Zinc and Copper Modulate Differentially the $P2X_4$ Receptor

Claudio Acuña-Castillo, Bernardo Morales, J. Pablo Huidobro-Toro

Abstract

The rat ATP $P2X_4$ receptor was expressed in *Xenopus laevis* oocytes to assess the effect of zinc and copper as possible regulators of purinergic mechanisms. ATP applied for 20 s evoked an inward cationic current with a median effective concentration (EC_{50}) of 21.4 ± 2.8 μ*M* and a Hill coefficient (n_H) of 1.5 ± 0.1. Coapplication of ATP plus 10 μ*M* zinc displaced leftward, in a parallel fashion, the ATP concentration-response curve, reducing the EC_{50} to 8.4 ± 1.8 μ*M* ($p < 0.01$) without altering the receptor n_H. The zinc potentiation was fast in onset, easily reversible, and voltage-independent and did not require metal preexposure. The zinc EC_{50} was 2-5 μ*M*, with a bell-shaped curve. At concentrations of 100-300 μ*M*, zinc produced less potentiation, and at 1 m*M*, it inhibited 50% the ATP current. The effect of zinc was mimicked by cadmium. In contrast, copper inhibited the ATP-evoked currents in a time- and concentration-dependent fashion, reducing the maximal current (I_{max}) without altering the EC_{50}. The copper-induced inhibition was slow in onset, slowly reversible, and voltage-independent. Whereas coapplication of 300 μ*M* copper plus ATP reduced I_{max} to 36.2 ± 5%, the coapplication of, or 60-s preexposure by, 10 μ*M* copper reduced I_{max} to 79 ± 9.2% ($p < 0.05$) and 39.6 ± 8.7% ($p < 0.01$), respectively. The inhibition was noncompetitive in nature and mimicked by mercury. Cobalt, barium, and manganese did not modify significantly the ATP-evoked current, demonstrating metal specificity. The simultaneous 1-min preapplication of both metals revealed that the 10 μ*M* zinc-induced potentiation was obliterated by 10 μ*M*copper, whereas 30 μ*M* copper not only reduced the potentiation, but inhibited the ATP response. Following coapplication of both metals for 20 s with ATP, at least 100 μ*M*copper was required to counteract the 10 μ*M* zinc-induced potentiation. The simultaneous preincubation with both metals provided evidence for a noncompetitive interaction. We hypothesize the existence of metal binding site(s), which are most likely localized in the extracellular domain of the $P2X_4$ receptor structure. These sites are selective and accessible to extracellular metal applications and bind micromolar concentrations of metals. The present results are compatible with the working hypothesis that trace metals, such as copper and zinc, are physiological modulators of the $P2X_4$ receptor. The modulation of brain purinergic transmission by physiologically and toxicologically relevant trace metal cations is highlighted.