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N-/R-cadherin Expression in Dorsal Root Ganglia and Spinal Cord

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Localizations of N- and R-cadherins in dorsal root ganglia and lumbar spinal cord of the chick were immunocytochemically examined. As a result, N- and R-cadherin appeared to be co-localized on plasmalemma of neurons in dorsal root ganglia that were in contact with satellite cells, whereas there was no expression in neurons of the ventral horn. These findings indicate the possibility that expression pattern of cadherins in the peripheral neuron differs with its functional property (e.g. sensory or motor).

Cadherins constitute a superfamily of calcium-dependent intercellular adhesion molecules (23) and are thought to play a role in cell recognition and segregation, morphogenetic regulation, and tumor suppression (22)(23)(7)(4)(5). Cadherin is a transmembrane glycoproteins and links to catenin at its cytoplasmic domain. Catenins are classified into alpha- and beta-catenins (12), and alpha E- and alpha N-catenins have been identified as subtypes of alpha-catenin (8)(7)(9)(11). Cadherin is linked directly to one or two molecules of beta-catenin, and to one molecule of alpha-catenin via beta-catenin (14)(10)(13). These proteins combine and form the cadherin-catenin complex to be able to bind to actin filaments, thus influencing the cytoskeletal system correlated to intercellular adhesion (14). 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)(15)(24).

In the previous studies, we identified the localization of N- and R-cadherin in the normal unmyelinated and regenerating chick sciatic nerve (18)(20), however it has not yet been clarified the distributions of those cadherin in the parent cell bodies localized in the dorsal root ganglia and spinal cord. In the study presented here, the distribution patterns of N- and R-cadherin in the dorsal root ganglia and spinal cord at the lumbar level where sciatic nerves originate were therefore examined. The results showed that N- and R-cadherin are co-localized in the dorsal root ganglia and dorsal horn of the spinal cord. On the other hand, these cadherins were not expressed in the cell bodies of neurons in the ventral horn. These findings suggest that N- and R-cadherin are distributed only in the sensory neuron, and different type of cadherins might be expressed in the motor neuron.

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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 $CaCl_2$, 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 ^oC. This segment was cryoprotected through a series 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 $CaCl_2$ (TBS-Ca) and incubated with TBS-Ca containing 5% skimmed milk for 30 min. These sections were incubated for 24 h at 4^oC with a rat monoclonal anti-N-cadherin antibody, NCD-2 (6), or a mouse monoclonal anti-R-cadherin antibody, RCD-2 (16) diluted 1:100 with TBS-Ca containing 1% skimmed milk. As a control, sections were incubated with rat and mouse serum instead of NCD-2 and RCD-2. After washing three times with TBS-Ca, the sections were incubated for 24 h at 4^oC with horseradish peroxidase (HRP)-labeled sheep anti-rat or anti-mouse IgG antibody [species-specific $F(ab')_2$ fragment (Amersham)] at a final dilution of 1:20. After washing with TBS-Ca, the sections were preincubated for 30 min in 50 μM Tris buffer containing 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB solution), and then reacted for 10 min in the DAB solution with 0.01% H_2O_2 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 NCD-2 antibody or the RCD-2 antibody, frozen sections were stained in an identical manner with normal rat or mouse serum being substituted for the monoclonal antibody. In these control sections no labeling was observed by light microscopy.

Dorsal root ganglia immunostained with NCD-2 and RCD-2

Intense immunoreactions of both NCD-2 and RCD-2 were found on the plasmalemma of neuron cell bodies and satellite cells in dorsal root ganglia (Figs.1 and 2). On the contrary nerve fibers showed weakly immunoreactive stripes in the negative background. Such weakly stainable structures were assumed to be unmyelinated fibers because of the finding by electron microscopy in our previous study (18,20).

Spinal cord immunostained with NCD-2 and RCD-2

In the spinal cord, intense immunoreactivities were distributed in Rexed's laminae I and II of the dorsal horn (Figs.3 and 4). On the other hand, the ventral horn including neuron cell bodies of motor neurons exhibited no immunoreactivity.

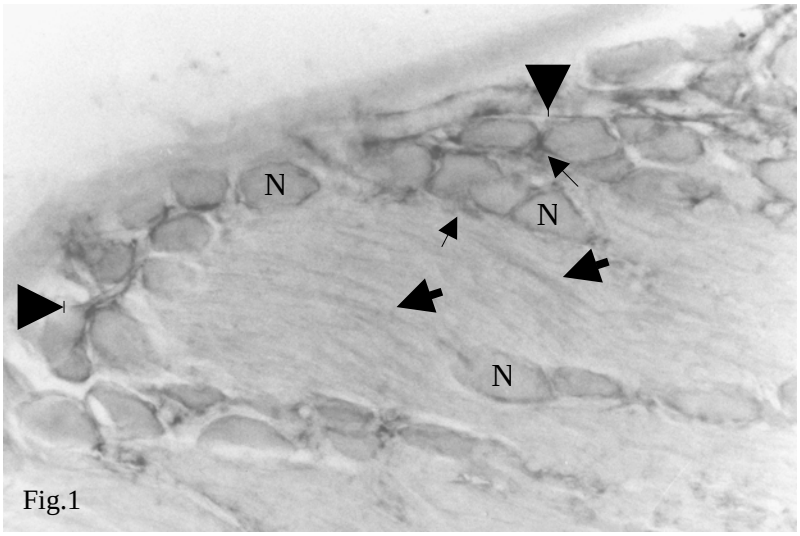


Figure 1. The chicken dorsal root ganglion of the lumbar level immunostained with a rat monoclonal antibody for N-cadherin (NCD-2). The plasmalemma of neuronal cell bodies (N) and satellite cells (small arrows) express NCD-2 immunoreactivity. Occasionally, intense immunoreactivities are found at the cell-cell contacts (arrowheads). Nerve fibers are weakly immunoreactive, and they appear as stripes in the negative background (large arrows).

