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Review Article

Hylonome

ATP P2 receptors and regulation of bone effector cells

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Abstract

ATP, signaling through P2 receptors, is one of the most important extracellular regulatory molecules in the skeleton. P2 receptors are divided into two subclasses, P2Y which are G-protein coupled and P2X which are ligand-gated ion channels. There is molecular and functional evidence for widespread expression of both subclasses of receptors by bone cells. Co-activation of P2Y and PTH1 receptors on osteoblasts, leads to synergistic expression of osteoblastic genes, providing a mechanism for integrating local and systemic regulatory signals in bone. Activation of $P2Y_1$ receptors on osteoblasts enhances expression of RANKL leading indirectly to an increase in osteoclast formation and resorption. Expression of $P2X_7$ inducible pores on osteoclast precursor cell membranes allows fusion to form multinucleated osteoclasts and blockade of this receptor inhibits resorption. Bone cells release nucleotides into the extracellular environment to provide highly localized and transient signals that regulate bone formation and bone resorption.

Keywords: Extracellular ATP, Extracellular Nucleotides, P2 receptors, Bone, Bone Remodeling, Osteoblasts, Osteoclasts, RANKL, Ultrasound.

Introduction

A trawl through any search engine, web site or dictionary to find a definition of ATP will yield a description approximating to '..the energy currency of the cell..' or '..the universal energy storage molecule..' confirming what biologists widely perceive as the major function of this molecule. Whilst these definitions are not incorrect they omit one of the major functions of ATP in biology - that of an an extracellular signalling molecule. No other endocrine or paracrine factor has such a wide array of cellular receptors distributed ubiquitously throughout tissues. And the skeleton is no exception; ATP and other nucleotides working through P2 receptors regulate all aspects of bone and cartilage biology including development, growth, turnover and repair.

The expression and function of P2 receptors in bone cells have been the subject of several recent reviews, including in this journal[1-4]. In this presentation, I intend to highlight some of the milestones that have contributed to our understanding of the roles of P2 receptors in bone, with specific emphasis on the results from my laboratory.

The first report that ATP could act as an extracellular signalling molecule was as long ago as 1929 when Drury and Szent-Györgyi described the physiological effects of adenine compounds on the mammalian heart[5]. However, P2 receptor research really began with the seminal work of Geoffrey Burnstock, who in the 1970s postulated that ATP was a neurotransmitter[6]. Over the past three decades, Burnstock has been at the helm of this field, which has seen the identification and elucidation of the function of the purinoceptor or P2 receptor family, and the detection of widespread expression of these receptors in mammalian tissues. The receptors are divided into two sub-families based on their mode of signal-transduction; P2Y, of which there are eight ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$), are G-protein coupled whereas P2X, of which there are seven ($P2X_{1-7}$), form a diverse group of ligand-gated ion channels. The $P2Y_4$ and $P2Y_6$ receptors are preferentially activated by the pyrimidines UTP and UDP, respectively.

The introduction of my laboratory into the P2 receptor field began in 1992 with the demonstration by Christof Schöfl that addition of ATP, at low micromolar concentrations, elevated intracellular free calcium in the osteosarcoma cell line SaOS-2 and in normal human osteoblasts[7] corroborating what others had found in rodent cells[8]. At this time the

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P2 receptors were pharmacological entities, but following advances in molecular cloning, Wayne Bowler confirmed the expression of $P2Y_2$ receptors in primary human osteoblasts and osteoclasts[9,10]. These initial studies were followed by a series of papers from our laboratory and others reporting conclusive functional and molecular evidence for the expression of specific P2Y and P2X receptors in bone-forming and bone-resorbing cells[11-15].

One of the key observations made in my research group by Wayne Bowler and Katherine Buckley was that activation of P2Y receptors in osteoblasts leads to induction of c-*fos*, an immediate early gene that plays a key role in the proliferation and differentiation of bone cells. Activation of osteoblastic P2Y receptors alone results in a moderate induction of c-*fos*, whereas dual activation of P2 receptors and PTH1 receptors leads, through multiple levels of interaction in downstream signalling, to a massive synergy in c-*fos* expression[16,17]. We believe this synergy provides a molecular mechanism whereby locally released extracellular nucleotides can sensitize cells to surrounding systemic PTH and thereby activate remodeling at discrete sites[3].

