Signal transmission within the P2X2 trimeric receptor and the voltage dependent structural rearrangements

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ATP receptor channel P2X2 is a trimer of two trans-membrane type subunits activated by extracellular ATP. It has been known that P2X2 has unique biophysical properties, such as ATP- and voltage-dependent gating in spite of the absence of canonical voltage-sensor. Two aspects of P2X2 activation have been studied in our recent work.

The crystal structures of ATP-unbound and -bound forms of zebra fish P2X4 have been solved, showing the trimeric structure and its structural rearrangements upon ATP binding. The crystal structure in the activated state is three-fold symmetric with 3 bound ATP, but it is known that binding of two ATP molecule is sufficient to activate P2X. The aim of our study was to clarify how the signal of asymmetric binding of two ATP molecules to the trimer is transmitted to open the channel pore. To approach this question, we utilized three tandem-repeat constructs to introduce mutations exactly controlling the number and position in the trimer. Our results showed that the signal of ATP binding at Lys308 is directly transmitted on the same subunit down to the level of Asp315 located at the linker between the ATP binding site and the pore region. The signal subsequently spreads equally to all three subunits at the channel pore level of Thr339, resulting in symmetric and independent contributions of the three subunits to pore opening.

We also approached the voltage-dependent structural rearrangements of P2X2 during the semi-steady state after ATP application, by comparing the speed of Cys modification. We introduced Cys residues at Asp315 and adjacent Ile67, and monitored the speed of current modification after application of Cd^{2+} in the presence or absence of ATP, and at hyperpolarized or depolarized membrane potential. We observed that current modification by Cd^{2+} bridging was much faster in the presence of ATP than the absence, and also at hyperpolarized than at depolarized potential. The results clearly demonstrate the presence of ATP-dependent and also voltage-dependent structural rearrangements of P2X2, in spite of the absence of a canonical voltage-sensor.