

PhD thesis by Federica Gasparri:

Ligand recognition in P2X receptors: from molecular determinants to physiologically relevant hetero-liganded states

Abstract

P2X receptors (P2XRs) are non-selective cation channels displaying a trimeric architecture. Each subunit forming the trimeric assembly comprises two membrane spanning helices, connected by a large extracellular loop. P2XRs are activated by adenosine 5'-triphosphate (ATP), which binds in the extracellular domain of the protein. They are expressed throughout the human body and mediate crucial physiological roles, such as modulation of synaptic transmission, inflammation and neuropathic pain. Despite their physiological relevance and recent breakthroughs in P2XR structure determination, numerous molecular aspects of ATP binding, as well as molecular pharmacology remain unclear.

This project therefore aimed to define the precise physico-chemical contribution of conserved amino acids in ATP recognition, the molecular determinants for agonist activity and P2XR potentiation by hetero-liganded states.

By using electrophysiology, conventional mutagenesis and insertion of unnatural amino acids (UAAs) in P2X2Rs expressed in *Xenopus laevis* oocytes, we revealed that a combination of side chain and backbone interactions is crucial for ligand recognition in this receptor family.

A range of ATP analogues was tested both through whole cell and single channel electrophysiological recordings. The data demonstrated that other nucleotide triphosphates, as well as subtle ATP analogues with either increased or decreased conformational flexibility of the ribose ring, act as partial agonists on P2X2Rs and lead to an open state identical to ATP. This suggests that the chemical nature and binding pose of an agonist might affect binding and gating, but does not change the open conformation of the pore.

Further, we showed that ATP-evoked responses are potentiated by co-application of a wide range of partial agonists at sub-saturating concentrations. This effect was also observed at the single molecule level and when mutations were introduced within the binding site or at the subunit interface. These data shed new light on how P2XRs can be modulated in the presence of multiple agonists, e.g. in a synaptic environment.

Overall, the results presented here give insights into some of the molecular determinants for ligand recognition, selectivity and modulation in P2XRs. We believe this information will improve our current knowledge on how to modulate this family of ligand-gated ion channels (LGICs) in the physiological environment.