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Extracellular Phosphate as a Non-competitive Antagonist of the Calcium-Sensing Receptor

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Introduction: In chronic kidney disease (CKD), declining renal function leads to hyperphosphataemia and to secondary hyperparathyroidism (SHPT). These contribute in turn to vascular calcification which is one of CKD's most life-threatening complications. The key controller of parathyroid hormone (PTH) secretion is the calcium-sensing receptor (CaSR), whose crystallised extracellular domain has revealed four putative phosphate-binding sites in the CaSR's inactive conformation [1]. This study aimed to investigate whether extracellular phosphate, at concentrations found in CKD, is capable of inhibiting the CaSR and thus increasing parathyroid hormone (PTH) secretion, and so providing a novel explanation for the aetiology of SHPT [2].

Method: Ca^{2+}_i mobilisation was measured in Fura2-loaded, CaSR stably-transfected HEK-293 cells (CaSR-HEK), by epifluorescence microscopy. Experimental buffer contained (mM) 20 HEPES (pH 7.4), 125 NaCl, 4 KCl, 0.5 $CaCl_2$, 0.5 $MgCl_2$ and 5.5 glucose with phosphate added as Na_2HPO_4 and KH_2PO_4 in a 4:1 ratio (pH 7.4). Extracellular signal-regulated kinase (ERK) activation was quantified by immunoblotting. PTH secretion was measured in 1.2mM Ca^{2+}-containing buffer in isolated human parathyroid cells obtained ethically following neck surgery.

Results: Raising phosphate concentration from a physiological 0.8mM to a pathophysiological 2mM (CKD-like) inhibited significantly CaSR activity by more than 50% in a Ca^{2+}_i assay (87±9 vs 42±4, mean±SEM, n=10, P<0.001 by T-test) and in ERK phosphorylation assay (94±6 vs 77±3, n=14, P<0.01 by T-test). Extracellular phosphate (2mM) significantly inhibited E_{max} in Ca^{2+}_o concentration-effect curves suggesting non-competitive antagonism (-32±3%, n≥7, P<0.0001 by F-Test), whereas EC_{50} was not altered. Further, in 1.5 mM Ca^{2+}_o (plus 1μM of the PAM, NPS-R568, used here to reduce the risk of precipitation) raising phosphate concentration attenuated CaSR activity with an IC_{50} of 1.3mM (95%CI 0.99 to 1.52). Mutation of $CaSR^{R62A}$ (a putative phosphate-binding site) substantially attenuated this inhibitory effect, whereas $CaSR^{R66A}$ (a second such site) retained it. Finally, pathophysiologic phosphate concentrations elicited a rapid and quickly-reversible increase, significant at 3mM phosphate, in PTH secretion in freshly-isolated human parathyroid cells endogenously expressing CaSR (N=6, P<0.05 by RM one-way ANOVA). The time course was consistent with phosphate eliciting a receptor-mediated action.

Conclusions: Extracellular phosphate appears to represent a non-competitive CaSR antagonist, acting at least at $CaSR^{R62}$, therefore enhances PTH secretion. In CKD, this could mean that lowering serum phosphate concentration might enhance the effectiveness of calcimimetics at attenuating excess PTH secretion.

References:

1. Geng Y, et al. (2016) eLife 5: e13662.
2. Rodriguez M et al. (2012) Am J Physiol Renal Physiol 288: F253-F264.