Extracellular histidine residues identify common structural determinants in the copper/zinc $P2X_2$ receptor modulation

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Abstract

To assess the mechanism of $P2X_2$ receptor modulation by transition metals, the cDNA for the wild-type receptor was injected to *Xenopus laevis* oocytes and examined 48–72 h later by the two-electrode voltage-clamp technique. Copper was the most potent of the trace metals examined; at 10 μM it evoked a 25-fold potentiation of the 10 μM ATP-gated currents. Zinc, nickel or mercury required 10-fold larger concentrations to cause comparable potentiations, while palladium, cobalt or cadmium averaged only 12- and 3-fold potentiations, respectively. Platinum was inactive. The non-additive effect of copper and zinc at 10–100 μM suggests a common site of action; these metals also shifted to the left the ATP concentration–response curves. To define residues necessary for trace metal modulation, alanines were singly substituted for each of the nine histidines in the extracellular domain of the rat $P2X_2$ receptor. The H120A and H213A mutants were resistant to the modulator action of copper, zinc and other metals with the exception of mercury. Mutant H192A showed a reduction but not an abrogation of the copper or zinc potentiation. H245A showed less affinity for copper while this mutant flattened the zinc-induced potentiation. Mutant H319A reduced the copper but not the zinc-induced potentiation. In contrast, mutants H125A, H146A, H152A and H174A conserved the wild-type receptor sensitivity to trace metal modulation. We propose that His120, His192, His213 and His245 form part of a common allosteric metal-binding site of the $P2X_2$receptor, which for the specific coordination of copper, but not zinc, additionally involves His319.