University of Western Ontario

CROSSTALK BETWEEN EXTRACELLULAR NUCLEOTIDE AND SPHINGOSINE-1-PHOSPHATE SIGNALING PATHWAYS IN OSTEOBLASTIC CELLS – IMPLICATIONS FOR ORTHODONTIC TISSUE REMODELING

M.E. Polymeris[1]*, S.J. Dixon[2]
Physiology and Pharmacology, Schulich School of Medicine & Dentistry
University of Western Ontario

Objective: Nucleotides are released from cells in response to mechanical stimuli. P2 receptors for nucleotides have been implicated in mechanotransduction and biological responses to orthodontic forces. Sphingosine-1-phosphate (S1P) is a potent lipid mediator that plays an important role in osteoclastogenesis and osteoblast-osteoclast coupling. Our aim was to investigate the interaction between nucleotide and S1P signaling in osteoblastic cells.

Methods: Both P2 receptors and S1P receptors signal in part through transient elevation of cytosolic calcium ($[Ca^{2+}]i$). To monitor $[Ca^{2+}]i$, UMR-106 rat osteoblastic cells were loaded with indo-1. Responses to S1P with and without pretreatment with nucleotides (ADP, ATP, CTP, TTP, UDP or UTP) were monitored by spectrofluorometry. Ca^{2+} responses were quantified as the area under the curve (AUC, $\Delta R \cdot s$).

Results: As previously reported, certain nucleotides (ADP, ATP, UTP) induced robust elevation of $[Ca^{2+}]i$ lasting ~1 min. In contrast, S1P alone induced a small $[Ca^{2+}]i$ elevation. However, pre-treatment with nucleotides (150-200 s) dramatically increased the amplitude of the S1P response, without markedly affecting its duration. AUC for S1P (1 µM) alone was 1.2 ± 0.3 $\Delta R \cdot s$; whereas, following ATP (100 µM), AUC for S1P was 10.5 ± 0.9 $\Delta R \cdot s$ ($p < 0.001$). Of the nucleotides tested, ADP, ATP, UDP and UTP significantly potentiated S1P responses. Conversely, CTP and TTP had no significant effect, indicating specificity.

Conclusions: This is the first report of crosstalk between nucleotide and S1P signaling in any cell type. Calcium controls important cellular functions, such as proliferation, differentiation and migration. Thus, this crosstalk may regulate osteoblast function in situations such as orthodontic tooth movement where high levels of nucleotides are present.