

Chris Robinson

Major: Cell and Molecular Biology

University: Southwest Missouri State University

Faculty Mentor: Dr. Gary Weisman

Mentor Department: Biochemistry

Funded by: NSF-REU Program in Biological Sciences & Biochemistry

Point mutation of an RGD sequence in the human P2Y₂ receptor to a QGD sequence conserves Go-mediated signal transduction

Chris Robinson, Sriparna Bagchi and Gary Weisman

The P2Y₂ nucleotide receptor is a G_{o/q} coupled receptor that is activated equipotently by extracellular nucleotides such as ATP or UTP and is upregulated in a variety of tissues in response to injury or stress. The biological effects of extracellular nucleotides are mediated through activation of P1 and P2 purinergic receptors. P1 receptors are responsive to adenine and P2 receptors are activated by a variety of nucleotides including ATP and UTP. The P2 receptors are subdivided into two distinct categories, the ionotropic ligand-gated channel (P2X) receptors and G-protein coupled P2Y-receptors, with seven transmembrane domains. Previous studies have shown that the human P2Y₂ nucleotide receptor contains an arginine-glycine-aspartic acid (RGD), integrin-binding domain. This domain is located in the first extracellular loop of the receptor and binds specifically to the $\alpha_v\beta_3/\beta_5$ group of integrins. The P2Y₂ receptor interacts with the α_v integrins by the RGD domain to activate G_o and induce cell migration. The human and murine P2Y₂R's have the RGD integrin-binding domain, whereas the rat homologue has a QGD domain. However, this change in the arginine position in the RGD integrin-binding domain is considered to be a conservative substitution that maintains integrin binding. In order to confirm this assumption we changed the RGD domain of the human P2Y₂ receptor into the QGD domain by in vitro mutagenesis. The wild-type and the QGD mutant P2Y₂ receptors were transiently transfected into P2 receptor null, 1321 N1 astrocytoma cell line. Preliminary data suggest that the QGD mutant can stimulate PLC dependent intracellular Ca²⁺ mobilization and also activate cofillin and extracellular signal-regulated kinases (Erk) with equal efficacy and agonist potency as the wild type receptor. Further tests need to be done to verify that integrin binding and signaling by the Rac and Rho pathways remain unaffected in the QGD mutant to induce integrin dependent cell migration in response to UTP and ATP.