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Differential targeting of dynamin-1 and dynamin-3 to nerve terminals during chronic suppression of neuronal activity

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ABSTRACT

Neurons express three closely related dynamin genes. Dynamin 1 has long been implicated in the regulation of synaptic vesicle recycling in nerve terminals, and dynamins 2 and 3 were more recently shown also to contribute to synaptic vesicle recycling in specific and distinguishable ways. In cultured hippocampal neurons we found that chronic suppression of spontaneous network activity differentially regulated the targeting of endogenous dynamins 1 and 3 to nerve terminals, while dynamin 2 was unaffected. Specifically, when neural activity was chronically silenced for 1–2 weeks by tetrodotoxin (TTX), the clustering of dynamin 1 at nerve terminals was reduced, while the clustering of dynamin 3 significantly increased. Moreover, dynamin 3 clustering was induced within hours by the sustained blockade of AMPA receptors, suggesting that AMPA receptors may function to prevent Dyn3 accumulation within nerve terminals. Clustering of dynamin 3 was induced by an antagonist of the calcium-dependent protein phosphatase calcineurin, but was not dependent upon intact actin filaments. TTX-induced clustering of Dyn3 occurred with a markedly slower time-course than the previously described clustering of synapsin 1. Potassium-induced depolarization rapidly de-clustered dynamin 3 from nerve terminals within minutes. These results, which have implications for homeostatic synapse restructuring, indicate that the three dynamins have evolved different regulatory mechanisms for trafficking to and from nerve terminals in response to changes in neural activity.

1. Introduction

The dynamins comprise a family of GTPases that are involved in membrane fission events (Ferguson and De Camilli, 2012; Mettlen et al., 2009). All three closely related dynamin genes are expressed in neurons (Cook et al., 1996), raising the possibility that they might participate in distinct cellular functions. At the synapse, the function of dynamin 1 (Dyn1) in presynaptic vesicle endocytosis has been well characterized. Dyn1 accumulates at clathrin coated endocytic pits, polymerizes into a collar-like structure at the neck of the endocytic bud, and mediates GTP hydrolysis dependent vesicle fission. This vesicle membrane retrieval, recycling of vesicle membrane, and subsequent re-loading of neurotransmitter, ensures the continuous supply of vesicles for sustained neurotransmission.

Dyn2 is ubiquitously expressed in tissue throughout the organism and is not up-regulated during synaptogenesis (Cook et al., 1996). Dyn2 is thought to support synaptic transmission in Dyn1 and Dyn3 double knock-out mice (Raimondi et al., 2011), and regulates the recycling of synaptic vesicles with kinetics and properties that distinguish it from Dyn1 and 3 (Tanifuji et al., 2013). Dyn2 is also implicated in additional functions, such as non-clathrin mediated endocytosis and microtubule related activities (Gonzalez-Jamett et al., 2013; Ishida et al., 2011).

Compared to dynamins 1 and 2, Dyn3 has been relatively less well characterized since its initial discovery in the brain (Cook et al., 1996). Dyn3 was proposed to be a component of the postsynaptic compartment of rat hippocampal neurons (Gray et al., 2003, 2005). However, studies using genetic knockdown and direct physiological measurements of neurotransmission support a presynaptic role for endogenous Dyn3 in synaptic vesicle recycling (Raimondi et al., 2011; Tanifuji et al., 2013). In Dyn1 knockout mice Dyn3 accumulates at nerve terminals, and defects in synaptic vesicle endocytosis are more severe in the Dyn1/Dyn3 double knockout mouse than in the Dyn1 single knockout mouse (Ferguson et al., 2007; Raimondi et al., 2011), suggesting that they have some redundant functions. Nevertheless, the degree to which Dyn3 functions overlap with those of Dyn1 remains unclear.

A recent study demonstrated that the three dynamin isoforms have distinguishable features at the synapse of rat superior cervical ganglion neurons (Tanifuji et al., 2013). Dyn1 regulated vesicle recycling to the readily releasable pool with fast kinetics and within in a relatively slow time window (>50 ms) in a manner dependent upon action

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potential frequency. By comparison Dyn3 resupplied the readily releasable pool of vesicles with slower kinetics but more rapidly, within 20 ms of the incoming action potential, and in a manner independent of firing frequency. Dyn2 displayed properties intermediate between the other two isoforms (Tanifuji et al., 2013).

In the present study we compared the endogenous subcellular distribution of the three dynamin isoforms in cultured rat hippocampal neurons following chronic suppression of network activity. We observed that silencing of synaptic activity resulted in opposite effects on the presynaptic accumulation of Dyn1 and Dyn3, while having no detectable effect on Dyn2. These results indicate that dynamic changes in action potential firing rate can alter the ratio of Dyn1 and Dyn3 in nerve terminals, a finding that may have implications for synaptic function and homeostatic mechanisms.

2. Results

2.1. Subcellular distribution of the three dynamin isoforms

Tissue-wide expression of all three dynamin isoforms in the brain was previously described using northern blot analysis (Cook et al., 1996) and immunodetection (Urrutia et al., 1997). We used dissociated cultures derived from embryonic rat hippocampus to examine the subcellular distribution of the three dynamins and their response to neural activity. All evaluations were performed on cultures fixed after 21 days in vitro, at which time cultures have established near maximal synapse density and their glutamatergic synapses are mainly present on dendritic spines. As described previously (Calabrese et al., 2006), presynaptic terminals appear as small varicosities distributed along the dendrites, and are frequently found in close apposition to dendritic spines. Other nerve terminals are present at GABAergic inhibitory synapses along the dendrite. Each of the antibodies used to specifically detect the three dynamin isoforms was previously reported to lack crossreactivity to the other two isoforms (Gray et al., 2003; Kurklinsky et al., 2011; Lu et al., 2007). We confirmed in hippocampal neurons the selectivity of the Dyn1 and Dyn3 antibodies (Supplemental Fig. S1). All neurons in culture displayed specific immunostaining with all three antibodies; astrocytes were variably labeled with the three antibodies and were not examined further in this study.

