ABSTRACT

Glutamate is the main excitatory neurotransmitter in the mammalian brain, and its transmission is responsible for higher brain functions, such as learning, memory and cognition. Glutamate action is mediated by variety of glutamate receptors, of which N-methyl-D-aspartate (NMDA) receptors are the most remarkable due to their high Ca^{2+} permeability and complex pharmacology. Despite the widespread expression of NMDA receptors in astroglial cells in different brain regions, they have been studied mostly in neurons. Therefore, the role of astroglial NMDA receptors under physiological conditions as well as in pathological states, such as cerebral ischemia, is not fully understood. The aim of this work was to elucidate the presence, composition and function of these receptors in astrocytes under physiological conditions and after focal cerebral ischemia. For this purpose, we used transgenic (GFAP/EGFP) mice, in which astrocytes express enhanced green fluorescent protein (EGFP) under the control of human promotor for glial fibrillary acidic protein (GFAP) enabling astrocyte isolation and their collection via fluorescence-activated cell sorting. We performed single-cell RT-qPCR analysis of astrocytes isolated from the cortex of adult mice. The analyzed cells were isolated from the uninjured brains of 50 days old mice (control) and mice 3, 7 and 14 days after middle cerebral artery occlusion (MCAo), a model of ischemic injury. For each cell, we analyzed the expression of genes specific for astrocytes, reactive astrocytes and individual subunits of NMDA receptors. Additionally, we have employed immunohistochemical analysis and intracellular Ca^{2+}-imaging technique to elucidate the functional properties of astroglial NMDA receptors. Gene expression profiling revealed that astrocytes express genes for all NMDA receptor subunits, of which expression was increased after ischemic injury. Nevertheless, our immunohistochemical analysis showed that astrocytes express only GluN1, GluN2C, GluN2D and GluN3A subunits. Furthermore, intracellular Ca^{2+}-imaging disclosed that under physiological conditions the activation of astroglial NMDA receptors results in significant Ca^{2+} entry into the cell, while under ischemic conditions the Ca^{2+} permeability of astroglial NMDA receptors is markedly reduced. Based on our findings, we conclude that astroglial NMDA receptors probably do not contribute to Ca^{2+}-mediated cell damage following ischemic injury.