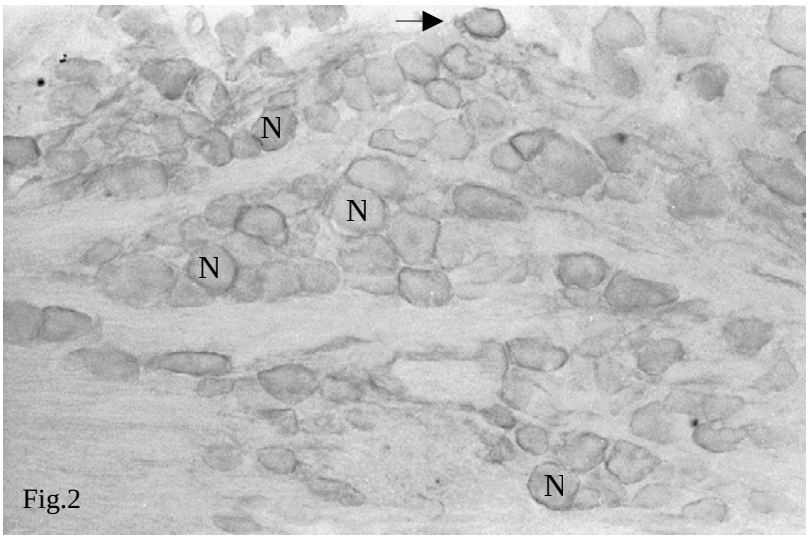


Figure 2. The dorsal root ganglion immunostained with a mouse monoclonal anti-R-cadherin antibody (RCD-2). Distribution pattern of RCD-2 immunoreactive products is almost the same as NCD-2, that is to say neuron cell bodies (N) and satellite cells (small arrow) exhibit RCD-2 immunoreactivities on the plasmalemma.



Figure 3.
The spinal cord of the lumbar level immunostained with NCD-2. In the Rexed's laminae I and II of the dorsal horn, the neuropile shows NCD-2-immunoreactivity (arrows). The cell bodies of neurons in the ventral horn exhibit no immunoreactivity (arrowhead).

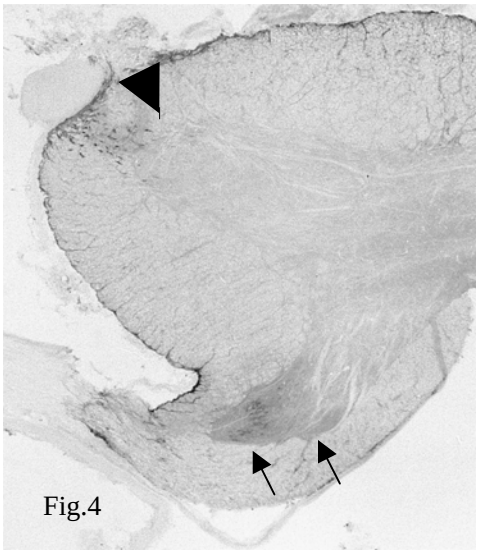


Figure 4.
The spinal cord of the lumber level immunostained with RCD-2. The Rexed's laminae I and II of the dorsal horn show RCD-2-immunoreactivity on the neuropile (arrows). The ventral horn including neuron cell bodies exhibit no immunoreactivity (arrowhead).

DISCUSSION

This study has demonstrated that cell bodies of sensory neurons in dorsal root ganglia express N- and R-cadherin on the plasmalemma, whereas motor neurons in the ventral horn did not express these cadherin.

This result indicates a possibility that sciatic nerve fibers can be distinguished between the sensory and the motor components by their expression pattern of cadherins. 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 (16). Fibers expressing the same type of cadherins can adhere to each other to make fasciculation so that this difference in cadherin expression is thought to be a nerve sorting function (17). It is thus hypothesized that even after development the different bundles of nerve fibers (e.g. sensory or motor) express the different type of cadherin. If the sensory nerve consists of only unmyelinated fibers, this hypothesis is supported by our previous finding that both of N- and R-cadherin are expressed not in myelinated but in unmyelinated axons (18)(20). This finding is also relevant for intense immunoreactivity in the Rexed's laminae I and II of the dorsal horn because this area is abundant in unmyelinated fibers. However, it is unlikely that all sensory nerves are unmyelinated. Our previous study (18) has demonstrated that regenerating axons after crush injury begin to express N-cadherin even from myelinated fibers, so that it is possible that parent neurons of myelinated fibers also show N- or R-cadherin expression. Anyway, further study at the electron microscopical level will be required before definite conclusions can be reached.

In our previous study, the motor neurons in the ventral horn showed an absence of alpha N-catenin, which can be associated with the cytoplasmic domain of N-cadherin. On the other hand, alpha E-catenin having the ability to link to N-, P- and E-cadherin has been demonstrated to be expressed in the motor neurons. This result implies that P- or E-cadherin instead of N- or R-cadherin might be present on the plasmalemma of motor neurons.

N- and R-cadherin are found on the plasmalemma of dorsal root ganglia, whereas alpha E- and alpha N-catenin have been shown in our previous study (21) not to be restricted to the area under the plasmalemma but to extend throughout the cytoplasm. These catenins are thus likely to be independent from the cadherin-catenin complex. Since it has been demonstrated that cadherin-mediated cell-cell adhesions are rearranged dynamically by the regulation of Rho-GTPases (3), N- and R-cadherin in dorsal root ganglia can be expected to have poor adhesive properties for the cell-cell contacts.

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