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Research Report

Actomyosin-dependent microtubule rearrangement in lysophosphatidic acid-induced neurite remodeling of young cortical neurons

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ABSTRACT

It has been shown that lysophosphatidic acid (LPA), a signaling phospholipid, induces neurite retraction and the formation of retraction fibers in young cortical neurons by actin rearrangement. This study examined the rearrangement of microtubules (MTs) during LPA-induced neurite remodeling by immunostaining with antibodies against several types of tubulin. The results showed that α-tubulin was present in growing neurites as well as in cell bodies with various localization profiles. Exposure of neurons to LPA resulted in neurite retraction, accompanied by the rearrangement of MTs in neurites and the accumulation of MTs in cell bodies, without significant changes in the total amount of MTs in the cytoskeletal fraction of cultured neurons. Similar findings were obtained when young neurons were stained for other types of tubulin, including β-tubulin type III and posttranslationally acetylated and tyrosinated tubulin. LPA-induced MT rearrangement was accompanied by accumulation of myosin IIB and polymerized actin at the base of retraction fibers. These effects of LPA on MTs and myosin IIB were blocked by pretreatment with inhibitors of the actomyosin and Rho pathways (cytochalasin D, blebbistatin, and Y27632), but not by an MT stabilizer (taxol), whereas taxol inhibited neurite retraction and MT depolymerization induced by nocodazole. Furthermore, neurofilaments also showed rearrangement in response to LPA, which was blocked by cytochalasin D and Y27632, but not taxol. Taken together, these results suggested that LPA did not induce MT depolymerization and that LPA-induced actomyosin activation produced MT and neurofilament rearrangement, leading to neurite remodeling.

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1. Introduction

After the final mitosis, neurons begin neuritogenesis and neurite outgrowth to form synaptic connections. These morphological changes require the function of the cytoskeleton, particularly actin microfilaments (MFs) and microtubules (MTs). MFs are thought to be the basic engine that generates forces for cell polarity establishment, cell migration, and neurite extension, whereas MTs are important for the maintenance of cell morphology and the speed of neurite

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outgrowth. Both cytoskeletal elements are rearranged during development in response to extracellular cues that regulate the direction and speed of neurite outgrowth (Dickson, 2002; Guan and Rao, 2003). These elements are also mechanically and functionally integrated with each other (Baas and Ahmad, 2001; da Silva and Dotti, 2002; Dent and Gertler, 2003). Neurofilaments (NFs), other cytoskeletal components, have also been demonstrated to contribute to axonal elongation. Recent studies have identified proteins that mediate physical and functional interactions between intermediate filaments and MTs or actin MFs in developing neurons (Lariviere and Julien, 2004). Therefore, three cytoskeletal components must be well interconnected each other during neuritogenesis and neurite outgrowth.

Lysophosphatidic acid (LPA) is an extracellular signaling phospholipid that produces actin rearrangement to influence cell morphology and motility in various cell types, including nonneuronal and neuronal cells (Fukushima et al., 1998, 2000, 2002a,b; Moolenaar, 1995; Ridley and Hall, 1992; Sayas et al., 1999, 2002; Tigyi et al., 1996a,b). For example, in fibroblasts, LPA is well known to stimulate actin stress fiber formation through the activation of the small GTPase, Rho (Ridley and Hall, 1992). LPA has also been shown to regulate MT rearrangements, including MT stabilization and microtubule-organizing center reorientation through stimulation of Rho pathways and Cdc42 (another type of the small GTPase), respectively (Cook et al., 1998; Palazzo et al., 2001). These effects are independent of actin polymerization because actin polymerization inhibitors have no effect on LPA-induced MT rearrangements.

We have previously demonstrated that LPA induces transient neurite retraction, accompanied by retraction fiber formation, in young cortical neurons commencing neuritogenesis (Fukushima, 2004; Fukushima et al., 2002b). LPA-induced neurite retraction or remodeling is mediated by actin polymerization downstream of the small GTPase Rho, as observed in fibroblasts and many other cell types (Fukushima et al., 2001, 2002a; Ishii et al., 2004; Moolenaar, 1995). These morphological changes led us to hypothesize that MTs and NFs also rearrange and collapse within neurites. However, studies on the mode by which LPA affects MTs and NFs in neurons are lacking. The present study pharmacologically examines interactions between actin and MTs or NFs during LPA-induced neurite remodeling in young cortical neurons.

