Microvesicles shed from microglia contribute to metabotropic glutamate receptor 5 effects on neuronal viability

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Metabotropic glutamate (mGlu) receptors 5 are known to be involved in the regulation of synaptic transmission and have long been implicated in mechanisms of neurodegeneration and neuroprotection. More recently, a role for these receptors in neuroinflammmation has been envisaged, suggesting a potential anti-inflammatory effect. However, a cooperating role of mGlu5 receptor in LPS-induced microglia activation has also emerged. To face this controversy, we analyzed the role of mGlu5 receptors in microglia using the BV2 cell line. mGlu5 receptors are expressed in microglial cells as well as in astrocytes and their levels are not modified upon treatment with LPS or the group 1 mGlu agonist, 3,5-dihydroxyphenylglycine (DHPG). Activation of mGlu5 receptors by DHPG or the more selective mGlu5 receptor agonist (RS)-2-chloro-5-hydroxyphenylglycine (CHPG), reduced intracellular content of TNF-Î± induced by exposure to LPS as assessed by Western blot and flow cytometry, suggesting a potential anti-inflammatory effect mediated by mGlu5 receptor. Cytokines released by microglia may affect neuronal viability but a different way of cell to cell communication involves shedding of microvesicles (MVs) from the plasma membrane. These have a diameter of 100-1000 nm and their formation has been described in several cell types, including microglia cells. MVs may in fact contain molecules that are transferred to neurons and/or surrounding non-neuronal cells. MVs shed from BV2 cells were obtained after stimulation of P2X7 receptor with the more stable ATP analog, benzoyl-ATP (100 μM, 20 min), and isolated by differential centrifugation. Formation of MVs could be monitored for at least 40 min by live cell imaging after P2X7 receptor stimulation in BV2 cells previously stained with the cell membrane dye FM1-43. Released MVs could be transferred to neurons around which they assembled before being internalized. This effect was prevented by preincubation with annexin-V that binds to phosphatidylserine, highly enriched in MVs. Neither of these effects, i.e. MVs shedding and internalization was markedly affected by pretreatment of BV2 cells with mGlu5 receptor agonists. However, mGlu5 receptor stimulation counteracted the increased expression of flotillin, a membrane-associated protein that concentrates in MVs, induced by treatment with LPS. When MVs were transferred to SH-SY5Y human neuroblastoma cells, the viability of neurons in response to a toxic insult such as β-amyloid$_{25-35}$ (25 μM) or rotenone (up to 10 μM) was not modified. In contrast, when the toxic insult was induced in the presence of MVs from DHPG-pretreated microglial cells, an increased neuronal death was observed. Interestingly, this effect was not present when MVs from LPS activated microglia were transferred. Potentiation of rotenone-induced SH-SY5Y neuronal death was also present upon stimulation of mGlu5 receptor expressed in neurons. The present results confirm that mGlu5 receptor stimulation can contribute to increased neuronal damage and suggest a role for microglia, through the shedding of MVs, in this effect.