Immunohistochemical Localization of Some Extracellular Molecules and Their Integrin Receptors in the Rat Pacinian Corpuscles

P Dubový, I. Svíženská, R. Jančálek and I Klusáková

Department of Anatomy, Division of Neuroanatomy, Medical Faculty, Brno, Czech Republic

Abstract. The present results suggest that laminin-1 and 3 are localized in the specialized Schwann cells of Pacinian corpuscles, in spite of incomplete deposition of the basal lamina on the surface of their cytoplasmic processes In addition, laminin-3 is concentrated and probably function as a stop protein not only in the neuromuscular junction, but also in the specialized Schwann cells enveloping the dendritic zone of the afferent axon No significant changes of immunostaining for both laminins and their integrin receptors following denervation of Pacinian corpuscles indicate that their synthesis is independent to afferent axon as a prerequisite for successful reinnervation

Key words: Laminin-1 and 3 — Integrin receptors — Specialized Schwann cells

Pacinian corpuscle, rapidly adapting mechanoreceptor, consists of the dendritic zone of the afferent axon surrounded by specialized Schwann cells (inner core) and connective tissue capsule The basal lamina and other components of the extracellular matrix maintain an ionic stability along the dendritic zone, and prepare conditions for restoration of reinnervation

The specialized Schwann cells and their cytoplasmic processes form a multilamellar complex named as the inner core The outermost lamellae and bodies of specialized Schwann cells are covered by distinct basal lamina while the surface of innermost lamellae is invested by incomplete basal lamina-like matrix The basal lamina of outer specialized Schwann cells is considered to be continuous with those covering the axon-Schwann cell units (nerve fibres) innervating the corpuscle (Idé 1986) As we described previously (Dubový and Svíženská 1990), the basal laminae of the specialized Schwann cells have also a specific function in maintaining the auxiliary structures of the sensory corpuscle The specialized Schwann cells and their extracellular matrix including the basal lamina play a role in cessation of the axonal growth and differentiation of the dendritic zone during development as well as after reinnervation

Laminins, a dominant group of glycoproteins in the basal lamina, synthesized by Schwann cells (Cornbrooks *et al* 1983) are referred as a potent stimulator of axon outgrowth (Manthorpe *et al* 1983) and Schwann cell migration (Bailey *et al* 1993) Laminin molecular isoforms produced by Schwann cells are heterogeneously distributed along the

Correspondence address P Dubový, Department of Anatomy, Division of Neuroanatomy, Medical Faculty, Komenského nám 2, 662 43 Brno, Czech Republic

nerve fibres Laminin-2 (merosin) is preferably localized in the basal lamina of Schwann cells in nerve trunk, while laminin-3 (s-laminin) is concentrated in the basal lamina of the neuromuscular junctions (Sanes *et al* 1990) Integrins are crucially important transmembrane receptor glycoproteins by which the cells bind to and respond to the extracellular matrix or neighbouring cells

In the present paper we have demonstrated the expression of laminin-1, laminin-3, tenascin-C, collagen-IV, and β1, α1, α5, α6 integrin chains in the rat intact and denervated Pacinian corpuscles by indirect immunohistochemical method at the light microscopic level Interosseal Pacinian corpuscles were removed from adult Wistar rats (both males and females) following transcardial perfusion with and subsequent immersion in Zamboni's fixative solution (Zamboni and deMartino 1967), washed in 10% sucrose in 0 1 M phosphate buffer (pH 7 2) for overnight Cryostat sections, 10 μm thick, were treated overnight with monoclonal or polyclonal antibodies against laminin-1 (1 1000, Sigma-Aldrich, a s , Prague), laminin-3 (1 50, Hybridoma Bank, Iowa, U S A), tenascin-C (1 100, Serotec, Ltd , England), collagen-IV (1 500, Sigma-Aldrich, a s , Prague), β1, α1, α5, α6 (1 100, Immunotech, a s , Prague) were used for indirect immunohistochemical method For visualization of the antibody binding we used high sensitive systems including biotin/streptavidin/HRP, biotin/streptavidin/ALPase or amplification of biotin/streptavidin/HRP with biotinyled tyramine (generously gifted by Dr J Mokry, Hradec Králové)

The best results of immunolabeling were obtained with binding of biotin/streptavidin/HRP and subsequent double amplification using biotinyled tyramine The dense immunostaining for laminins, tenascin-C and integrin receptors was observed in the transitional zone between the inner core and capsule A distinct immunostaining for all investigated antigens was also found in the specialized Schwann cells surrounding the tips of dendritic zone of afferent axon The proper space of inner core displayed positive immunostaining of a medium intensity for laminins, tenascin-C and integrins In addition, a line of immunostaining for tenascin-C set off the surface of dendritic zone Fine immunostaining for collagen-IV was seen in the capsule lamellae and at the surface of the inner core in both, intact and denervated corpuscles The staining intensity for all above-mentioned antigens did not change within 70 days from denervation

Our results indicate that laminins (1 and 3) are localized in the specialized Schwann cells of Pacinian corpuscles in spite of incomplete deposition of the basal lamina on the surface of their cytoplasmic processes Laminin-3 is concentrated in the neuromuscular junction (Sanes *et al* 1990) where may function as a stop protein for motor axons during differentiation or regeneration of the neuromuscular junction (Porter *et al* 1995) The present findings suggest that laminin-3 is concentrated and probably function as a stop protein not only in the neuromuscular junction but also in the specialized Schwann cells enveloping the dendritic zone of the afferent axon The transitional zone between the inner core and capsule is enriched by laminins and their integrin receptors that may have a role in functional integration of the inner core and capsule in intact Pacinian corpuscles

Tenascin-C, an extracellular matrix multimodular glycoprotein with very restricted tissue distribution is involved in the control of cell adhesion, neuron migration and neurite outgrowth (Faissner 1997) The present results suggest that this glycoprotein may be involved in contacts of the dendritic zone of afferent axon in mature Pacinian corpuscles

Immunostaining of integrin α5 chain which makes with β1 chain a receptor for fibronectin (Lefcort *et al* 1992) and L1 (Ruppert *et al* 1995), was confined to the specialized Schwann cells of Pacinian corpuscles where corresponding ligands were demonstrated earlier (Idé and Tohyama 1984, Nolte *et al* 1989)

No significant changes of immunostaining for laminins, tenascin-C and integrins in

the auxiliary structures following their denervation indicate that their synthesis is independent to afferent axon, what is prerequisite for successful reinnervation

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