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Ultrastructural Localization of Alpha E-catenin in the Rat Sciatic Nerve

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Alpha E-catenin is included in the same category as alpha N-catenin, and both of them have been identified as subtypes of alpha-catenin. A previous study issued from our laboratory demonstrated that alpha N-catenin is localized in the axoplasm of unmyelinated peripheral nerves, whereas the localization of alpha E-catenin in the peripheral nervous system has not yet been reported. The present study was focused on the distribution of alpha E-catenin in the rat sciatic nerve. By electron microscopy using immunocytochemical methods, alpha E-catenin immunoreactivities were generally detected in the Schwann cell cytoplasm of unmyelinated nerve and the outer loop of myelinated nerve. Those findings suggest that alpha E-catenin may be associated with Schwann cell-Schwann cell or axon-Schwann cell contacts.

Cadherin, a transmembrane glycoprotein, constitutes a superfamily of calcium-dependent intercellular adhesion molecules (27) that are thought to be involved in cell recognition and segregation, morphogenetic regulation and tumor suppression (4-7, 13, 26, 27, 28, 30, 32). Our previous studies have demonstrated that N- and R-cadherin are expressed on the plasmalemma of axons and Schwann cells in sciatic nerves, and they are likely to function at the axon-axon and axon-Schwann cell contact (22, 24). The distal portion of the cytoplasmic tail of cadherin binds beta-catenin, which in turn is associated with alpha-catenin, and thereby links the adhesive complex to the actin cytoskeleton (20, 29, 31). Alpha E- and alpha N-catenins have been identified as subtypes of alpha-catenin (7-9, 17), and alpha N-catenin is localized in the axoplasm of unmyelinated sciatic nerves (23). N-cadherin can associate not only with alpha N-catenin but also with alpha E-catenin, and we have also detected expression of alpha E- and alpha N-catenins in dorsal root ganglia and spinal cord at the lumbar level, where sciatic nerve originates (25). However, the ultrastructural localization of alpha E-catenin in peripheral nerves has not yet been identified. In the present study, the distribution of alpha E-catenin in the rat sciatic nerve was therefore examined by means of electron microscopy using immunocytochemical methods.

MATERIALS AND METHODS

Animals and Fixation

White Wistar rats (female, 42-70 days of age) were used in the present study. All animal experiments were conducted to the Guidelines for Animal Experimentation at Kobe University School of Medicine. The animals were anesthetized by intraperitoneal injection

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of Nembutal (sodium pentobarbiturate 50mg/kg body weight) and fixed by transcardiac perfusion with a fixative containing 4% paraformaldehyde in 0.15M NaCl in 100mM phosphate-buffered saline (PBS) at pH7.5, supplemented with 1mM $CaCl_2$ and 8% sucrose. Nerve segments were excised from the sciatic nerve and immersed in the same fixative as described above for 4h at 4°C.

Immunoblotting analyses

Immunoblotting analyses were performed in a similar manner as previously reported (14, 18). Fresh nerve segments dissected out from the sciatic nerve were homogenized with ultrasonic disruptor, and solubilized with lysis buffer [50mM Tris-HCl (pH7.4), 0.5% (v/v) NP-40, 150mM NaCl, 5mM EDTA, 1mM phenylmethyl sulphonyl fluoride (PMSF), 10μg/ml leupeptin and 10μg/ml aprotinin]. As positive control, homogenates of rat brain were used. The cell lysates were prepared by centrifugation at 12,000 rpm for 15 min to remove insoluble materials. The supernatants were eluted with Laemmli sample buffer. The whole cell lysates were then separated by SDS-PAGE, and transferred to Polyvinylidene fluoride (PVDF) membrane filters (Immobilon, Millipore). The membranes were immunoblotted with goat anti-alpha E-catenin polyclonal antibodies (catalogue no. sc-1495) (Santa Cruz Biotechnology, CA), using chemiluminescence reagent (Renaissance, NEN).

