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Research report

Voltage-gated calcium and sodium channels mediate Sema3A retrograde signaling that regulates dendritic development

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ABSTRACT

Growing axons rely on local signaling at the growth cone for guidance cues. Semaphorin3A (Sema3A), a secreted repulsive axon guidance molecule, regulates synapse maturation and dendritic branching. We previously showed that local Sema3A signaling in the growth cones elicits retrograde retrograde signaling via PlexinA4 (PlexA4), one component of the Sema3A receptor, thereby regulating dendritic localization of AMPA receptor GluA2 and proper dendritic development. In present study, we found that nimodipine (voltage-gated L-type Ca^{2+} channel blocker) and tetrodotoxin (TTX; voltage-gated Na^{+} channel blocker) suppress Sema3A-induced dendritic localization of GluA2 and dendritic branch formation in cultured hippocampal neurons. The local application of nimodipine or TTX to distal axons suppresses retrograde transport of Venus-Sema3A that has been exogenously applied to the distal axons. Sema3A facilitates axonal transport of PlexA4, which is also suppressed in neurons treated with either TTX or nimodipine. These data suggest that voltage-gated calcium and sodium channels mediate Sema3A retrograde signaling that regulates dendritic GluA2 localization and branch formation.

1. Introduction

Intraneuronal transport is fundamental for neuronal survival, morphogenesis, and function. Proteins, mRNAs, and organelles are selectively transported to either axons or dendrites by kinesin superfamily motor proteins and adaptor or scaffolding proteins (Hirokawa and Takemura, 2005; Hirokawa et al., 2010). Although the mechanism of selective transport by motor/adaptor proteins has been clarified, regulation of this mechanism by extracellular environmental cues remains unclear.

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Sema3A is a secreted protein identified as a factor that collapses and paralyzes the neuronal growth cone by acting through the receptor complex of neuropilin1 (NRP1) and plexinAs (PlexAs), ligand-binding and signal-transducing subunits of the class 3 semaphorin receptor complex, respectively (Luo et al., 1993; Takahashi et al., 1999; Tamagnone et al., 1999). Disruption of Sema3A in mice leads to several abnormalities in neuronal development and function (Behar et al., 1996; Chen et al., 2008; Morita et al., 2006; Nakamura et al., 2009; Polleux et al., 2000; Sasaki et al., 2002; Schlomann et al., 2009; Taniguchi et al., 1997, 2003; Yamashita et al., 2007). We previously showed that Sema3A facilitates dendritic as well as axonal transport in cultured hippocampal neurons. Sema3A initiates signals at axonal growth cone, and these signals are propagated to the somatodendritic compartment by retrograde axonal transport of Sema3A and PlexA4. PlexA interacts with the AMPA receptor GluA2 at the immunoglobulin-like Plexin-transcription-factor domain (Plex-IPT). PlexA4 induces dendritic localization of GluA2 through *cis*-interaction with GluA2 at the Plex-IPT domain in the somatodendritic compartment. The GluA2 in dendrites is necessary for dendritic branching (Saglietti et al., 2007). Hence, the retrograde axonal transport is an essential cellular response to Sema3A, and it is involved in the regulation of dendritic development (Yamashita et al., 2014).

Ion signaling also plays a role in Sema3A-facilitated axonal transport in cultured dorsal root gangliaon (DRG) neurons (Yamane et al., 2012). The facilitation of axonal transport by Sema3A is blocked by inhibitors of L-type voltage-gated calcium ion (Ca^{2+}) channels (VGCCs) and tetrodotoxin (TTX)-sensitive voltage-gated sodium ion (Na^{+}) channels (VGSCs) (Yamane et al., 2012). However, the role of ion signaling in Sema3A-regulated dendritic development is still unknown. In the present study, we examined the local effect of nimodipine, an L-type VGCC inhibitor, and TTX in Sema3A-treated cultured hippocampal neurons. We found that local ion signaling in distal axons mediates retrograde transport of Sema3A, which in turn regulates dendritic GluA2 localization, thereby contributing to dendritic development.

