Differential role of extracellular histidines in copper, zinc, magnesium and proton modulation of the P2X7 purinergic receptor

Claudio Acuña-Castillo, Claudio Coddou, Paulina Bull, Jocelyn Brito and J. Pablo Huidobro-Toro

Abstract

The P2X7 receptor is a non-selective cationic channel activated by extracellular ATP, belonging to the P2X receptor family. To assess the role of extracellular histidines on the allosteric modulation of the rat P2X7 receptor by divalent metals (copper, zinc and magnesium) and protons, these amino acid residues were singly substituted for corresponding alanines. Wild-type and mutated receptors were injected to Xenopus laevis oocytes; metal-related effects were evaluated by the two-electrode voltage-clamp technique. Copper inhibited the ATP-gated currents with a median inhibitory concentration of 4.4 +/- 1.0 micromol/L. The inhibition was non-competitive and time-dependent; copper was 60-fold more potent than zinc. The mutant H267A, resulted in a copper resistant receptor; mutants H201A and H130A were less sensitive to copper inhibition ($p < 0.05$). The rest of the mutants examined, H62A, H85A, and H219A, conserved the copper-induced inhibition. Only mutants H267A and H219A were less sensitive to the modulator action of zinc. Moreover, the magnesium-induced inhibition was abolished exclusively on the H130A and H201A mutants, suggesting that this metal may act at a novel cationic modulator site. Media acidification inhibited the ATP-gated current 87 +/- 3%; out of the six mutants examined, only H130A was significantly less sensitive to the change in pH, suggesting that His-130 could be involved as a pH sensor. In conclusion, while His-267 is critically involved in the copper or zinc allosteric modulation, the magnesium inhibitory effects is related to His-130 and His-201, His-130 is involved in proton sensing, highlighting the role of defined extracellular histidines in rat P2X7 receptor allosteric modulation.