STRUCTURAL BASES OF P2X4R AND P2X2R ALLOSTERIC MODULATION

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P2X4R and P2X2R are trimeric functional ATP gated ligand channels which share similar binding and gating mechanisms but have different allosteric behavior. According with D. rerio P2X4R crystal, there are three pockets in the interphases between two adjacent subunits. Our ATP docking is in agreement with the experimental data from site directed mutagenesis that identified essential residues, but also suggest new residues that were not previously implied in this function (Fig 1A).

One key visual characteristic is rendered by the domain that covers the ATP-pocket. This domain, called “head”, corresponds to a structural domain according to TOPDomain analysis. This domain also has a high degree of collectivity in our Normal Mode Analyses with different servers. All these data suggest that the movement of the “head” let the ATP stay in the pocket.

The transmembrane domain has six alpha helixes, two by each subunit, where the TM2 makes a gate that can be opened to let the cation pass across the membrane (Fig 1B). In our comparative analysis of the two conformations in crystals from Gallus gallo ASIC1 using Normal Mode Analyses with MinMaxPath server, TM2 makes a translational movement opening the pore, and TM1 makes mainly a rotational movement. This is in agreement with the experimental data that shows a similar behavior in the TM1 and TM2 P2XR.

Figure 1. General model analysis. A. rP2X4R ATP docking, the two subunits which makes the pocket are shown in different colors. B. General model of the trimeric mechanism of intraprotein transduction from binding to gating.
In P2X4R, Cu2+ is a negative allosteric modulator. According to experimental data, H140 is an essential residue in the allosteric modulation, but also a residue implied in the ATP binding. The substitution of this residue also implies a decrease in the IC50. We proved that the Cu2+ tetrahedral planar complex between H140, D138 and H2O (Fig 2B) interferes with the ATP pharmacophore explaining the non-competitive effect.

In P2X2R, Cu2+ is a positive allosteric modulator. Based on the identification of the three residues described above as involved in the allosteric effect, we proposed that the tetrahedral complex favors the connection of the “head” of one subunit with the other adjacent subunit “body”, leaving the ATP trapped in the pocket and thus increasing the currents (Fig 2C).

Additionally the neurosteroid and IVM only modulate P2X4R. According with our docking study, Q36 and T335F are essentials residues (Fig 2D). The electrophysiological study of T335F shows a lack of effect of the positive allosteric effect of alphaxolone and a change from positive to negative modulation of allopregnanolone.