Influence of metal cation binding on the conformation of ependymin cell adhesion molecules

Rupert Schmidt

Zoologisches Institut der J.W.Goethe-Universität
Siesmayerstraße 70
W-6000 Frankfurt am Main

As components in cell membranes and as constituents of the extracellular matrix, cell adhesion molecules are known to guide the migration of axons and neurectodermal cells and were proposed to modulate the efficacy of neuronal connections during epigenetic differentiation (compare Schmidt, 1989; Schachner, 1992). An appealing hypothesis suggests that synaptic changes induced by learning in vertebrates may in part depend on a replay of such early neurodevelopmental adhesion and recognition events.

A secretory CNS specific glycoprotein, first detected by its increased metabolism after behavioural conditioning and named ependymin according to its early immunohistochemical localization in goldfish brain, was recently shown to provide a good substrate for outgrowth of retinal ganglion cell axons in vitro (J. Schmidt et al., 1991) in accordance with demonstration of the L2/HNK-1 cell adhesion epitope in its N-linked carbohydrate chains. Quantitative *in situ* hybridization revealed that rapid induction of ependymin mRNA after shock avoidance conditioning was significantly different from that following a stressful pseudo-conditioning paradigm (Schmidt et al., 1991).

Ependymin forms a hetero-dimer and the two homo-dimers from 31 and 37 kDa subunits, respectively. ^{45}Ca and ^{65}Zn binding were studied by autoradiography after electrophoretic transfer to nitrocellulose membranes. For quantitative analysis, purified ependymin was incubated with ^{45}Ca or ^{65}Zn and separated from unbound radioligands by filtration techniques. Ion specificity was tested by addition of other metal cations. Both, calcium and zinc, displayed specific, saturable, reversible, high affinity binding to ependymin at independent binding sites. Stoichiometric calculations suggest that two subunits chelate one Zn^{2+}, whereas only that portion of ependymin molecules which is present in the form of monomers binds Ca^{2+}. Ependymin polymerizes on addition of Ca^{2+} chelating agents (Shashoua, 1988).

Tetanic, synchronous, and contiguous stimulation of neuronal circuits decrease the extracellular calcium concentrations in the vicinity of the activated synaptic connections and may polymerize ependymin in the extracellular matrix of activated synapses. The effect of the metal cation concentrations on the conformation of this cell adhesion molecule may thus convert the specificity of neuronal activity into local ultrastructural differentiations.

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