Immunocytochemical staining

Fixed nerve segments were cryoprotected through a series of increasing sucrose concentrations (10, 15, 20, and 25%) in 0.2M NaCl in 50mM Tris-buffered saline (TBS) at pH7.5, embedded in OCT compound (SAKURA, USA), quick-frozen, and sectioned at 8μm thickness on a cryostat. Those frozen sections were mounted on slides, washed in TBS supplemented with 1mM $CaCl_2$ (TBS-Ca) and incubated with blocking solution (DAKO Protein Block Serum-Free, DAKO) for 30 min. These sections were further incubated for 24h with anti-alpha E-catenin antibody diluted 1:100 with PBS containing 0.005% blocking solution. As a control, some sections were incubated with goat serum instead of anti-alpha E-catenin antibody. After washing them three times with PBS, all of the sections were incubated for 24h with HRP-labeled swine anti-goat IgG antibody (ACI3404) (Biosource, California) at a final dilution of 1:20. Then, after washing them with PBS, the sections were incubated for 10 min in PBS containing 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB) and 0.02% H_2O_2. The sections were subsequently embedded with 50% glycerine in PBS and examined by light microscopy. Sections showing intense immunoreactivity were briefly washed in 0.1M sodium cacodylate buffer and postfixed at 4°C in 2% O_SO_4 in 0.1M sodium cacodylate buffer for 2h. After washing in 0.1M sodium cacodylate buffer, the sections were dehydrated in a graded series of ethanol, followed by n-butyl glycidyl ether (QY-1), and embedded in Epon812. Ultrathin sections were cut on a LKB Ultrotome, and examined with a HITACHI H-7100 electron microscope.

RESULTS

Immunoblotting analyses

On immunoblots of homogenates from the rat sciatic nerve, as well as brain homogenates used as a positive control, anti-alpha E-catenin antibody (sc-1495) detected a band of 102kD (Fig. 1).

Immunocytochemical staining

As a control for nonspecific labeling by the anti-alpha E-catenin antibodies, frozen sections were stained in an identical manner with normal goat serum. In these control sections any immunoreactive labeling could not be observed on myelinated or unmyelinated fibers by electron microscopy (Fig. 4b). On the other hand, the light microscopic analysis

with labeling by the anti-alpha E-catenin antibodies revealed that the normal sciatic nerve was seen to be immunoreactive stripes in the negative background (Fig. 2). Of these stripes, thin types were observed to give an outline of myelinated fibers characterized by nodes of Ranvier, while wide types were assumed to be unmyelinated fibers because of the electron microscopic findings as described below. Electron microscopic analysis elucidated that alpha E-catenin immunoreactivity was localized in the cytoplasm of Schwann cells ensheathing both myelinated and unmyelinated fibers (Figs. 3, 4a, 4b). Such immunoreactivities were not concentrated under the plasmalemma but distributed diffusely throughout the cytoplasm without any relationship with the cell organelle. In the myelinated fibers, alpha E-catenin immunoreactivity was detected in the outer loop of Schwann cells and the Schwann cell tongues of nodes of Ranvier but not in the inner or lateral loops (Fig. 4a, 4b). The immunoreactivity was generally absent in the axoplasm of myelinated and unmyelinated fibers, however the axoplasm of the nodal region exhibited slight immunoreaction (Fig. 4b).

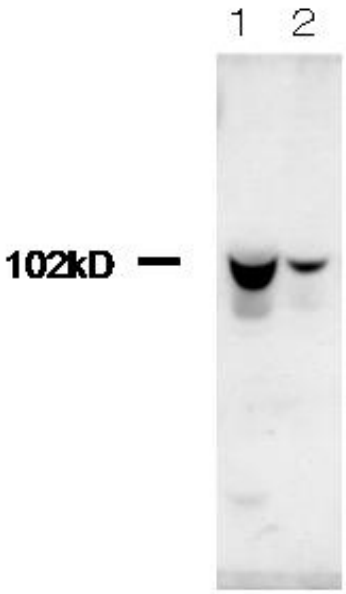


Fig. 1. Immunoblotting of homogenates of rat sciatic nerves in lane 2, and of brain as positive control in lane 1. Anti-alpha E-catenin antibody detects a single band of 102 kD on both lanes.

