Neuroscience 160 (2009) 1–5

RAPID REPORT

NEURONAL DEPOLARIZATION MODIFIES MOTOR PROTEIN MOBILITY

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Abstract—Active neuronal transport along microtubules participates in the targeting of mRNAs, proteins and organelles to their sites of action. Cytoplasmic dynein represents a minus-end-directed microtubule-dependent motor protein. Due to the polarity of microtubules in axonal and distal dendritic compartments, with microtubule minus-ends pointing toward the inside of the cell, dyneins mainly mediate retrograde transport pathways in neurons. Since dyneins transport synaptic proteins, we asked whether changes in neuronal activity would in general influence dynein transport. KCl-induced depolarization, a condition that mimics the effects of neuronal activity, or pharmacological blockade of neuronal action potentials, respectively, was combined with neuronal live cell imaging, using an autofluorescent dynein intermediate chain fusion (monomeric red fluorescent protein [mRFP]–dynein intermediate chain [DIC]) as a model protein. Notably, we found that induced activity significantly reduced dynein particle mobility, as well as both the total distance and velocity of movements in mouse cultured hippocampal neurons. In contrast, blockade of neuronal action potentials through TTX did not alter any of the parameters analyzed. Neuronal depolarization processes therefore represent candidate mechanisms to regulate intracellular transport of neuronal cargoes.

Key words: , neuron, transport, microtubule, motor protein, dynein, neuronal activity.

Neurons change the number and composition of various molecules in response to activity changes (Ehlers, 2000; Kennedy and Ehlers, 2006). For instance processes of synaptic plasticity, the ability of synapses to change in strength and/or efficiency, require the delivery and removal of molecules on a fast time scale (Carroll et al., 2001; Dresbach et al., 2003; Shapira et al., 2003; Malenka and Bear, 2004; Nicoll and Schmitz, 2005). Active intracellular transport along microtubules represents a key process for long-distance movement of cellular cargo and both kinesin and dynein motor protein complexes have been identified to mediate a number of neuronal transport processes in anterograde or retrograde directions, respectively (Vallee et al., 2004; Hirokawa and Takemura, 2005; Caviston and Holzbaur, 2006). The polarity of microtubules in axons and distal dendrites is thought to be uniform with microtubule minus-ends being oriented towards the inside of the cell, however microtubules in proximal dendrites of hippocampal neurons might display a mixed polarity (Baas et al., 1988), with local consequences for the direction of transport in initial neurite segments. Prominent neuronal cargoes with respect to plasticity include excitatory and inhibitory neurotransmitter receptors, known to undergo postsynaptic delivery, surface membrane internalization and recycling (Setou et al., 2000, 2002; Maas et al., 2006). Cargo adapters and accessory proteins participate in the regulation of motor protein transport and directionality along microtubules (Setou et al., 2002) and calcium signals have been shown to provide a cue for local cargo release in dendrites (Guillaud et al., 2008). Activity-dependent processes and neuronal depolarization contribute to the transport regulation of different molecules including the RNA-binding proteins FMRP (fragile × mental retardation protein) (Antar et al., 2005) and Sam 68 (Ben Fredj et al., 2004) as well as the protein semaphorin 3A (de Wit et al., 2006) and the brain-derived neurotrophic factor (BDNF) (Kohara et al., 2001). However, which mechanisms determine the mobility parameters of the underlying motor cargo complexes in polarized neurons and how such processes contribute to the regulation of transport in response to activity changes are currently barely understood.

EXPERIMENTAL PROCEDURES

Constructs/antibodies

The plasmid monomeric red fluorescent protein (mRFP)–DIC was generated by subcloning the dynein intermediate chain (DIC) 2B cDNA as BglII/KpnI fragment into pmRFP. Antibodies used for immunostaining were anti–dynein heavy chain (DHC) (1:50, Santa Cruz, Heidelberg, Germany) and anti-DIC (1:100, Millipore, Schwalbach/Ts., Germany).

Cell culture/transfection/immunostaining

Primary cultures of hippocampal neurons were prepared from mice at postnatal days 0-1, as described (Loebrich et al., 2006; Maas et al., 2006). Cells cultured for 7–10 days *in vitro* (DIV) were transfected using a calcium phosphate coprecipitation protocol (Loebrich et al., 2006). Immunostaining was performed as previously described (Loebrich et al., 2006; Maas et al., 2006).

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Abbreviations: DHC, dynein heavy chain; DIC, dynein intermediate chain; DIV, days *in vitro*; mRFP, monomeric red fluorescent protein; MTOC, microtubule-organizing center.

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doi:10.1016/j.neuroscience.2009.02.034

Live cell imaging

Live cell imaging (time-lapse video microscopy) using DIV 11–14 neurons was performed with an inverted fluorescent microscope Zeiss Axiovert 200 M (Zeiss, Göttingen, Germany) combined with a Sony CCD-Kamera (Visitron Systems, Puchheim, Germany), as described (Maas et al., 2006). Neurons were first imaged under control conditions. The HEPES-buffer was subsequently removed and exchanged with Hepes-buffer containing either 10 mM KCl or 500 nM TTX, 10 min prior to further image acquisition. Images were acquired using the MetaVue 6.2r6 software connected to the digital camera. Movies were taken at 15 s intervals over 480 s, each. Cells at the microscope stage were temperature controlled and kept in HEPES-buffer at all times.

