et al., 2008). An increase in intracellular Ca+2subsequent to receptor stimulation was both necessary and sufficient to stimulate serotonylation and activation of Rac1 (Dai et al., 2011). Together, these findings lead us to hypothesize that multiple receptor subtypes increase TGase-catalyzed transamidation and activation of small G proteins which can alter dendritic spine morphology.

5-HT2A receptors are widely distributed in most forebrain regions. Disrupted function of 5-HT2A receptors has been identified in various neurological and psychiatric disorders such as schizophrenia, Alzheimer's disease (Fehér et al., 2013), autism, depression and anxiety (Gray and Roth, 2007; Berg et al., 2008; Hervás et al., 2014). 5-HT2A receptors are also the target for several antidepressants, anxiolytics, atypical antipsychotics and hallucinogens (González-Maeso et al., 2007; Mestre et al., 2013; Amodeo et al., 2014). 5-HT2A receptors localize to dendrites, dendritic shafts, and dendritic spines (Cornea-Hebert et al., 2002; Peddie et al., 2008). Initiation of 5-HT2A receptor expression coincides with the period of synaptogenesis (Roth et al., 1991). 5-HT2A receptor activation alters dendritic spine area via a kalirin-7 dependent pathway (Jones et al., 2009). Stimulation of 5-HT2A receptors also changes the density of specific subtypes of dendritic spines (Yoshida et al., 2011). Those studies suggest that 5-HT2A receptors play a role in the regulation of dendritic spine architecture and actin cytoskeleton. However, the underlying mechanisms by which the 5-HT2A receptor signaling regulates dendritic spines and the role of serotonylation of Rac1 and possibly other members of the Rho family in the process are not clear.

NMDA receptors can mediate synaptic plasticity such as long-term potentiation (Collingridge et al., 1983). NMDA receptor activation causes an influx of $Ca^{2+}$and activation of calmodulin-dependent kinase II. The Ca^{2+}influx and activation of calmodulin-dependent kinase II result in recruitment of AMPA receptors to the synapse and dendritic spine enlargement both of which are determinants of synaptic strength (Matsuzaki et al., 2004). The Ca^{2+}influx is necessary for activation of small G proteins in the dendritic spines and subsequent increase in spine volume (Murakoshi et al., 2011). We hypothesize that the NMDA receptor-dependent influx of Ca^{2+}increases TGase-catalyzed transamidation and activation of small G proteins. Muscarinic receptor signaling has also been shown to be involved in synaptic plasticity and can produce long-term potentiation (Dennis et al., 2015), perhaps also by a mechanism involving TGase-catalyzed transamidation and activation of small G proteins.

In the present study, we used rat primary cortical cultures which develop dendritic spines to test whether activation of TGase via stimulation of 5-HT2A/C receptors induces transamidation and activation of Rac1, Cdc42 and RhoA, and whether the transamidation results in changes of dendritic spine architecture. SH-SY5Y and N2A cell lines were used for biochemical studies to examine muscarinic, NMDA and AMPA receptors. Our results indicate that both G protein-coupled and ligand-gated ion channel receptors can stimulate transamidation and activation of Rac1 and that transamidation can regulate dendritic spine size.

2. Materials and methods

2.1. Reagents

1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI), *N*-Methyl-D-aspartic acid (NMDA), (S)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and (2-hydroxyethyl) trimethylammonium chloride carbamate (carbachol) (Sigma-Aldrich, St. Louis, MO) and 2-aminoethyl disulfide dihydrochloride (cystamine) (MP Biomedicals, Solon, OH) were dissolved in saline or water and further diluted before application to cell cultures. SB 242084 (Sigma-Aldrich, St. Louis, MO) was dissolved in ethanol (the final concentration of ethanol exposure to cells was 0.1%). MDL100907 was kindly provided by Sanofi Aventis (Bridgewater, NJ) and dissolved in DMSO (the final concentration of DMSO exposure to cells was 0.01%).

2.2. Cell culture and transfection

A1A1v cells, SH-SY5Y and N2a cells were cultured and transfected as previously described for all of the biochemical experiments (Yu et al., 1990; Dai et al., 2008; Coleman et al., 2013). In addition, A1A1v cells were transfected with either of the following mammalian expression plasmid constructs: TGase1 (TGM1, clone ID: OHu23436, Genscript, NJ, USA) or TGase3 (TGM3, clone ID: OHu31856, Genscript, NJ, USA) with a common pcDNA3.1+-DYK vector backbone. Before each experiment, cells were maintained for 48 h in Dulbecco's modified Eagle medium (Fisher Scientific, Pittsburgh, PA) containing 10% fetal bovine serum (Atlanta Biologicals, Flowery Branch, GA) treated with charcoal to remove > 99% of endogenous 5-HT (Unsworth and Molinoff, 1992). Cells used as non-transfected control cells were incubated in charcoal filtered FBS but were otherwise untreated.

2.3. Primary cortical culture

Animals were used in accordance with the National Institute for Health Guide for the Care and Use of Laboratory Animals as approved by the University of Kansas Institutional Animal Care and Use Committee. Primary cortical neurons were isolated from E18 Sprague-Dawley rat embryos as described previously with minor modifications (Srivastava et al., 2011; Beaudoin III et al., 2012). The cortical tissues from feti from each pregnant rat were harvested and the cells from each litter were then pooled. Neurons were plated at a density of 5×10^5 cells/ml on 22 mm diameter round cover glass (Neuvitro Corporation, Vancouver, WA) or at a density of 2.7×10^6 cells/ml on T25 cell culture flasks (Fisher Scientific, Lenexa, KS) coated with poly-L-lysine (Sigma-Aldrich, St. Louis, MO). At 21 days in vitro (DIV), cultures were randomly chosen for treatment with 3 μM DOI or vehicle and in some experiments pretreatment with either 1 mM cystamine or a selective 5-HT receptor antagonist or vehicle.

2.4. Immunocytochemistry

Primary neurons were double-labeled with Alexa Fluor® 568 Phalloidin (Life Technologies, Grand Island, NY) for labeling F-actin, antibodies against microtubule-associated protein 2 (Map2) clone HM-2 (Sigma-Aldrich, St. Louis, MO) for labeling dendrites, antibodies against 5-HT2A receptors (Singh et al., 2007), and antibodies against PSD95 (6G6-1C9) (Life Technologies, Grand Island, NY). Alexa Fluor® 488 donkey anti-rabbit IgG (H + L) antibody and Alexa