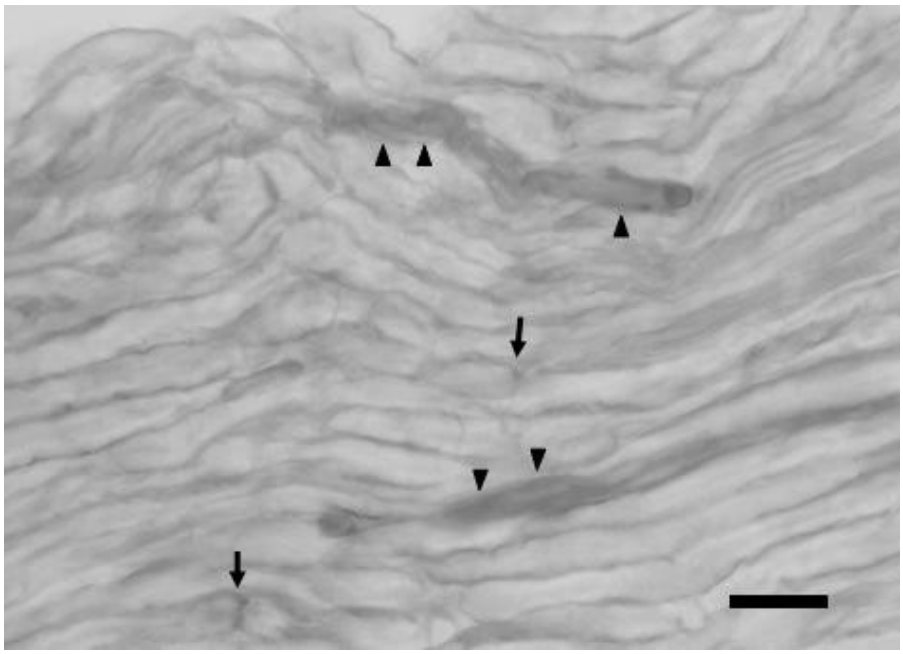


Fig. 2. Light micrograph showing a longitudinal section of a rat sciatic nerve immunostained with anti-alpha E-catenin antibody. Immunoreactivities appear to be stripes in the negative background. Thin stripes correspond to outlines of myelinated fibers characterized by nodes of Ranvier (arrows), while thick stripes are assumed to be unmyelinated fibers (arrowheads) based on the electron microscopic findings. (Bar=500μm).

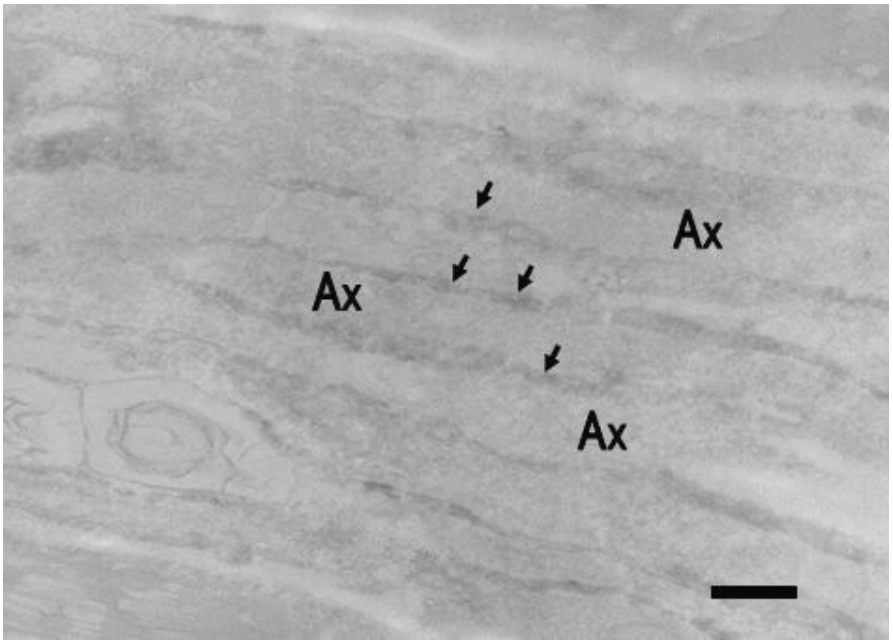


Fig. 3. Electron micrograph showing a longitudinal section of unmyelinated fibers immunostained with anti-alpha E-catenin antibody. Immunoreactivities are detected in the cytoplasm of Schwann cells, not concentrated under the plasmalemma but distributed diffusely (arrows). No immunoreaction is detected in the axoplasm (Ax). (Bar =1μm).

