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Calcium Signaling and the Control of Dendritic Development

Minireview

Sila Konur and Anirvan Ghosh*
Division of Biological Sciences
Neurobiology Section
University of California, San Diego
La Jolla, California 92093

Dendrites serve a critical role in neuronal information processing as sites of synaptic integration. The morphological diversity of dendritic architecture reflects specialized strategies that neurons have evolved to detect and process incoming information. Recent observations suggest that calcium signals exert an important influence on neuronal morphology by regulating the growth and branching of dendrites and the formation of dendritic spines. Calcium signals appear to influence branch dynamics by affecting the cytoskeleton near the site of calcium entry, whereas calcium-dependent dendritic growth involves activation of a transcriptional program.

The establishment of the dendritic tree is a highly dynamic process that is characterized by extension and retraction of branches, followed by stabilization and growth. The early development of dendrites is regulated by cell-intrinsic programs as well as by molecular signals that regulate various aspects of dendritic development (reviewed in McAllister, 2000; Scott and Luo, 2001; Cline, 2001; Whitford et al., 2002a; Miller and Kaplan, 2003; Jan and Jan, 2003). In the developing cortex, these signals include Semaphorin 3A, which regulates apical dendrite orientation (Polleux et al., 2000), neurotrophins, which regulate layer-specific dendritic growth and branching (McAllister et al., 1996; McAllister et al., 1997), Notch1, which restricts dendritic growth (Redmond et al., 2000; Redmond and Ghosh, 2001; Sestan et al., 1999), Slit-1, which acts as a dendritic branching factor (Whitford et al., 2002b), and classical cadherins and their signaling partner β-catenins, which play a role in the stabilization of dendrites and spines (Abe et al., 2004; Togashi et al., 2002; Yu and Malenka, 2003).

In addition to molecular signals, several observations support a role for neuronal activity in regulating dendrite morphology. The time of maximal dendrite growth and remodeling is concurrent with that of afferent innervation (Wise et al., 1979; Wu et al., 1996; Wu et al., 1999). During normal development of the somatosensory and visual systems, dendrites dramatically reorganize in response to synaptic/afferent input. Activity deprivation experiments also suggest that loss of normal activity during development leads to lasting deficits in dendritic development. For example, pharmacological blockade of synaptic activity in vitro and in vivo leads to deficits in dendritic growth (McAllister et al., 1996; Rajan and Cline, 1998; Redmond et al., 2002). Loss of sensory input alters dendritic development in both the lateral geniculate nucleus and the visual cortex (Coleman and Riesen, 1968; Wiesel and Hubel, 1963), and increasing neuronal activity by exposing animals to enriched environments enhances dendritic branching and spine density of cortical pyramidal neurons (Holloway, 1966; Greenough et al., 1973; Volkmar and Greenough, 1972). In the developing retinotectal system, sensory input enhances dendritic growth in tectal neurons (Sin et al., 2002). Recent observations suggest that these effects of activity on dendritic patterning are mediated by calcium signaling. Here, we review our current understanding of the mechanisms by which local and global changes in calcium dynamics regulate dendritic growth and branching.

Regulation of Dendritic Growth by Calcium Signaling Pathways

Many regions of the CNS, including the retina, the hippocampus, and the cortex, show spontaneous calcium transients before the onset of sensory experience (Ben-Ari et al., 1997; Feller et al., 1996; Garaschuk et al., 2000; Yuste et al., 1995). In the neocortex, domains of spontaneous coactivation are observed during the first postnatal week and involve the diffusion of IP3 through gap junctions (Yuste et al., 1995). At the same time, TTX-sensitive calcium waves traveling in the anterior-posterior axis are also detected in the developing cortex (Garaschuk et al., 2000). This period of spontaneous network activity corresponds to a period of the rapid dendritic growth, and emerging evidence suggests that dendritic growth and branching during this period are dependent on calcium signaling.

Calcium levels in neurons are regulated by influx through calcium channels as well as by release of calcium from intracellular stores. Calcium influx is mediated mainly by voltage-sensitive calcium channels (VSCC) and NMDA receptors. Release from stores principally involves calcium-induced calcium release (CICR) or activation by ligands that lead to the production of IP3, which acts on internal stores. Two major signaling targets of calcium influx are calcium/calmodulin-dependent protein kinases (CaMKs) and mitogen-activated kinase (MAPK). Upon calcium entry via VSCC or NMDA receptors, calmodulin binds multiple calcium ions and can activate various intracellular effectors, including CaMKs (reviewed in Ghosh and Greenberg, 1995).

