PURINERGIC CURRENT IN MICROGLIA IS ENHANCED BY METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION

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Introduction: Microglia have been described as important key regulators of neuroinflammation, influencing the activity of other cells in the brain. Such role is mediated by release of several agents, including ATP released by both microglia and astrocytes. During neuronal activity, glutamate levels in brain are elevated. Since microglia express both metabotropic glutamatergic (mGlu) receptors such as mGlu5and ionotropic P2X7receptors, both involved in the progression of neuroinflammatory conditions, we explored the possibility of interaction among pathways underlying activation of these two different receptor subtypes.

Materials and methods: ATP and glutamate interaction has been investigated by monitoring current elicited by P2X7receptor in patch-clamp experiments in murine microglia BV2cell line. Cells were activated by the selective agonist BzATP (100 mM). In different experiments, cells were exposed to mGluR5selective agonist, CHPG (200 mM) for a short incubation (5s) and then both agonists were applied. The same protocol was repeated in BV2cells transiently transfected with M3receptors. To test the effective transfection of this receptor subtype, live cell imaging experiments were performed in order to monitor the translocation of the pleckstrin homology (PH) domain, tagged with mCherry, upon Gq activation. Cells transfected only with PHmCherry did not show any translocation.

Results: To test the presence of P2X7channels, cells were stimulated with selective agonist BzATP (100 mM) and the current elicited after this treatment was blocked by the selective antagonist A438079(10 mM). On a separate set of experiments, we observed a robust increase in current elicited by P2X7during co-stimulation with BzATP and CHPG compared to BzATP application alone. Multiple stimulation of BzATP showed sensitization of P2X7receptor current up to three applications. Moreover, such increase was further potentiated following co-stimulation with CHPG and BzATP. To confirm that the response was selectively determined by mGlu5receptor stimulation, we tested activation of different Gq coupled receptors. BV2cells transfected with M3muscarinic receptor were exposed to carbachol (100 mM). P2X7current elicited during co-stimulation with carbachol and BzATP did not significantly differ from that activated by BzATP alone.

Discussion and conclusion: Taken together, these data suggest a strong interaction between ATP and glutamate, supported also by the lack of involvement of a different Gq coupled receptor, such as M3receptor. It is known that P2X7, during its activation, has the capability to increase the size of its pore and the increasing current observed after repeated stimulation with BzATP indicates such effect. Our data suggest that the current elicited after selective activation of P2X7can be increased by the simultaneous presence of mGlu5activation. P2X7and mGlu5receptors separately have important roles during neuroinflammation. Therefore, their interaction may play a role in conditions that promote microgliosis and that impact on the progression of several neurodegenerative diseases.