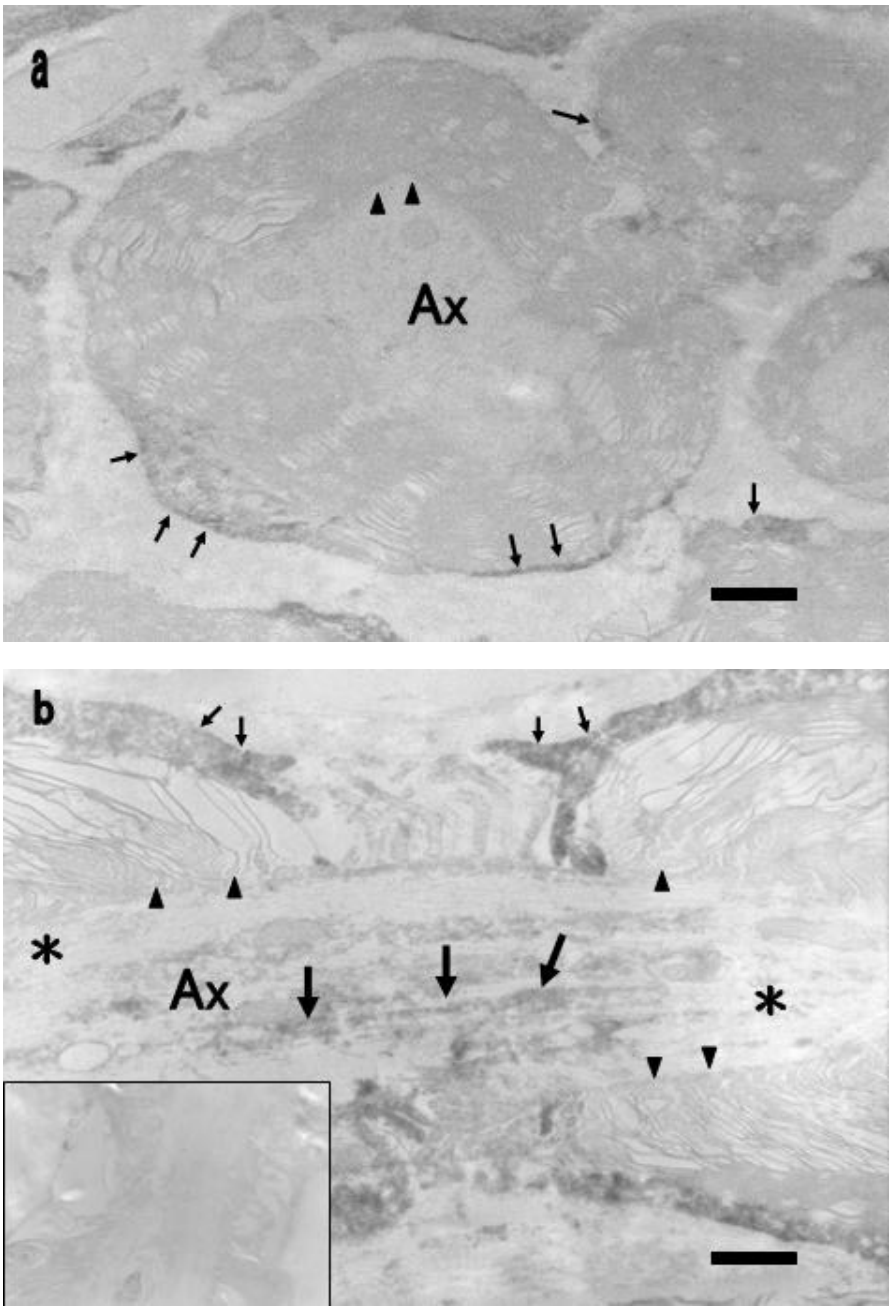


Fig 4. Electron micrographs showing myelinated fibers immunostained with anti-alpha E-catenin antibody. **a.** A cross section of myelinated fibers. Immunoreactivity is detected throughout the outer loop of Schwann cells (arrows), while no immunoreaction is observed either in inner loop of Schwann cells (arrowheads) or in the axoplasm (Ax). (Bar=1μm). **b.** A longitudinal section of node of Ranvier. Schwann cell tongues show immunoreactivity (small arrows), but the lateral loops of Schwann cell exhibit no immunoreaction (arrowheads). There seems to be slight immunoreactivities in the axoplasm of nodal region (large arrows), whereas no immunoreactivity in the paranodal region of the axon (asterisks). Inset with black frame shows a longitudinal section of node of Ranvier stained with goat serum as a control; no immunoreaction is detected in the axon or Schwann cells. (Ax; axon, Bar=1μm).

DISCUSSION

In this study, we have demonstrated that alpha E-catenin is distributed in the cytoplasm of Schwann cells of both myelinated and unmyelinated fibers in the rat sciatic nerve.

In unmyelinated fibers, previous studies (22-24) demonstrated that the plasmalemma of axons and Schwann cells expressed both N- and R-cadherins, and the axoplasm expressed alpha N-catenin, but in this study there is no alpha E-catenin expression in the axoplasm. These findings indicate that the localization at the axon-Schwann cell contact of unmyelinated fibers is homophilic as to the types of cadherins, but heterophilic as to the types of alpha-catenins.

In myelinated fibers, Schwann cells form a highly specialized architecture consisting of myelin and surrounding thin cytoplasmic channel network. This network shows physiological activity and is organized into several well-defined morphological regions including the inner and outer loops, Schmidt-Lanterman incisures and the paranodal channels (21). The paranodal channels appear to be a series of loops by electron microscopy that are often referred to as "paranodal loops or lateral loops". Interestingly alpha E-catenin is detected only in the outer loop but not in the inner or lateral loop. Our previous studies have demonstrated that N- and R-cadherin are located on the mesaxons of outer loops or paranodal loops, and E-cadherin or beta-catenin has been also reported to be expressed at the same regions (15). At least alpha E-catenin can be assumed to be contact with these cadherins or catenin in the outer loop of Schwann cells, and to contribute to Schwann cell-Schwann cell contacts at the mesaxons.