Of the CaMKs, CaMKII has been most extensively studied in relation to a role in dendritic development and function. Two isoforms of CaMKII, CaMKIIα and CaMKIIβ, mediate contrasting outcomes on dendrites. CaMKIIα has been reported to stabilize or restrict dendritic growth of frog tectal neurons in vivo and mammalian cortical neurons in vitro (Wu and Cline, 1998; Redmond et al., 2002). CaMKIIβ, however, has a positive effect on filopodia extension and fine dendrite development mediated by direct interaction with cytoskeletal actin (Fink et al., 2003). CaMKI has also recently been shown to alter cerebellar granule cell dendrite growth and process formation in hippocampal neurons (Wayman et al., 2004). Unlike CaMKI, CaMKIIα, and CaMKIIβ, CaMKIV is predominately localized in the nucleus. Mice

*Correspondence: aghosh@ucsd.edu

lacking CaMKIV have a defect in dendritic development (Ribar et al., 2000). Pharmacological blockade of CaMKs inhibits calcium-induced dendritic growth in cortical neurons, and expression of an activated form of CaMKIV mimics the dendritic growth effects induced by calcium influx (Redmond et al., 2002). The nuclear localization of CaMKIV suggests that it mediates its effect on dendrite growth via transcriptional events.

MAPKs are also activated by calcium influx via NMDA receptors and VSCCs. Repeated depolarization can lead to sustained MAPK activation and influences the formation and stability of dendritic filopodia (Wu et al., 2001). In addition, MAPK has been implicated in mediating growth of SCG dendrites in response to activity by phosphorylating MAP2 (Vaillant et al., 2002). MAPK signaling has also been implicated in mediating the effects of calcium influx on dendrite growth in cortical neurons (Redmond et al., 2002). Thus, CaMKs and MAPKs appear to be key mediators of calcium-dependent dendritic growth.

Calcium-Dependent Transcription and the Control of Dendritic Growth

Regulation of dendritic growth by calcium influx via VSCCs appears to involve calcium-dependent transcription. Redmond et al. (2002) showed that CaMKIV is required for calcium-dependent growth of cortical dendrites. The best-characterized target of CaMKIV is the transcription factor CREB, which is phosphorylated by CaMKIV at Ser-133. The effects of calcium influx and constitutively active CaMKIV on cortical dendrites are suppressed by dominant-negative mutants of CREB, suggesting that CREB-dependent transcription is required for activity-dependent dendritic growth (Redmond et al., 2002). The small GTP binding protein Rap1 also appears to be an important effector of calcium-dependent activation of CREB and activity-dependent dendrite development. Rap1 is rapidly activated by calcium influx, and inhibition of Rap1 suppresses activity-induced CREB phosphorylation and dendritic growth (Chen et al., 2005).

Although CREB is required for calcium-dependent dendritic growth, activation of CREB is not sufficient to induce dendritic growth (L. Redmond and A.G., unpublished data). This suggests that other transcription factors are likely to be involved in mediating activity-dependent dendritic growth. A novel approach for identifying activity-induced transcription factors in cortical neurons led to the discovery of CREST, a calcium-activated transactivator that is required for dendritic growth (Aizawa et al., 2004). CREST is a CBP-interacting protein that is expressed at high levels in the early postnatal cortex. Dendritic growth is severely compromised in the cortex and hippocampus in *crest* knockout mice, indicating that CREST function is required for dendritic development in vivo. Importantly, depolarization-induced dendritic growth is abolished in cortical neurons from CREST mutant animals, supporting a key role for CREST in calcium-dependent dendritic growth. These observations suggest that activity-induced dendritic development in early postnatal life requires activation of a transcriptional program, which is likely to be regulated by CREB, CREST, and CBP.

Regulation of Dendritic Branch Dynamics and Stability by Local Calcium Signals

Spontaneous activity in the brain is gradually replaced by sensory-evoked activity during postnatal development. Work in the retinotectal system suggests that much of the effect of sensory stimulation on dendritic branching may be mediated by NMDA receptor activation. Cline and colleagues have shown that NMDA receptor activation is required for activity-dependent dendritic development in tectal neurons (Rajan and Cline, 1998; Sin et al., 2002). Analysis of mice lacking the NR1 gene suggests that NMDA receptor signaling also plays an important role in regulating at least certain aspects of dendritic development in cortical neurons. Layer 4 neurons in the barrel cortex initially develop symmetric dendritic trees that undergo activity-dependent remodeling to develop arbors that are oriented toward the barrel centers, where the thalamic axons terminate. This remodeling of cortical dendrites fails to take place in mice lacking NR1 subunits in the cortex (Datwani et al., 2002). One possibility is that mechanisms that stabilize dendrites at sites of synaptic contact are compromised in the NR1 null mice.