2. Results

2.1. *Ion signals at the growth cone are necessary but not sufficient for Sema3A-induced effects*

Sema3A induces dendritic localization of GluA2 in cultured hippocampal neurons by enhancing retrograde axonal transport (Yamashita et al., 2014). In cultured DRG neurons, Sema3A-facilitated axonal transport depends entirely on extracellular Ca^{2+} and Na^{+} (Yamane et al., 2012). The facilitation of axonal transport is suppressed by L-type VGCC blockers such as nimodipine and nifedipine and by TTX, a VGSC blocker (Yamane et al., 2012). To investigate whether the same ion signaling is involved in Sema3A-induced dendritic localization of GluA2, we treated cultured hippocampal neurons with nimodipine or TTX before Sema3A treatment and examined immunocytochemical localization of GluA2 in dendrites. Fixation was done 30 min after the Sema3A treatment because the effect of facilitated axonal and dendritic transport return to the basal level after 30 min (Yamashita et al., 2014). We found that both nimodipine (1 μM) and TTX (100 nM) suppressed Sema3A-enhanced immunostaining of GluA2 in MAP2-positive dendrites (Fig. 1). This result suggests that Sema3A induces the localization of GluA2 in dendrites through nimodipine- and TTX-sensitive ion signals in the hippocampal neurons.

To further investigate possible role of ion signals in these biological activities of Sema3A, we examined the effect of Sema3A on dendritic branching in cultured hippocampal neurons with or without nimodipine or TTX. Bath application of Sema3A increased the number of branching points in cultured hippocampal neurons. Pretreatment with nimodipine or TTX attenuated Sema3A-induced dendritic branching (Fig. 2). This result suggests that both L-type VGCCs and TTX-sensitive VGSCs are required for Sema3A-induced dendritic branching.

To examine whether the activation of the voltage-dependent ion channels mimicked the effect of Sema3A, we applied a depolarizing stimulus to cultured hippocampal neurons by raising the extracellular concentration of KCl in culture media. Bath application of KCl (10 mM) enhanced the immunostaining of both GluA1 and GluA2 in dendrites (Fig. 3). In contrast, Sema3A has been found to enhance the immunofluorescence levels of GluA2 but not GluA1 (Yamashita et al., 2014). To further understand the difference between Sema3A and KCl stimulation, we next investigated the site of action of KCl by using a microfluidic culture system. This system enabled us to isolate the axonal or somatodendritic regions and stimulate these two parts separately (Park et al., 2006). When Sema3A was locally applied to the axonal areas, but not the somatodendritic areas, the immunostaining of GluA2 in the dendrites increased (Yamashita et al., 2014). In contrast, local application of KCl (10 mM) to the somatodendritic areas, but not to the axonal areas, increased the immunostaining of GluA2 (Fig. 4). These findings indicate that depolarization by KCl at the distal axons is not sufficient to induce dendritic GluA2 localization. In order to check that 10 mM KCl surely causes depolarization at distal axons, we electrophysiologically tested the effect on antidromic population spikes in mouse hippocampal slices (Uchida et al., 2012). Depolarization of distal axons is expected to increase the magnitude of antidromic population spikes due to recruitment of subthreshold fibers to a certain stimulus intensity. In consistent with depolarization of distal axons by 10 mM KCl, the amplitude of the antidromic population spike was initially increased (to 135±14% of the control, $p<0.05$, $n=9$, paired two-tailed *t*-test), and then decreased (to 56±24 of the control, $p=0.13$) (Fig. 5), possibly due to steady state inactivation of axonal voltage-dependent Na^{+} channels, since it initially became larger than control values during washout (to 146±10% of control, $p<0.01$), then recovered to the baseline. Bi-directional sequence of enhancement and suppression of antidromic action potentials is in consistent with the notion of depolarizing action of 10 mM KCl at distal axons.