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P2X7R, β_3-integrin and Cx-43 mediate interaction between astrocytes and adjacent autoreactive immune cells

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Astrocytes form a dense meshwork throughout the central nervous system (CNS) which qualifies them to perform interactive maintenance functions with neighboring cells. In neuroinflammation, this astroglial cell-to-cell interaction varies which can either promote or lessen pathological processes (1,2). In multiple sclerosis (MS), astrocytes engage in an interaction with immune cells which drives neurodegeneration by creating and sustaining an inflammatory CNS environment (3). Previously, we showed that CNS-infiltrated immune cells (CNS-IICs) in the experimental autoimmune encephalomyelitis (EAE) rat, rapidly alter the activity pattern of astrocytes by activating glial P2X7 receptor (P2X7R)(4). In the present study, we aimed to identify the properties of astroglial P2X7R in EAE and to identify mechanisms responsible for astrocyte activation in the presence of CNS-IICs ($CD4^+$ T cells). In this respect, spinal cords from rats at the peak of EAE and age-matched healthy controls were isolated and protein expression of P2X7R and connexin-43 (Cx-43) were investigated. P2X7R protein expression was decreased in the lumbar spinal cord, while Cx-43 did not change. Next, we found that P2X7R and Cx-43 proteins interact in the lumbar spinal cord since both the monomer and the dimer Cx-43 co-immunoprecipitate with P2X7R. Even though the colocalization of P2X7R and Cx-43 was decreased in EAE compared to the control, the analysis of the distribution of astroglial P2X7R and Cx-43 and their colocalization in the radius of 20 μm from the infiltrated $CD4^+$ T cell center showed that astroglial P2X7R and Cx-43 are specifically associated and concentrated in the proximity of CNS-IICs in the EAE spinal cord. Subsequently, to achieve an unambiguous analysis of astrocyte-immune cell interaction, we monitored Ca^{2+} dynamics in Fluo-4 labeled cultured naïve astrocytes following brief bath-application of CNS-IICs isolated and purified from spinal cords of EAE rats. Our data suggest that astroglial $\alpha_v\beta_3$-integrin acted upstream of P2X7R activation and is likely involved in establishing initial contact of astrocytes with CNS-IICs since astrocytic $\alpha_v\beta_3$-integrin block reduced the astrocytic Ca^{2+} response to CNS-IIC application. Furthermore, astrocytes challenged with CGP31157 (blocker of mNCLX and HCX) exhibited a prolonged intracellular Ca^{2+} elevation and higher ATP release after brief exposure to CNS-IICs, indicating a regulatory function of mitochondria on this intracellular astrocyte Ca^{2+} response. Collectively these data describing integrin-relevant cellular mechanisms of astroglial P2X7R activation could help to expand integrin-inhibiting therapeutic approaches currently in use for MS treatment toward control of astrocyte purine-based interaction with immune cells.

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