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BRAIN RESEARCH

Research Report

Regional and cellular distribution of ephrin-B1 in adult mouse brain

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ABSTRACT

The membrane-bound proteins ephrins and their receptors, Eph receptor tyrosine kinases, are known for their key role during development of the central nervous system (CNS). Ligand/receptor interactions as a result of cell–cell contacts activate intracellular signalling pathways which mediate specific cellular responses. Activation can occur bidirectionally in both the receptor and the ligand-bearing cells. Eph receptor and ephrin families have been implicated in synaptic plasticity in the mature brain: effects include long-term potentiation/depression of excitatory transmission (LTP/LTD) and an action on the structure and number of synaptic contacts. However, due to the redundancy of binding between receptors and ligands, the role of individual proteins has not yet been completely elucidated. Ephrin-B1 has been suggested to play a role in synaptic plasticity in the hippocampus, but its expression and localization at pre- or post-synaptic sites has been poorly documented, most likely due to the apparent low activity of the corresponding gene in mature brain. Here we present immunohistochemical data demonstrating a broad but highly regulated cellular distribution of ephrin-B1 in the mature mouse brain. We show that ephrin-B1 is expressed post-synaptically on dendritic spines in the cortex, supporting a role in synaptic plasticity in this region. However, the prevalent extra-synaptic distribution in regions such as the hippocampus and cerebellum suggests an additional structural role, perhaps at the neuron/glia interface.

1. Introduction

Ephrins and their receptors, the Eph tyrosine kinases are membrane-bound proteins that are key elements in the regulation of and response to cell–cell interactions in the immature tissue environment. The primary function of ephrins during development consists of the patterning of cell and axonal populations. During the earliest stages of nervous system development, ephrin expression forms boundaries for tissue formation and cell migration (Klein, 1999; Coulthard et al., 2002). At later stages, as cell mobility is reduced, ephrins direct the formation of organized axonal projections, through the formation of expression gradients across interconnected brain regions (Palmer and Klein, 2003). In addition to their role as topographic guidance cues, there is strong evidence that Eph receptor–ephrin interactions contribute to the formation of synaptic contacts (Dalva et al., 2000; Rodenas-Ruano et al., 2006).

Cellular responses are triggered by signalling events elicited via the receptor as a consequence of ligand binding.

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However, there is also evidence for receptor induced signalling via the ligand, known as reverse signalling (Lim et al., 2008). Ephrins are divided into two sub-families, namely the A-type and the B-type: ephrin-As are anchored to membranes by a phosphatidyl-inositol (PI) linkage, while ephrin-Bs are transmembrane proteins and are primarily responsible for reverse signalling events (Aoto and Chen, 2007). Within families, specificity of the ephrin/receptor interaction is relatively low, since receptors bind all ligands of the same group. In addition, there is evidence for some cross-group binding (EphA4 and EphB2; Pasquale, 2004; Himanen et al., 2004).

In contrast to strong and widespread expression of ephrins and receptors during brain development, their levels are low in the adult, in accordance with the decrease of their guiding role, which is maintained only in the germinative subventricular zone (SVZ) and the corresponding path to the olfactory bulb, the rostral migratory stream (RMS) (Conover et al., 2000): however, some genes for B-type ligands and receptors maintain high expression in specific areas (Liebl et al., 2003), implying a role in adult brain function, the most likely being structural modification of mature synapses. In adult hippocampus, ephrin-A3 located on the membrane of glial cells could interact with the EphA4 receptor on dendritic spines to trigger a reduction in spine density (Murai et al., 2003). It has been also shown that, at hippocampal CA1–CA3 synapses, post-synaptic ephrin-B3 can have both a receptor-mediated influence on the pre-synapse protein composition and a receptor-independent (reverse) effect on the number of the excitatory contacts (Rodenas-Ruano et al., 2006). Another current idea on the contribution of ephrins/receptors to synaptic plasticity is related to the activity-driven molecular mechanisms involved in long term potentiation and depression of excitatory transmission (LTP and LTD), particularly in the hippocampal area (rev. by Aoto and Chen, 2007). It has been shown that the genetic deletion of the EphB2 receptor affects postnatal LTP-related activity of the n-methyl-D-aspartate (NMDA) receptor at CA1 hippocampal subfield and dentate gyrus synapses (Henderson et al., 2001); furthermore, post-synaptic B-type ephrins have been linked to LTP/LTD at the CA3/CA1 connections (Grunwald et al., 2001), whereas post-synaptic EphB receptors and pre-synaptic ephrin-Bs have been implicated in NMDA-independent LTP at mossy fibres (Contractor et al., 2002). These findings demonstrate pre- and post-synaptic effects of forward and reverse signalling suggesting the existence of multiple mechanisms of ephrin/receptor interplay across specific synaptic structures.

