

Alpha E- and Alpha N-catenin Expression in Dorsal Root Ganglia and Spinal Cord

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The localization of alpha E- and alpha N-catenin in dorsal root ganglia and spinal cord was examined immunocytochemically. Alpha E- and alpha N-catenin appeared to be co-localized in cell bodies of neurons in dorsal root ganglia, but alpha N-catenin was not expressed in cell bodies of neurons in the ventral horn that showed alpha E-catenin expression. These findings indicate the possibility that the type of alpha catenin expressed in neurons differs with its functional property (e.g. sensory or motor).

Cadherins constitute a superfamily of calcium-dependent intercellular adhesion molecules (22) and are thought to play a role in cell recognition and segregation, morphogenetic regulation, and tumor suppression (21)(22)(5)(3)(4). Cadherin is a transmembrane glycoproteins and link to catenin at its cytoplasmic domain. Catenins are classified into alpha-, beta-, and gamma-catenins (13), and alpha E- and alpha N-catenins have been identified as subtypes of alpha-catenin (6)(5)(7)(11). Cadherin is linked directly to one or two molecules of beta-catenin, and to one molecule of alpha-catenin via beta-catenin (15)(8)(14). Gamma-catenin is associated loosely with the alpha- and beta-catenin complex (13)(14). These proteins combine and form the cadherin-catenin complex that can bind to actin filaments, thus influencing the cytoskeletal system correlated to intercellular adhesion (15). Recently, proteins of the p120 family have been identified as a regulator of cadherin-based cell adhesion, and those are also binding to the cytoplasmic domain of cadherins (1)(2)(16)(23).

At present, it is known that alpha E-catenin can associate with N- and P-cadherins in addition to E-cadherin, and alpha N-catenin with E-cadherin as well as N-cadherin. In previous studies, we identified the localization of N-cadherin and alpha N-catenin in the normal unmyelinated and regenerating chick sciatic nerve (19)(20). Alpha E-catenin can also bind to N-cadherin, and we have clarified the nature of alpha E-catenin expression in the peripheral nerve (data not shown). The parent cell bodies of sciatic nerve fibers are located in the dorsal root ganglia and spinal cord, but their alpha E- and alpha N-catenin expression has not yet been clarified. In the study presented here, the distribution patterns of alpha E- and alpha N-catenin were therefore examined in the dorsal root ganglia and spinal cord at the lumbar level where sciatic nerves originate. The results showed that alpha E- and alpha N-catenin are co-localized in the dorsal root ganglia and dorsal horn of the spinal cord. On the other hand, alpha E- and alpha N-catenin showed a different distribution in the ventral horn of the spinal cord. Alpha E-catenin was expressed in the cell bodies of neurons in the

ventral horn where alpha N-catenin was absent. These findings suggest that catenin expression may be differentially regulated depending on the functional properties of neurons such as sensory or motor properties.

MATERIALS AND METHODS

Materials

White Leghorn chickens (female, 11~27 days of age) were obtained from a commercial poultry farm (Kakogawa City, Japan). All animal experiments were conducted according to the "Guidelines for Animal Experimentation at Kobe University School of Medicine." The animals were anesthetized with halothane and fixed by transcardiac perfusion with a fixative containing 3% paraformaldehyde in 0.15 M NaCl and 100 mM phosphate-buffered saline (PBS) at pH 7.5 supplemented with 1 mM CaCl₂, and 8% sucrose. The spinal cord of the lumbar level where sciatic nerves derived was excised with dorsal root ganglia and immersed in the same fixative as described above for 4 h at 4 °C. This segment was cryoprotected through a range of increasing sucrose concentrations (10, 15, 20 and 25%) in 0.2 M NaCl and 50 mM Tris-buffered saline (TBS) at pH 7.5, embedded in OCT compound, quick-frozen, and sectioned 8-µm thick in a cryostat.

Immunohistochemistry

The frozen sections mounted on slides were washed in TBS supplemented with 1 mM CaCl₂ (TBS-Ca) and incubated with TBS-Ca containing 5% skimmed milk for 30 min. These sections were incubated for 24 h at 4°C with a rat monoclonal anti-alpha E-catenin antibody, alpha-18 (12), or a rat monoclonal anti-alpha N-catenin antibody, NCAT-2 (6) diluted 1:200 with TBS-Ca containing 1% skimmed milk. As a control, sections were incubated with rat serum instead of alpha-18 or NCAT-2. After washing three times with TBS-Ca, the sections were incubated for 24 h at 4°C with horseradish peroxidase (HRP)-labeled sheep anti-rat IgG antibody [species-specific F(ab')₂ fragment (Amersham)] at a final dilution of 1:20. After washing with TBS-Ca, the sections were incubated for 30 min in 50 µM Tris buffer containing 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB solution), and then incubated for 10 min in the DAB solution with 0.01% H₂O₂ added. The sections were embedded with 50% glycerine in TBS-Ca and examined by light microscopy.

RESULTS

Control

As control for non-specific labeling by the alpha-18 antibody or the NCAT-2 antibody, frozen sections were stained in an identical manner with normal rat serum being substituted for the monoclonal antibody. In these control sections no labeling was observed by light microscopy.

