

## Metabolic acidosis induces bone resorption via proton receptor-mediated activation of inositol phosphate-dependent calcium signaling.

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Metabolic acidosis (Met) increases urine calcium (Ca) excretion without an increase in intestinal Ca absorption, resulting in net loss of bone mineral. In vitro, Met stimulates net Ca efflux from neonatal mouse calvariae by stimulation of a prostaglandin E<sub>2</sub>-dependent increase in RANKL, leading to osteoclastic bone resorption. However, the pathway by which increased extracellular [H<sup>+</sup>] transduces an intracellular signal is not clear. G protein-coupled proton sensing receptors (PSRs) provide a potential mechanism for transduction of extracellular acidosis into intracellular responses. Transcripts for the 4 known PSRs, OGR1, GPR4, TDAG8, and G2A, are detectable in total RNA isolated from cultured calvariae. To determine if OGR1, which is coupled to inositol phosphate (IP), modulates Met-stimulated bone Ca efflux, we utilized the OGR1 inhibitor CuCl<sub>2</sub> (100 μM) and found that it significantly inhibited Met-induced net Ca efflux from calvariae. Increased intracellular metabolites of IP lead to an increase in intracellular Ca (Ca<sub>i</sub>). We measured Ca<sub>i</sub> by fluorescent imaging of fura-loaded primary bone cells. The cells were analyzed in a closed chamber with entry and exit ports to facilitate rapid medium change at a fixed pH, Pco<sub>2</sub> and [HCO<sub>3</sub><sup>-</sup>]. Infusion of physiologic Met medium (pH = 7.11, Pco<sub>2</sub> = 45 mmHg, [HCO<sub>3</sub><sup>-</sup>] = 14 mM) induced a marked, rapid, flow-independent, transient increase in Ca<sub>i</sub> in individual cells, which was inhibited by CuCl<sub>2</sub>. We then tested the effect on bone resorption of several inhibitors that block different steps within the IP<sub>3</sub> pathway: 2-aminoethoxydiphenyl borate (2-APB), which inhibits IP<sub>3</sub> receptors and the subsequent increase in Ca<sub>i</sub>; thapsigargin (TG) an ER Ca-ATPase inhibitor which depletes Ca<sub>i</sub> stores; and TMB-8, which blocks Ca release from ER. Neonatal mouse calvariae were incubated for 48h in Met (pH ~7.11) or neutral (Ntl, pH ~7.40) medium in the absence or presence of each of these inhibitors (inh). Medium was changed at 24 h. All three inhibitors significantly decreased the net Ca efflux which was induced by incubation in Met at 24-48h.

Inhibitor	Conc	Ntl	Met	Ntl + inh	Met + inh
APB	100 μM	131±55	670±101*	9±38 <sup>+</sup>	264±138 <sup>+</sup>
TG	100nM	174±45	866±103*	122±32 <sup>+</sup>	290±25 <sup>**°</sup>
TMB-8	100 μM	326±68	933±62*	1±26 <sup>**</sup>	319±80 <sup>°</sup>

Data are nmoles Ca released/bone/24 hr (mean ± SE); n =6-8 for each group; \* p< 0.05 vs Ntl; <sup>+</sup> p< 0.05 vs Met; <sup>°</sup> p< 0.05 vs Ntl+inh.

These results are consistent with Met activation of OGR1 to induce IP<sub>3</sub>-dependent Ca<sub>i</sub> transients, which may then modulate osteoblastic activity and lead to the subsequent increase in osteoclastic bone resorption.