

Abstract

Ischemic injury leads to a sequence of pathophysiological events, which are accompanied by a release of growth factors and morphogens that significantly affect cell proliferation, migration and also their differentiation. Following ischemia, besides enhanced neurogenesis and gliogenesis in subventricular zone of the lateral ventricles and gyrus dentatus of the hippocampus, neurogenesis/gliogenesis also occurs in non-neurogenic regions, such as cortex or striatum. Recently, the attention was turned to a new glial cell type, termed polydendrocytes or NG2 glia. Under physiological conditions, these cells are able to divide and differentiate into mature oligodendrocytes due to they have often been equated with oligodendrocyte precursor cells. Based on recent reports, polydendrocytes are also able to generate protoplasmic astrocytes (Zhu et al., 2008) and neurons *in vitro* (Belachew et al., 2003), however their ability to differentiate into astrocytes or neurons under physiological or pathological conditions is still highly debated.

Therefore, we have investigated the effect of different growth factors and morphogens, specifically brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and a morphogen sonic hedgehog (Shh), on differentiation potential of polydendrocytes *in vitro* after their isolation from non-injured cortex. Furthermore, we have investigated polydendrocyte differentiation potential *in vitro* following focal cerebral ischemia. We have used transgenic mice, B6;FVB – Tg(Cspg4-cre)1Akik/J, in which, after cross-breeding with reporter mice, the polydendrocytes were labeled by enhanced green fluorescent protein (EGFP). A middle cerebral artery occlusion (MCAO) was employed to induce focal cerebral ischemia. For all experiments, we used EGFP⁺ cells isolated from the adult mouse cortex. Following cell isolation, either from non-injured or ischemic brains, the cells were cultured for 4 weeks and electrophysiologically characterized using patch clamp technique in whole-cell configuration. Recorded cells were identified immunocytochemically. According to their current profile, the EGFP⁺ cells were divided into three cell types; namely passive, complex and precursor type of cells. Passive cell type typically expressed symmetrical, time- and voltage-independent passive currents and low input resistance. Complex cell type displayed inwardly rectifying K⁺ currents, delayed outwardly rectifying K⁺ currents and fast activating and inactivating K⁺ currents. Some cells also showed small inwardly rectifying Na⁺ currents. The current pattern of precursor cell type

included delayed outwardly rectifying K^+ currents and in some cases, also fast activating and inactivating K^+ currents. Our results revealed that Shh maintained EGFP⁺ cells in precursor stage and other factors influenced electrophysiological properties of precursor cell type, but not complex type of cells. BFGF had the most significant effect on precursor type of cells resulting in depolarized resting membrane potential, increased membrane capacitance and high current density of delayed outwardly rectifying K^+ currents. Moreover, focal cerebral ischemia promoted incidence of passive cell type, which also occurred in cell culture isolated from non-injured cortex after exposure to BDNF. Thus we can conclude that factors BDNF, bFGF, VEGF and Shh influence membrane properties of precursor cell type and that focal cerebral ischemia increases proportion of passive cell type originating from EGFP⁺ polydendrocytes.