In normal epithelial cells, alpha E-catenin is concentrated under the plasmalemma and binds cadherins to actin filaments to mediate strong cell-cell adhesion (10, 16, 19). On the contrary, the present study showed no distinct difference in alpha E-catenin density under the plasmalemma between the cell-cell and cell-extracellular matrix contacts. This finding is similar to that of alpha N-catenin in the unmyelinated axons in our previous study, in which the expression was distributed diffusely throughout the axoplasm with both the HRP method and the immunogold method (23). Actually the cadherin-mediated cell-cell adhesions can be rearranged dynamically by the small guanosine triphosphatases (GTPases), Rac1 and Cdc42 (2), or IQGAP1. IQGAP1 is localized at the sites of cell-cell contact and constitutes a target of Rac1 and Cdc42. The interaction of IQGAP1 with beta-catenin ejects alpha-catenin from the cadherin-catenin complex, so that this interaction leads to weak adhesive activity and diffuse alpha-catenin distribution (1, 3, 11, 12). If the diffuse HRP distribution in this study shows the proper alpha E-catenin localization, it suggests that alpha E-catenins are dissociated from the cadherin-catenin complex. Whereas, DAB stain with pre-embedding method is possible to disperse on the electron microscopic level, therefore it cannot be draw concluded.

Parental cell bodies of sciatic nerves are located in the dorsal root ganglia or the spinal cord at the lumbar level. In the previous study, those cell bodies of chickens showed alpha E-catenin expression (25), therefore distribution of alpha E-catenin in the peripheral neuron might be distorted, that is to say, alpha E-catenin is concentrated in cell bodies but not in axons or dendrites except for nodes of Ranvier. However, we must think over an influence of the difference in species of animals used in studies (i.e., chicken and rat), and need further study for a conclusion.

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