2. Results

2.1. *Effects of LPA on MT rearrangement*

This study employed dissociated cell cultures consisting of single, young cortical neurons. We have already shown that more than 90% of the total viable cells in these cultures are neurons at 18 h after plating, and that they possess short, growing neurites, and lamellae (designated here as neurites for both) (Fukushima et al., 2002b). We have also demonstrated that LPA induces retraction of these extending neurites and some portions of neurites remain attached to the substrate, producing retraction fibers characterized by fine spikes accumulating in one or two areas (Fukushima et al., 2002b). During this retraction response, actin MFs are rearranged, resulting in their accumulation at the fiber base, and a portion of the MFs remains within the fibers (Fukushima et al., 2002b) (see also Fig. 1h).

To observe whole MTs, we immunocytochemically examined α-tubulin with the DM1A antibody, which has been widely used in many studies. Whole MTs were distributed throughout the cell, from cell bodies to neurites (Figs. 1a and c–e). Approximately 28% of neurons possessed bundled MTs, which were present in neurites and protruded from cell bodies (see the legends of Fig. 1). The rest of neurons were morphologically immature and possessed very short MT filaments. Similar percentages and distribution patterns were obtained when β-tubulin type III, known as an early neuronal marker, was stained (Table 1). Closer immunostaining analysis of the MT distribution revealed that a few MT fragments penetrated into the neurite edge (Fig. 1e). Exposure of these neurons to LPA (1 μM, 15 min) induced retraction of protrusive MTs and cell rounding (Figs. 1b and f–h). The rearrangement of MTs resulted in their enrichment at the cell cortex, including the retraction fiber base. However, no remarkable overlap of MTs with accumulated MFs was observed, and only a few MTs were present in the retraction fibers (Fig. 1h).

A decrease in the number of neurons with protrusive MTs was observed as early as 3 min after LPA addition and maintained at least for 60 min (Fig. 1i). Western blot analyses showed no marked difference in α-tubulin content in both soluble (i.e., unpolymerized) and cytoskeletal (i.e., polymerized) MT fractions during LPA exposure (Fig. 2a). The relative amounts of soluble and cytoskeletal α-tubulin to total α-tubulin were also unchanged by LPA exposure (Fig. 2d).

2.2. *Effects of LPA on posttranslationally modified tubulin*

Tubulin is posttranslationally modified, such as acetylation and tyrosination (Dent and Gertler, 2003; Luduena, 1998). These modifications produce MT heterogeneity and alter the ability of MTs to interact with each other or with MT-associated proteins. Acetylated tubulin is present in old, less dynamic MTs, whereas tyrosinated tubulin is in newly formed, more dynamic MTs. During neurite sprouting from sympathetic neuronal cell bodies, tyrosinated MTs are transported into sprouts prior to acetylated MTs (Smith, 1994).

The distribution of these posttranslationally modified tubulins was immunocytochemically examined in young neurons. When cortical neurons were immunostained with anti-tyrosinated tubulin antibody (TUB-1A2), a wide distribution throughout the cell and MT penetration into the neurite edge were observed (data not shown). The number of neurons bearing protrusive tyrosinated MTs was similar to that for α-tubulin, and LPA exposure expectedly resulted in rearrangement of tyrosinated MTs (Table 1). Although there was accumulation of these MTs at the cell cortex, they were not frequently detected within retraction fibers (data now shown). Tyrosinated tubulin amounts in soluble and cytoskeletal MT fractions were consistent during LPA exposure (Figs. 2b and d). These results were quite similar to those observed for MTs revealed by α-tubulin immunostaining with DM1A.

Acetylated tubulin, less dynamic tubulin, was also detected in most neurons when visualized with anti-acetylated tubulin

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