ROLE OF P2X7 IN MICROGLIAL FUNCTION

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Shedding of membrane particles into the extracellular medium represents an important mean of intercellular communication in hematopoietic cells. We have recently demonstrated that also microglia cells, just like monocytes, their systemic counterpart, are able to form and release membrane vesicles into the extracellular medium and that shed vesicles are used by these cells to release the pro-inflammatory cytokine Il-1beta upon P2X7 receptor activation (Bianco et al., 2005). Aim of the present study was to get further insights into the mechanism of P2X7-induced vesicle shedding from microglia. A biochemical analysis by western blotting of the protein components present in shed vesicles, as compared to microglial homogenates, revealed that the enzyme acid sphingomyelinase (aSMase) is significantly enriched in vesicles relative to homogenates. aSMase catalyzes the hydrolysis of sphyngomyelin into ceramide and its enzymatic activity can change the fluidity of the plasma membrane. To investigate whether aSMase can be involved in ATP-induced vesicle release, we measured aSMase activity in microglial cells exposed to the P2X7 agonist BzATP (100 μM) and found a peak of aSMase activity 2 min after agonist exposure. An involvement of aSMase in P2X7-induced vesicle shedding was also indicated by a spectrophotometric evaluation of the amount of vesicles present in the supernatant of FM1-43 labelled microglia preincubated with two inhibitors of aSMase, imipramine (20 μM) or D609 (25 mg/ml). A significant lower amount of vesicles was indeed detected in the medium of microglial cells exposed to aSMase blockers. To evaluate whether the ability of P2X7 receptor to trigger large pore formation is relevant for aSMase activation, microglial cells were pretreated with a specific MAP kinase p38 inhibitor, SB203580 (400 nM), which inhibits the opening of membrane pores induced by P2X7 receptor activation. The analysis of aSMase activity and the quantitation of vesicle shedding by the spectrophotometric assay indicated that SB203580 strongly inhibited aSMase activation and completely blocked vesicle shedding induced by P2X7 receptor activation. All together our results indicate that both p38 MAP kinase and aSMase act downstream the P2X7 receptor and that their activation is necessary for P2X7-induced vesicle shedding to occur. In line with this hypotesis, a 85-90% reduction of P2X7-dependent IL 1-beta release was assayed in microglial cells pre-treated with either aSMase or p38 MAP kinase inhibitors. Overall our results indicate that inhibition of vesicle shedding is an efficient way to reduce the release of the IL 1-beta from microglia, thus opening new strategies for the pharmacological treatment of neurodegenerative diseases characterized by an inflammatory component.

Bianco F, Pravettoni E, Colombo A, Schenk U, Moller T, Matteoli M, Verderio C. (2005) Astrocyte-Derived ATP Induces Vesicle Shedding and IL-1beta Release from Microglia. J Immunol**.** 174:7268-77.