P2Y receptor activation inhibits the formation and proliferation of primary mouse sub-ventricular-derived neurospheres

M.R. Stafford, P.F. Bartlett and D.J. Adams, School of Biomedical Sciences and The Queensland Brain Institute, University of Queensland, Brisbane QLD 4072 Australia.

Purinergic receptors mediate a variety of biological effects in response to extracellular nucleotides. In the brain, astrocytes and neurovascular endothelial cells release nucleotides such as ATP and may have regulatory roles in the stem cell micro-environment. Extracellular ATP also mediates Ca^{2+} wave propagation between astrocytes. Further, a correlation between Ca^{2+} wave intensity and cortical neuronal production in the ventricular zone of the embryo suggests a potential role for extracellular ATP and Ca^{2+} waves in early neurogenesis. In the present study we show that (i) sub-ventricular zone (SVZ) stem cells express purinergic receptors, (ii) ATP evokes intracellular Ca^{2+} transients in SVZ-derived neurospheres, and (iii) purinergic agonists can affect primary neurosphere formation and proliferation. $HSA^{lo}PNA^{lo}$ stem cells were purified from the SVZ of adult mice using flow cytometry. The stem cell population expressed mRNA for P2Y1, 2, 6, 12 and 14 receptor subtypes. In primary neurospheres loaded with Fura-2AM, ATPγS (1-30 μM) and ADPβS (1-30 μM) evoked Ca^{2+} transients in the presence and absence of external Ca^{2+}. Transients were reversibly attenuated by PPADS (20 μM) and completely abolished by the P2Y1 antagonist MRS2179 (30 μM), suggesting the presence of functional metabotropic P2Y1 receptors. To study purinergic effects on sphere formation, single cell suspensions derived from primary SVZ tissue were treated with purinergic agonists/antagonists and grown under sphere forming conditions. ATPγS and ADPβS (10-30 μM) but not UTP, UDP, UDP-glucose or αβ methylene ATP (100 μM) reduced both the size and frequency of primary neurospheres. This inhibitory effect was partially antagonized by MRS2179 (30 μM) and completely reversed by the P2Y12 antagonist MRS2395 (10 μM). Taken together, these data demonstrate that ATP and ADP evoke P2Y1 mediated Ca^{2+} transients in SVZ-derived neurospheres and that the inhibitory effect of adenine nucleotides on neurosphere formation and proliferation involves P2Y1 and P2Y12 receptor activation. Modulation of either stem cell proliferation or differentiation by purinergic receptor mediated G-protein signalling pathways may therefore represent a potential modulatory mechanism within the stem cell niche.