NMDA receptor signaling also appears to be important in the emergence of dendritic spines, which act as sites of synaptic contact. During postnatal development, spine density doubles, spine motility decreases, and the vast majority of spines acquire mature mushroom spine morphology (Konur and Yuste, 2004; Miller and Kaplan, 2003; Miller, 1981; Miller and Peters, 1981). The formation of new spines can be triggered in vitro by long-term potentiation (LTP)-inducing stimuli, suggesting that NMDA receptor activation may trigger cytoskeletal changes that underlie spine formation (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Lendvai et al., 2000).

Several proteins have been implicated in mediating the effects of NMDA receptor activation on spine morphogenesis. SPAR, a Rap-specific GTPase-activating protein, forms a complex with NMDA receptors and PSD-95 and causes an enlargement of spine size in transfected neurons (Pak et al., 2001). NMDA receptor activation also leads to recruitment of cortactin, an actin binding protein, to the synapse. Overexpression of cortactin leads to spine elongation, and knockdown of cortactin leads to spine depletion, indicating that cortactin may be an important mediator of activity-dependent spine morphogenesis (Hering and Sheng, 2003).

Both activity-dependent and -independent mechanisms of spine formation appear to involve activation of Rho family GTPases (Irie and Yamaguchi, 2002; Penzes et al., 2003). A recent study suggests that Tiam1, a Rac1 GEF, may play a crucial role in coupling NMDA receptors to Rho family proteins to regulate dendritic growth and spine formation (Tolias et al., 2005). Tiam1 coprecipitates with NMDA receptors and is activated by phosphorylation upon NMDA receptor-mediated calcium influx, causing Rac1 activation. Inhibiting Tiam1 by RNAi or dominant-negative proteins results in decreased dendritic branching and spine density, revealing a requirement for Tiam1 in activity-dependent dendritic development. APV withdrawal experiments show that NMDAR-dependent spine formation induced by this paradigm also requires Tiam1. Tiam1-depen-

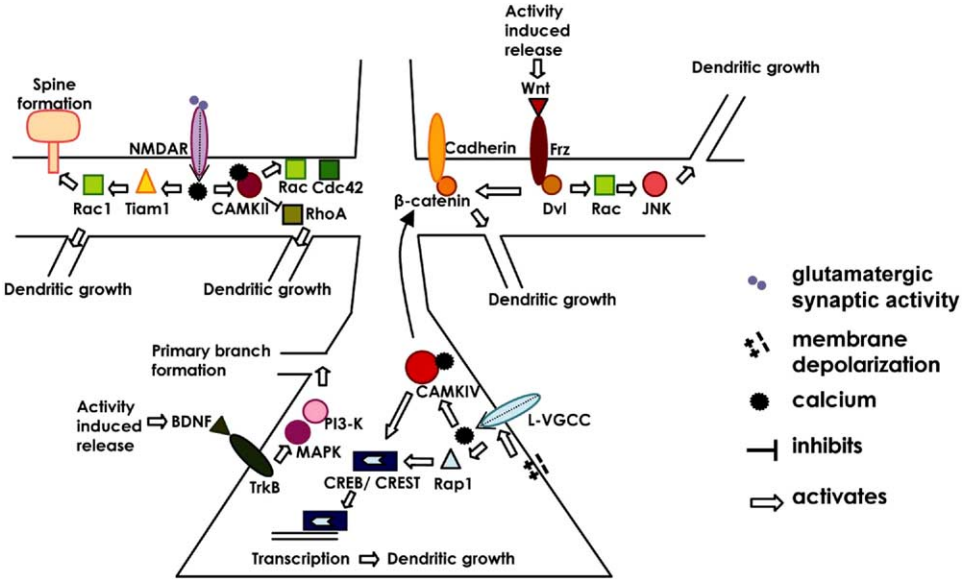

Figure 1. A Summary of Some of the Signaling Pathways by which Neuronal Activity Influences Dendritic Development

The effects of neuronal activity on dendritic development are mediated by calcium influx via voltage-gated calcium channels (VGCCs) and NMDA receptors, as well as release from internal stores. Local calcium signals act via Rho family proteins to regulate dendritic branch dynamics and stability, while global calcium signals recruit transcriptional mechanisms to regulate dendritic growth.