Under control conditions we detected endogenous Dyn1 immunoreactivity in axons and nerve terminals, but also to some extent in dendrites and cell bodies (Fig. 1A, *left*), in agreement with previous findings (Faire et al., 1992; Noda et al., 1993). Dyn1 was distributed in a punctate manner along the dendritic arbor. Dyn1 puncta were strongly colocalized with the synaptic vesicle marker synapsin 1 (Syn1) (Fig. 1B), consistent with the known enrichment of both proteins within nerve terminals (McPherson et al., 1994).

In comparison to Dyn1, Dyn2 immunoreactivity appeared more evenly and diffusely distributed along axons and dendrites, and, although detectable, it lacked strong enrichment at synapses (Fig. 1A, *control*). Consistent with this observation, Dyn2 immunoreactivity was only weakly colocalized with Syn1, (Fig. 1B, *control*).

Similar to Dyn1, Dyn3 immunoreactivity in control cultures was characterized by a punctate staining pattern; however, for Dyn3 the puncta size was highly variable (Fig. 1A, *control*). Although a few large puncta colocalized with Syn1 (Fig. 1B, *control*) most puncta appeared to be small in size, and mainly distributed along the axon shaft, rather than being concentrated in axonal varicosities.

2.2. Chronic silencing of action potentials by TTX differentially alters Dyn1 and Dyn3 distribution patterns

All three dynamin isoforms have a role in the recycling of neurotransmitter vesicles at synapses (Raimondi et al., 2011; Tanifuji et al., 2013). Changes in the levels of action potential firing can alter the concentrations of synaptic proteins, including those that regulate vesicle retrieval (Cremona and De Camilli, 1997; Gundelfinger et al., 2003). Here we asked whether suppression of spontaneous electrical activity for various times would regulate the distribution of Dyn1, Dyn2, and Dyn3 at synapses. We chronically silenced spontaneous network activity by culturing the neurons for two weeks in the presence of tetrodotoxin (TTX, 1 μM), during which time synapses are being established and stabilized. In agreement with previous reports (Robinson et al., 1994), Dyn1 immunoreactivity became less prominent at synapses (Fig. 1A, *bottom row*) and became more diffusely distributed along axons. In opposite fashion, chronic TTX caused Syn1 to accumulate at synapses, as reported previously (Chi et al., 2001). Therefore, chronic TTX induced a prominent decrease in the colocalization of Dyn1 and Syn1 at synapses. We detected no change in the distribution of Dyn2.

Interestingly, this same prolonged block of action potential firing that decreased clustering of Dyn1 instead increased the clustering of Dyn3. TTX induced a redistribution of Dyn3 from a more diffusely punctate pattern to one where Dyn3 became concentrated in larger clusters (Fig. 1A, *bottom row*). These larger Dyn3-positive clusters strongly colocalized with Syn1, indicating that Dyn3 accumulated within nerve terminals (Fig. 1B, *bottom row*). The increase in colocalization was further enhanced because Syn1 itself also accumulated within nerve terminals.

We took a closer look at these unanticipated results for Dyn3. Qualitatively it appeared that in control conditions the majority of Dyn3 puncta were very small and diffusely distributed throughout the region where axons come into close proximity to dendrites and form synaptic contacts. These small puncta mostly did not overlap with the MAP2 signal, which is confined to the dendrite shaft. Instead, the majority of puncta appeared to be present within the meshwork of fine-caliber axons that typically wrap around the dendritic arbor of cultured hippocampal neurons (Fig. 2A, B control). Upon incubation with TTX for 2 weeks, these small puncta were greatly diminished in number, while at the same time larger puncta became more prominent (Fig. 2A, B TTX).

These qualitative observations were corroborated by quantitative analyses. First, the average coefficient of variation in pixel intensity over the image field was significantly increased in the presence of TTX (Fig. 2C). This result is consistent with the observation that after TTX there is a greater variation in signal intensity across the image compared to the control condition, where signal intensity is more uniform throughout each field of view, due to the diffuse distribution of small Dyn3-positive puncta throughout the axonal meshwork. Second, the average area occupied by individual puncta (defined as being between 6 and 18 pixels in size) was dramatically increased following TTX (Fig. 2D). This is again consistent with the qualitative observation of a reduction in small puncta concomitant with an increase in large puncta. Indeed, examination of a frequency histogram plotting the number of puncta per field across a range of sizes revealed a clear rightward shift in the size distribution, with TTX favoring puncta greater than 10 pixels at the expense of those under 10 pixels (Fig. 2E).

Taken together, these observations suggest that TTX induces a relocalization of Dyn3 from clusters within the axonal shaft to larger clusters within nerve terminals. The aggregated Dyn3 clusters now colocalize strongly with Syn1, which also accumulates within terminals after chronic action potential silencing.

2.3. TTX-induced Dyn3 clusters are located presynaptically

The above observation that Dyn3 can become highly concentrated in nerve terminals in an activity-dependent manner was somewhat unexpected, firstly because Dyn3 behaved oppositely to its close homolog Dyn1, and secondly because an earlier paper had indicated that Dyn3 was specifically enriched in dendritic spines, the postsynaptic compartment of excitatory synapses, rather than in the presynaptic terminal (Gray et al., 2003). We therefore evaluated more precisely which compartment (presynaptic or postsynaptic) corresponded to