Dorsal root ganglia and spinal cord immunostained with alpha-18 and NCAT-2

Immunoreactions of both alpha-18 and NCAT-2 in dorsal root ganglia were distributed throughout the cytoplasm of neuron cell bodies (Fig.1). In the spinal cord intense immunoreactivities for both alpha-18 and NCAT-2 were found in Rexed's laminae I and II of the dorsal horn (Fig.2). In the ventral horn, on the other hand, immunoreactivity for alpha-18 was observed in neuron cell bodies that seemed to be motor neurons where no immunoreactivity for NCAT-2 was seen (Fig.2). In general, there was no indication of accumulation of catenin beneath the plasmalemma of neuron cell bodies in the dorsal root ganglia and ventral horn.

ALPHA E/N-CATENIN IN DRG AND SPINAL CORD

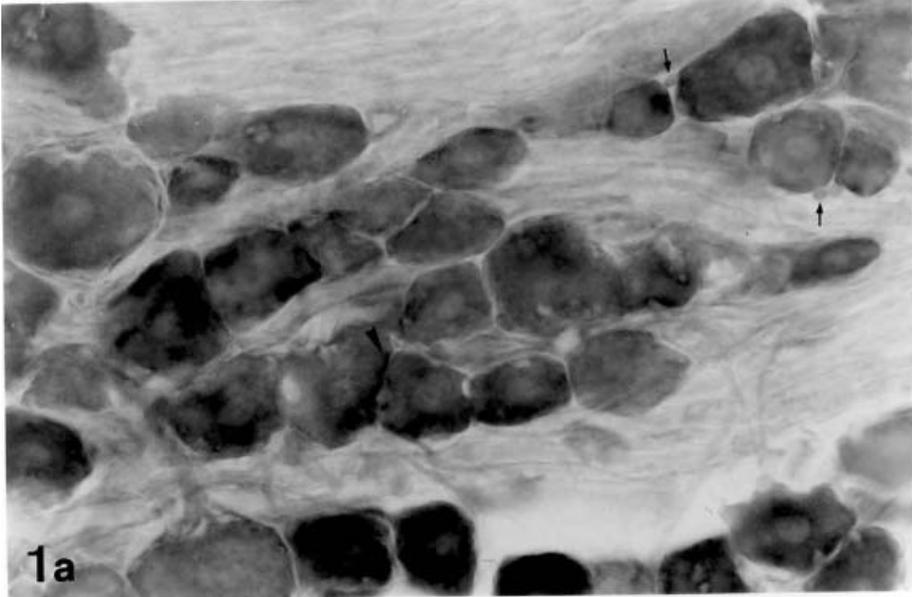


Figure 1. a. The dorsal root ganglion immunostained with alpha-18. Immunoreaction is shown throughout the cytoplasm of neuron cell bodies including nucleus. This immunoreaction is distributed uniformly in general but concentrated under plasmalemma at a few intercellular contacts (arrowhead). Some of the satellite cells also show immunoreactivity (arrows).

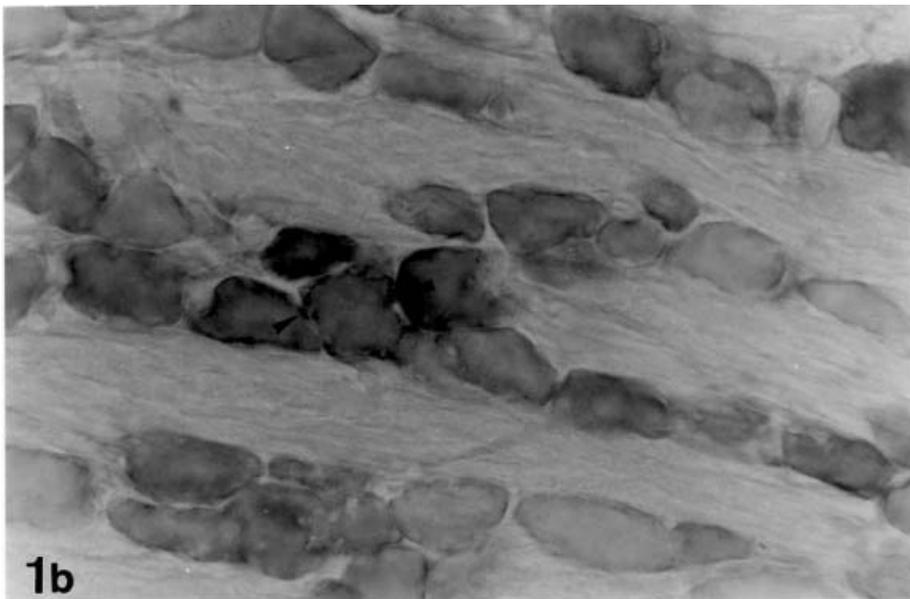


Figure 1. b. The dorsal root ganglion immunostained with NCAT-2. Distribution pattern of NCAT-2 immunoreactive products is almost the same as alpha-18. The intense immunoreactivity is occasionally found under plasmalemma at a few intercellular contacts (arrowheads).

