BIDIRECTIONAL REGULATION OF HETEROMERIC ACID-SENSING ION CHANNEL 1A/3 BY ZINC

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Introduction

Acid-sensing ion channel 1a and 3 subunits are all expressed in sensory neurons, where they are though to play critical roles in pain perception associated with tissue acidosis. Our previous studies have shown that both homomeric ASIC1a and 3 channels are inhibited by physiological concentrations of zinc. ASIC1a and 3 can form functional channels in heterogonous system, which is believed to be expressed in sensory neurons. Here, we found that heteromeric ASIC1a/3 channels are sensitive to zinc with bidirectional effects and histidine residues in the extracellular domain of the ASIC1a/3 channels are critical for zinc-mediated effects. Our studies provide novel insights into structural basis of zinc regulation of heteromeric ASIC1a/3 channels.

Methodology

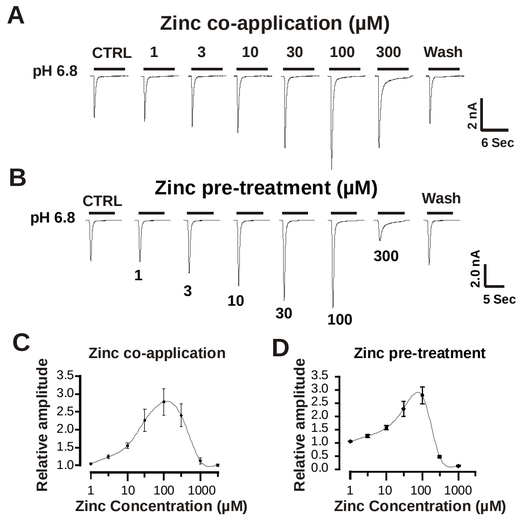
Cell Culture: CHO cells were grown in F12 medium supplemented with 10% FBS. Cells were used for transfection 1-2 days after plating. Expression of ASIC1a/3 in CHO cells: CHO cells at near 50 to 70 % confluence were co-transfected with cDNA for rat ASIC1a, rat ASIC3 and green-fluorescent protein (GFP) in the pcDNA 3 vector (Invitrogen), using X-tremeGENE DNA transfection reagent (Roche Molecular Biochemicals). 0.75 µg of DNA for ASIC1a, ASIC3 and 0.25 µg of DNA for GFP were used for each 35 X 35 mm culture dish. GFP positive cells were viewed under fluorescent microscope for patch-clamp 48h later.

Electrophysiology: Whole-cell voltage-clamp recordings were performed using Axopatch-200B amplifier and pClamp 10 software (Axon Instruments).

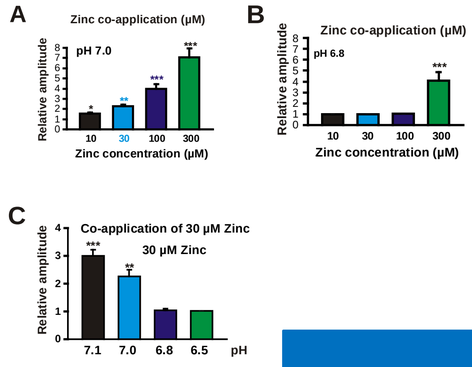
Solutions: Extracellular solutions contained (in mM): 140 NaCl, 5.4 KCl, 25 HEPES, 10 Glucose, 2.0 CaCl2, 1.0 MgCl2; pH was adjusted with NaOH or HCl. For pH <6.0, HEPES was replaced with 10 mM MES. Patch electrodes contained (in mM): 140 K Gluconate, 2.0 MgCl2, 1.0 CaCl2, 10 HEPES, 11 EGTA, 5 MgATP; pH 7.3.

Results

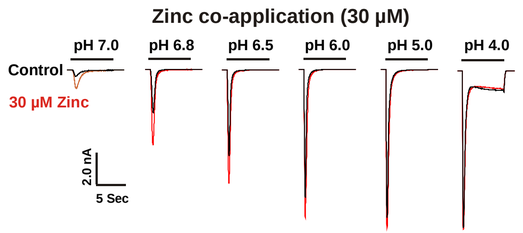
1 Bidirectional effects of zinc on peak amplitude of heteromeric ASIC1a/3 currents.



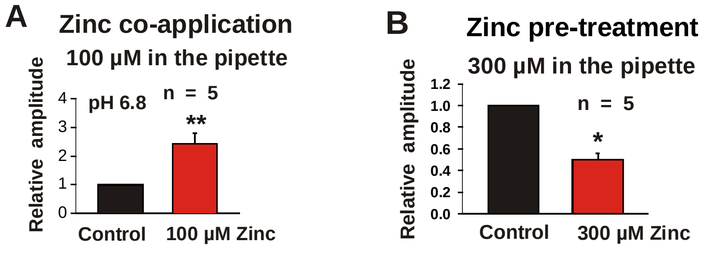
2 Effect of zinc on sustained component of the ASIC1a/3 channel.



3 Zinc potentiation of ASIC1a/3 current is dependent on pH activation.



4 Extracellular zinc is responsible for the effects of the ASIC1a/3 currents.



Summary/Conclusion

1. Heteromeric ASIC1a/3 channels are potentiated and inhibited by zinc in different treatment manners, respectively;
2. Zinc-mediated potentiation of heteromeric ASIC1a/3 channels is pH-dependent;
3. Extracellular zinc is responsible for the effects of the ASIC1a/3 current.

References

1. Chu et al., J Neurosci., 24 (40): 8678 - 8689, 2004 .
2. Jiang et al., Neuroscience. 169 (2): 574 - 583, 2010.
3. Chu et al., Trans Stroke Res., 5(1): 69 - 78, 2014.