dent Rac1 activation could provide a mechanism for local dendritic stability in response to NMDA receptor activation. The involvement of Tiam1 in NMDA receptor-mediated dendritic development supports a series of observations that indicate that Rho family GTPases are major effectors of dendritic growth and remodeling. Alterations in the activity of Rho family proteins affect various aspects of dendritic growth (reviewed in Luo, 2000), Rho family proteins are activated by sensory stimuli, and activity-dependent dendritic growth requires activation of Rac and Cdc42 (Sin et al., 2002).

Intracellular calcium stores represent another source of calcium signals that might act locally to influence dendrite dynamics. Lohmann et al. (2002) recorded spontaneous elevations in intracellular calcium levels in developing retinal ganglion cells and found that, along with global increases in calcium levels induced by action potentials, there were local increases in intracellular calcium that were mediated by CICR and were restricted to short segments of dendrites. Blockade of local, but not global, events results in a rapid retraction of dendrites, indicating that release from intracellular stores can regulate dendritic branch stability. In support of this interpretation, photo-uncaging of caged calcium prevented retraction of dendritic branches.

Such mechanisms are likely to contribute to dendritic dynamics in other systems. Neonatal hippocampal CA1 neurons spontaneously exhibit elevations in intracellular calcium that spread through short lengths of dendrites, occurring most frequently at branch points (Koizumi et al., 1999). The rise in calcium levels is due to the release of calcium from IP3 and ryanodine-sensitive stores and may influence dendritic branch stability. A recent paper from Lohmann et al. (2005) suggests a role for local calcium signaling in regulating filopodial dynamics. They report that extension of dendritic filopodia in hippocampal slice cultures are associated with increases in calcium transients and that local uncaging of calcium in filopodia can arrest their motility. Such a mechanism may be involved in coupling synaptic activation to restrictions in filopodial motility.

BDNF and Wnts as Mediators of Activity-Dependent Dendritic Development

While much of the emphasis on mechanisms of activity-dependent dendritic development has focused on intracellular mechanisms, several observations suggest that neuronal activity might also regulate dendritic patterning by regulating the production and release of factors that in turn affect dendritic growth. One such mediator is brain-derived neurotrophic factor (BDNF). BDNF expression is regulated by calcium signaling in cortical neurons, and BDNF exerts a major influence on cortical dendrite development (Gorski et al., 2003; Shieh et al., 1998; Tao et al., 1998). While initial studies had focused on the role of BDNF in the development of excitatory pyramidal neurons (McAllister et al., 1996; McAllister et al., 1997; Horch et al., 1999), recent observations suggest that BDNF also plays an important role in regulating dendritic development in interneurons (Jin et al., 2003). A depolarizing effect of GABAergic stimulation during early postnatal development appears to induce release of BDNF from pyramidal neurons and causes dendritic maturation of interneurons (Marty et al., 1996). The effects of BDNF on dendritic growth are mediated by MAP kinase and PI3-kinase signaling (Dijkhuizen and Ghosh, 2005).

Another potential mediator of activity-dependent dendritic growth is Wnt signaling. Yu and Malenka (2003) observed that induction of dendritic growth by membrane depolarization requires a cadherin-β-cat-

enin interaction. Expressing dominant-negative proteins that interfere with cadherin-β-catenin signaling blocks depolarization-induced dendritic growth, and overexpression of β-catenin or cadherin increases the total dendritic tip number. Depolarization increases Wnt secretion in these cultures, and Wnt treatment results in a similar increase in dendritic growth, suggesting that activity-dependent release of Wnt proteins and recruitment of Wnt signaling may provide another mechanism for dendritic growth control (Abe et al., 2004; Murase et al., 2002; Togashi et al., 2002; Rosso et al., 2005).

Concluding Remarks

There is now compelling evidence that dendritic development is a highly dynamic process that is regulated by both activity-independent and activity-dependent signals (Figure 1). During postnatal development, neuronal activity is a key determinant of dendritic growth and branching. These effects involve calcium-dependent regulation of the local cytoskeleton via Rho family GTPases, which influence branch dynamics and stability, as well as calcium-dependent transcription, which appears to regulate the expression of genes that regulate dendritic growth. By recruiting both local and global calcium signaling mechanisms to regulate dendritic development, neuronal activity exerts a critical influence on the architecture of the developing brain.

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