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BRAIN RESEARCH

Research Report

The immunolocalization of the synaptic glycoprotein neuroplastin differs substantially between the human and the rodent brain

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ABSTRACT

Neuroplastin is a cell adhesion molecule of the immunoglobulin superfamily that exists in two splice isoforms, np65/np55, and that was reported to play a prominent role in synaptic plasticity processes. The splice isoform np65 associates with synapses in an activity-dependent manner and has been shown to play a role for the induction of hippocampal long-term potentiation in rodents. We have therefore analyzed the distribution of neuroplastins in human brain. Neuroplastin is present in many neuronal cell types of the forebrain and cerebellum and immunoreactive label covers the cell soma, neurites and also puncta in the neuropil were visible. Interestingly, we found some remarkable species differences in the expression patterns of neuroplastins between the human and the rodent brain. In human brain np65 is prominently present in cerebellum while np55 is the predominant isoform in mouse and rat cerebellum. Moreover, the parasagittal stripe-type of staining seen with np55 in mouse cerebellum is not found in human brain. In addition we found no segregation of np65 immunolabel in hippocampal subregions like it was reported previously for the rat. These results might indicate different cellular functions of the molecule in different species.

1. Introduction

Synaptic cell adhesion proteins mediate the physical contact between pre- and postsynaptic neurons, which is one of the primary events in synaptogenesis as well as plasticity processes including learning and memory (Washbourne et al., 2003; Yamagata et al., 2003). Cell adhesion molecules of the immunoglobulin (Ig) superfamily are among this group of molecules. The immunoglobulin domains of the corresponding family members have been shown in many cases to support stable homophilic adhesion between cell membranes and it is tentatively assumed that similar processes will also occur at the synapse (Washbourne et al., 2003). Neuroplastin is a glycoprotein of the Ig superfamily that is abundant in brain

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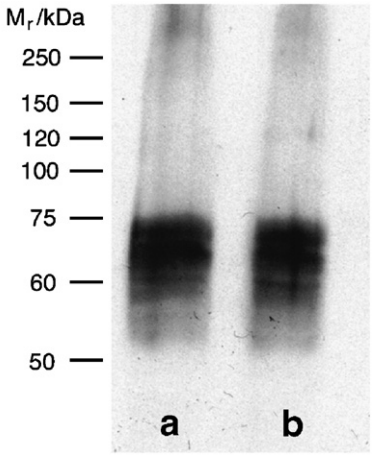

Fig. 1 – The monospecific np65 antiserum detects np65 after immunoblotting in crude membrane fractions prepared from postmortem human cortex (lane a) and cerebellum (lane b). 20 μg of protein was loaded in each lane.

and enriched in rat forebrain synaptic membrane preparations (Hill et al., 1988; Willmott et al., 1992; Langnaese et al., 1997, 1998). Neuroplastin is expressed in two isoforms produced by alternative splicing, np55 (a widely expressed two Ig domain form) and np65 (a neuron-specific three Ig domain form, tightly associated with synaptic membranes). Both isoforms are glycosylated (Langnaese et al., 1997, 1998) but only the three-domain form of neuroplastin, np65, and not the two-domain form np55, displays homophilic interaction (Smalla et al., 2000). Previous work has shown that np65 plays an important role in synaptic plasticity and it was suggested that it might be involved in learning and memory processes (Smalla et al., 2000). Further studies combining cellular aspects of learning and memory processes with behavioral experiments have to be done in rodent models. Especially with regard to the generation and interpretation of genetic neuroplastin mouse models it is imperative to compare the expression of the protein in rodent and human brain. To address this problem we therefore performed immunostainings on human brain sections.

2. Results

Immunoblotting experiments with a polyclonal neuroplastin antiserum revealed the presence of np65 in human brain protein homogenates (Fig. 1). The immunoreactive bands have a fuzzy appearance, which might be due to differential glycosylation of the protein. Interestingly, however, and in contrast to rat and

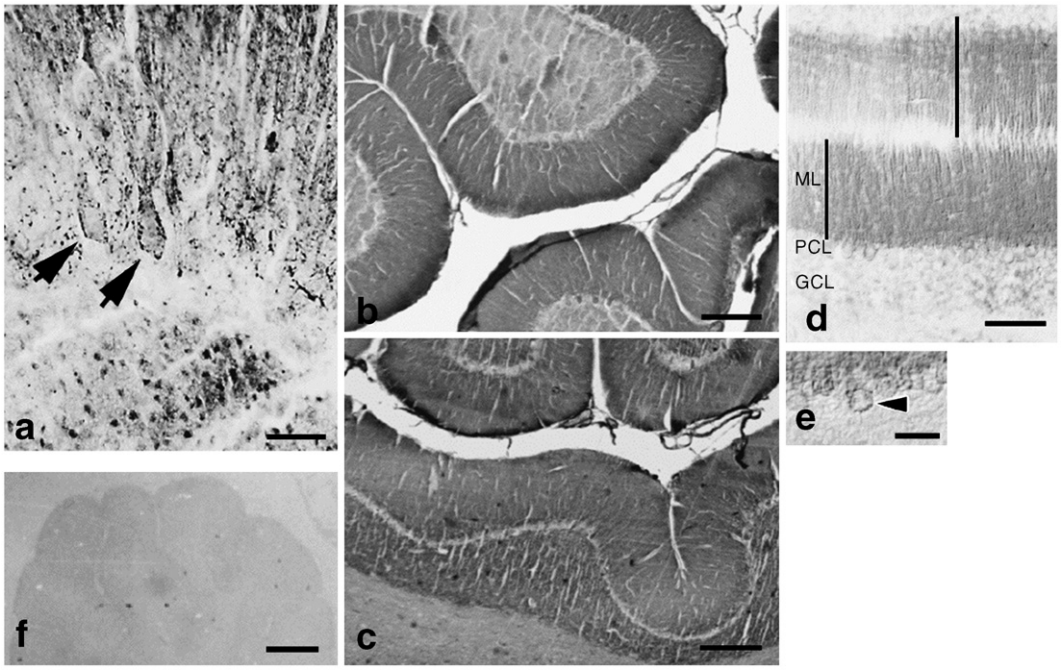

Fig. 2 – Immunolocalization of neuroplastin in human and mouse cerebellum. (a) Neuroplastin immunoreactivity in human cerebellar cortex. Outer cell membranes of Purkinje cells (arrows) are as well as multiple granule cells are labeled. Scale bar=40 μm. (b) Low power microphotograph showing the regional distribution of neuroplastin in the cerebellar anterior lobe. Please note the absence of parasagittal stripes of np65 immunoreactivity. Scale bar=0.5 mm. (c) Low power microphotograph demonstrating the distribution pattern of np65 immunoreactivity in the inferior posterior lobe. Parasagittal stripes do not appear. Scale bar=0.5 mm. (d) Stripes of neuroplastin immunoreactivity in the mouse cerebellum. Borders are indicated by vertical lines. ML: molecular layer; PCL: Purkinje cell layer; GCL: granular cell layer. Scale bar=200 μm. (e) Neuroplastin-immunopositive Purkinje cell in the mouse cerebellum exhibiting strong membranous label (see arrow) Scale bar=20 μm. (f) Control section from mouse cerebellum after preabsorption of the antibody with recombinant neuroplastin protein fragments. Scale bar=0.5 mm.