P2 receptors are also important regulators of bone resorption. Katherine Buckley has used the *in vitro* systems in which functional human osteoclasts can be generated from peripheral blood monocytes either by use of a stromal layer of UMR-106 cells or by supplementation with RANKL and MCSF. When ATP or UTP is added to stromal-cell free, RANKL-supplemented cultures, there is no obvious effect on the extent of lacunar resorption. In contrast, ATP at low μmol concentration greatly enhances the excavation of resorption pits in co-cultures containing UMR-106 cells, but UTP is without effect. The explanation for these results is that ATP, but not UTP, acting via the $P2Y_1$ receptor, upregulates RANKL expression by stromal cells and this results in enhanced osteoclast formation and activation of resorption[18].

Alison Gartland has demonstrated that the $P2X_7$ receptor also plays a role in osteoclast formation. In common with other members of the P2X family, this is an ATP-gated ion channel but in addition, the receptor has the unique ability to form pores that are permeable to molecules of up to 900Da, when exposed to repeated or prolonged application of nucleotide agonist. This receptor is expressed by human osteoblasts[14] and osteoclasts and its potential functions include regulation of cytokine release, induction of apoptosis and fusion of osteoclasts. It is likely that formation of $P2X_7$ inducible pores allows fusion of osteoclast precursor cell membranes to form multinucleated osteoclasts. Blockade of the receptor with the antagonist, oxidised ATP, or a specific blocking monoclonal antibody inhibits formation of multinucleate osteoclasts in RANKL-supplemented cultures of human peripheral blood monocytes. The mononuclear cells differentiate and become adherent but do not form multinucleate cells presumably because of a specific failure in the fusion process[19]. Given the apparent importance of this receptor to osteoclast survival and activity, we examined mice deficient in the $P2X_7$ receptor but found that they appeared normal and displayed no overt skeletal abnormalities[20]. Furthermore, we were able to prove their ability to form multinucleated osteoclasts *in vitro* and *in vivo*. The explanation for these findings is not clear but indicates that there may be some redundancy in the fusion mechanisms in osteoclasts[21].

In order to activate P2 receptors, nucleotides must be released into the bone microenvironment. ATP is present in mmol concentrations in cells and can be released by cell lysis, cell trauma or physiological mechanisms, possibly through ABC transporters. Katherine Buckley has demonstrated using luciferin-luciferase luminometry that ATP is present in SaOS-2 cell conditioned medium, and furthermore the concentration correlates with cell number, indicating these cells constitutively release ATP[22]. Shear forces arising from perturbation of the medium enhance the accumulation of ATP in the medium[3]. Interestingly, we found that introduction of ADP and/or UTP into the cultures resulted in increased ATP concentrations in the medium, which was found not to be receptor mediated. Nucleotide inhibition and substrate specificity studies revealed an ecto-nucleoside diphosphokinase (ecto-NDPK) was responsible for the ADP-->ATP conversion; PCR and immunocytochemistry confirmed its presence. These data establish the co-existence of ATP-consuming, and importantly for the first time, ATP-generating activities on the osteoblast cell surface, the discovery of which has significant implications for studies involving activation of P2 receptor subtypes in bone[22].

More recently, we found that following exposure of osteoblasts to ultrasound *in vitro*, cellular ATP release was enhanced, with subsequent effects on gene expression and cell proliferation. In the clinical situation, ultrasound stimulation of bone fractures results in a decreased time to fracture healing. This could be explained, in part, by our observations of increased release of ATP from bone cells leading to increasing expression of RANKL and decreasing expression of OPG by osteoblasts to promote osteoclastogenesis, and enhancing the proliferation of osteoblasts[23].

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