However, interpretation of these studies is complicated by the lack of specificity in ephrin/Eph receptor binding and a first step towards understanding this possibly redundant

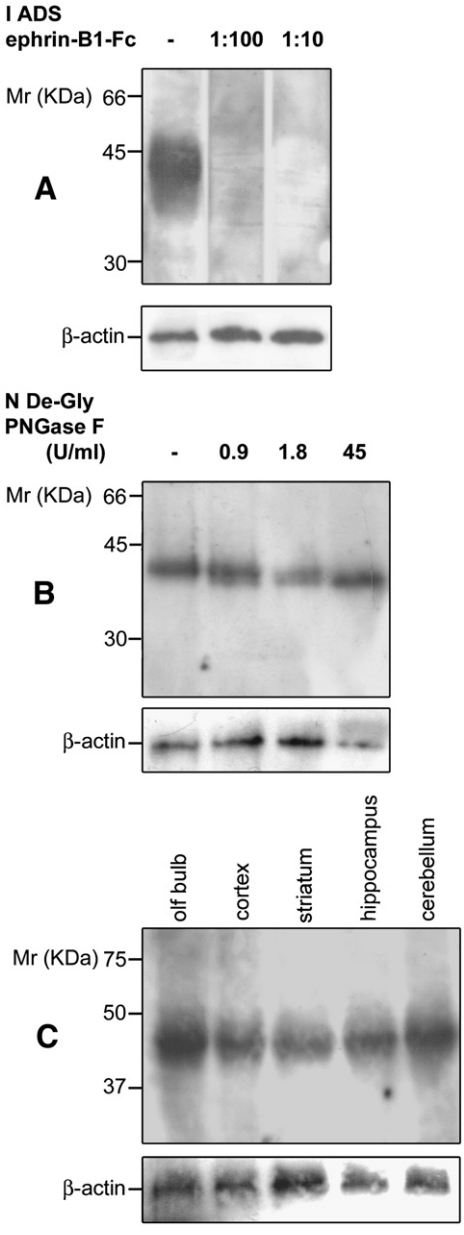

Fig. 1 – Western blot analysis of ephrin-B1 in proteins from adult mouse brain. A: Proteins from the whole cerebral cortex were extracted, separated by electrophoresis and bound to a membrane. Membrane lanes from duplicate samples were cut into strips and separately incubated with the anti-ephrin-B1 antibody (first lane) or with the same antibody which had been immunoadsorbed (I ADS) with the ephrin-B1-Fc chimera protein, diluted one hundred times (second lane) or ten times (third lane) from the original solution (0.25 mg/ml). Marks for the molecular weight (Mr) were obtained from a standard protein mixture, run on a separate lane. Membrane lanes were probed with a peroxidase-linked secondary antibody and the immunopositive bands evidenced by peroxidase-driven luminescence. A similar system was used to detect β-actin, as a loading control (small panels). B: Proteins from mouse cortex were incubated with different amounts of a de-glycosylating enzyme, to yield different amounts of the de-glycosylated product. Labels above each lane refer to different amounts of de-glycosylation enzyme added, in International Units/ml. Samples were then processed for western blotting with the anti-ephrin-B1 antibody as described for panel A. C: The main zones of the adult mouse brain were separated and their proteins processed in separated samples. The band lanes of the different zones were immunostained in the same membrane with the anti-ephrin-B1 antibody, as described for panel A.