Quantitative analysis

For analysis of mobile particles, unprocessed image stacks of five independent neurons per experimental condition were followed throughout individual frames using MetaMorph 7.1 (Molecular Devices Corp., Sunnyvale, CA, USA). To compensate strong light intensities in cell bodies, brightness was eventually reduced to track mobile puncta in somata. Due to the inability of MetaMorph to reliably track crossing particles, the program's "Automatic track function" was excluded and mobile particles were instead manually tracked per mouse click throughout each individual frame. Statistical significance was evaluated with the Student *t*-test according to the following definition: $P>0.05$ not significant; $P=0.01-0.05$ significant (*); $P=0.001-0.01$ very significant (**); $P<0.001$ extremely significant (***).

RESULTS

To gain insight into the role of neuronal activity in the regulation of microtubule transport, we aimed to perform live cell imaging with a fluorescent motor protein component. Fusion of autofluorescent green fluorescent protein (GFP)-derived tags to heavy chains of motor proteins, which mediate ATP-dependent tubulin binding, often compromises transport, most likely due to steric hindrance in the fusion protein. To circumvent similar problems, we therefore fused mRFP to a DIC, representing an essential and exclusive component of dynein motor complexes. Immunostaining of mRFP-DIC-expressing cultured hippocampal neurons with an antibody specific for the dynein heavy chain (DHC) revealed that a large number (>90%) of mRFP-DIC puncta colocalized with endogenous DHC signals (Fig. 1A), indicating that mRFP-DIC fusion proteins associate with dynein motor complexes. Furthermore, DIC-specific antibodies recognized the fusion protein (Fig. 1B). Upon expression of mRFP-DIC particles in untreated control neurons, fluorescent puncta were found in both

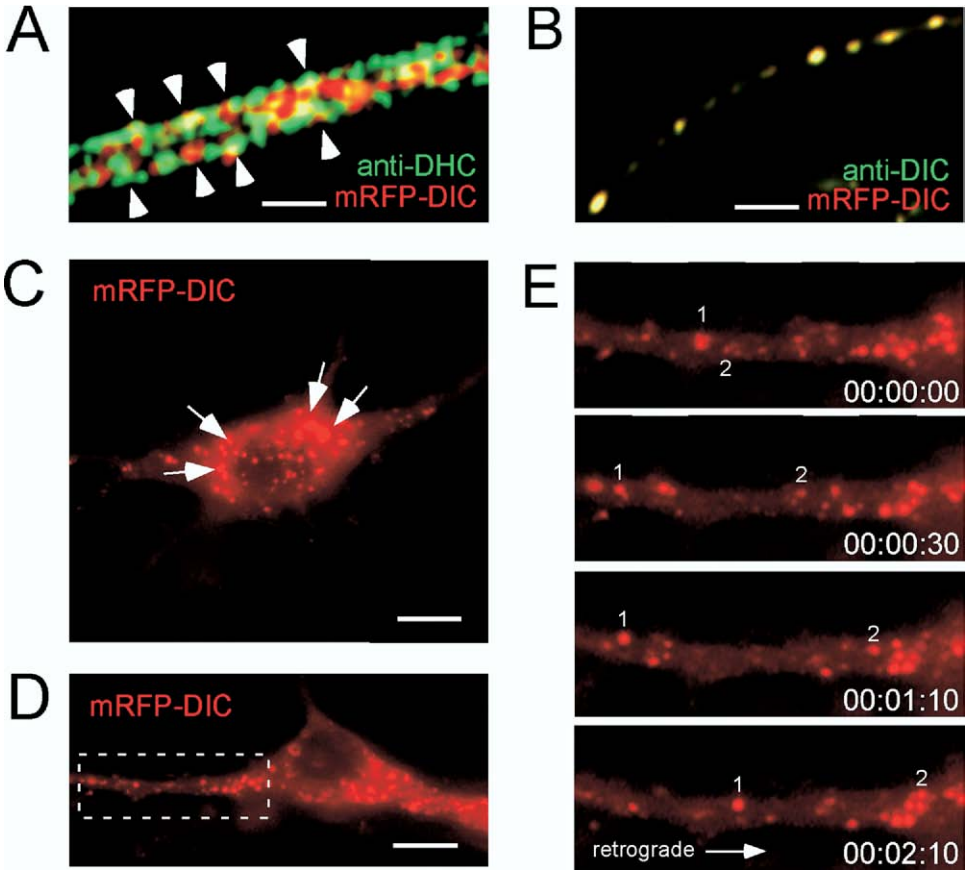

Fig. 1. Cultured hippocampal neurons transfected with mRFP-DIC. (A, B) High power magnification of neurite regions. (A) mRFP-DIC particles (red) colocalize with endogenous DHC-positive signals (green). (B) DIC-specific antibodies (green) detect the mRFP-DIC fusion protein (red). (C, D) Autofluorescent particles, representing dynein motor complexes, are distributed across soma and neurites and display high concentrations at the perinuclear region (arrows), represented through the MTOC. (E) High power magnification of the selected area shown in D. Autofluorescent particles are highly mobile over time. Time units represent hours: minutes: seconds. Scale bars=5 μm (A, B); 15 μm (C, D).