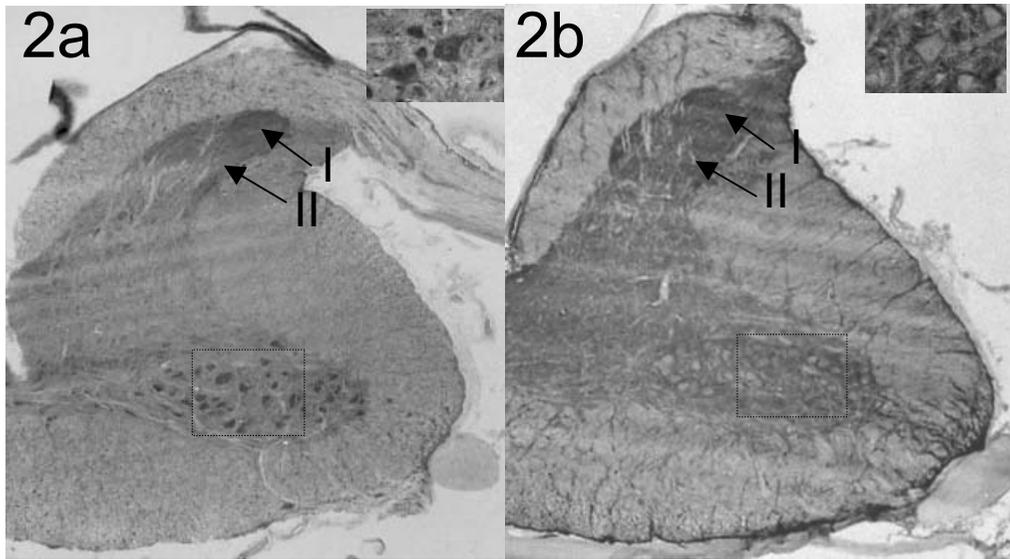


Figure 2. a. The spinal cord of the lumbar level immunostained with alpha-18. The intense immunoreactivity is observed in the cytoplasm of neuron cell bodies in the ventral horn (inset). The neuropile in the Rexed's laminae I and II of the dorsal horn also shows alpha-18 immunoreactivity (arrows).

Figure 2. b. The spinal cord of the lumbar level immunostained with NCAT-2. The neuropile in the Rexed's laminae I and II of the dorsal horn shows NCAT-2-immunoreactivity (arrows), but there is no immunoreactivity in cell bodies of neurons in the ventral horn (inset).

DISCUSSION

Our study has demonstrated that cell bodies of sensory neurons in dorsal root ganglia express not only alpha E-catenin but also alpha N-catenin, whereas motor neurons in the ventral horn have alpha E-catenin without alpha N-catenin expression. This finding indicates the possibility that the motor fibers in sciatic nerves can be distinguished from the sensory fibers by their immunoreactivity for anti-alpha N-catenin antibody. If the sensory nerve consists of unmyelinated fibers, this hypothesis is supported by our previous findings that alpha N-catenin is expressed not in myelinated but in unmyelinated nerve fibers (20). However, it is doubtful that all sensory nerves are unmyelinated. Considering that cadherins were not present in myelinated fibers based on our previous finding and that catenins are used to link to cadherins, there is no need for myelinated fibers to express catenins even in sensory neurons. On the contrary, the Rexed's laminae I and II of the dorsal horn are shown to be intense immunoreactive region for anti-alpha E- and alpha N-catenin antibodies, since this area is rich in unmyelinated fibers. Our previous studies also indicated that cadherins and alpha N-catenin are present in some of the growth cones sprouting from myelinated fibers. These findings imply that growth cones without myelination need cadherins and alpha catenin which function at the axon-axon or axon-Schwann cell interaction. Once myelination has started, cadherins and alpha N-catenin of growth cones are likely to disappear. Even among growth cones, there are those with intense immunoreaction and very faint immunoreaction to anti-alpha N-catenin antibody. It is hypothesized that the former may be derived from the sensory and the latter from the motor neurons.

ALPHA E/N-CATENIN IN DRG AND SPINAL CORD

During the development, the general somatic sensory nerve, like the trigeminal nerve, shows N-cadherin expression at embryonic days 6 to 11, while R-cadherin is expressed in the visceral motor fibers of the vagus and glossopharyngeal nerves (17). Fibers expressing the same type of cadherins can adhere to each other for fasciculation so that this difference in cadherin expression is thought to have a nerve sorting function (18). Catenins could also affect intercellular adhesion by forming complexes with cadherins and may contribute indirectly to nerve fasciculation. It is thus hypothesized that the different expression patterns of alpha N-catenin in the spinal cord may be associated with peripheral nerve sorting during development or regeneration. Another hypothesis is that alpha N-catenin may not be distributed uniformly in the motor neurons: the alpha N-catenin expression is concentrated in the neurites or under the plasmalemma but not in the perikaryon. However, this hypothesis argues against the observation of alpha N-catenin in the dorsal root ganglion. Since Mizoguchi et al. detected that alpha N-catenin is located under the plasmalemma in the central nerve (10), further study at the electron microscopical level will be required before any definite conclusions can be reached.

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