MHR

N. R. JØRGENSEN, ET AL. [2006] MED HYPOTHESES RES 3: 615-622.

ROLE OF P2 PURINERGIC RECEPTORS IN BONE METABOLISM AND THEIR THERAPEUTIC POTENTIAL

NIKLAS RYE JØRGENSEN*, MARIE SOLGAARD AND PETER SCHWARZ

DEPARTMENT OF CLINICAL BIOCHEMISTRY, COPENHAGEN UNIVERSITY HOSPITAL HVIDOVRE, HVIDOVRE, DENMARK (N.R.J., M.S.), AND RESEARCH CENTER OF AGING AND OSTEOPOROSIS, DEPARTMENT OF GERIATRICS, GLOSTRUP UNIVERSITY HOSPITAL, GLOSTRUP, DENMARK (N.R.J., P.S.)

REVIEW

ABSTRACT. THE ROLE OF purinergic P2 receptors in bone biology has been evaluated over the last decade. These receptors have proven to be an interesting target for new pharmacological agents with the ability to regulate bone metabolism. As the prevalence of osteoporosis increases new efficient agents to treat the disease are sought for. The P2 receptors are activated by nucleotides and recently, studies have shown a possible role for these in bone turnover and metabolism. P2 receptors can be divided further into P2X and P2Y subtypes with strikingly different mechanisms of action. They are expressed both by osteoblasts and by osteoclasts, and agonist binding affects cell proliferation, differentiation, activity, and apoptosis of the cells. With the increasingly knowledge of the function and role of these receptors in bone biology, they will undoubtedly be a potential target for designing new drugs, which can be used for treatment of metabolic bone diseases, including osteoporosis. This article is reviewing the studies documenting the effects of nucleotides and P2 receptors in bone and bone cells.

*ADDRESS ALL CORRESPONDENCE TO: DR. NIKLAS RYE JØRGENSEN, DEPARTMENT OF CLINICAL BIOCHEMISTRY, COPENHAGEN UNIVERSITY HOSPITAL HVIDOVRE, KETTEGÅRD ALLÉ 30, DK-2650 HVIDOVRE. PHONE: +45 36 32 23 65. FAX: +45 36 75 09 77. E-MAIL: niklas@dadlnet.dk

• MEDICAL HYPOTHESES AND RESEARCH • THE JOURNAL FOR INNOVATIVE IDEAS IN BIOMEDICAL RESEARCH •

INTRODUCTION

Osteoporosis is an increasing problem both as an economical burden to society and to the patient experiencing osteoporosis-related fractures. A search for new agents influencing bone metabolism in the use for treating metabolic bone disorders such as osteoporosis is continuously ongoing. Within the last few years several studies have shed light on the role of P2 purinergic receptors in bone biology and have raised the question as to whether agents acting via these receptors could possibly modulate bone turnover. Thus P2 receptors seem to be a pharmacological target for the regulation of bone turnover and could very well turn out to be beneficial in the treatment of metabolic bone diseases.

Adenosine 5'-triphosphate (ATP) is an important co-factor for enzymes as well as it serves as the cells' vital source of energy. Additionally, it also serves as a potent extracellular messenger molecule, exerting its effects on specialized receptors on the cell surface. In 1972 Burnstock [1] proposed the existence of a separate receptor binding ATP but not adenosine. This family of receptors was termed P2 purinergic receptors in contrast to the adenosine sensitive P2 purinergic receptors.

P2 PURINERGIC RECEPTORS

P2 receptors primarily respond to ATP, adenosine 5'-diphosphate (ADP), uridine 5'-triphosphate, and uridine 5'-diphosphate (UDP). Based on differences in the molecular structure and differences in signalling mechanisms P2 receptors are divided in two substantially different types, P2X and P2Y receptors [2] but both are localized to the plasma membrane.

P2X receptors are ligand gated ion channels. Based on the pharmacological profile using receptor agonists seven distinct subtypes have been characterized and subsequently cloned. These are termed P2X1-P2X7. The protein contains from 379 to 472 amino acids with two membrane-spanning domains connected by a long extracellular loop (FIG. 1A) [3]. The ion channel is supposedly constituted by two to three structurally homologous receptor proteins [2]. ATP binding is followed by a membrane depolarization due to influx of sodium through the ion channel. The depolarization activates voltage dependent calcium channels in the cell membrane, resulting in a rapid influx of calcium from the extracellular space and thus increase in the intracellular calcium concentration (FIG. 1B) [4]. The coupling between ligand binding and the intracellular events is a rapid process with a duration of only milliseconds , which is beneficial as the P2X receptors are widely expressed in nervous tissues and in muscles where rapid signalling is crucial to the correct function of the tissues [2].

P2Y receptors are G-protein coupled receptors and 8 subtypes have been characterized (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11-14) based on agonist potencies [4]. The protein consists of 7 transmembrane domains connected by intra and extracellular loops (FIG. 2A). The inositol 1,4,5-triphosphate (IP3) pathway is activated upon ligand binding. IP3 works as a second messenger and mediates mobilization of calcium from intracellular stores (FIG. 2B). As for P2X, receptor activation leads to increase in the intracellular calcium concentration and subsequently intracellular effects depending on the cell type tested [2,5]. In contrast to the P2X receptors the time lag between ligand binding and intracellular effect for the P2Y receptors is substantially longer. The primary cause of this is that activation of the signalling cascade involves synthesis of second messengers [2].

NUCLEOTIDES

Nucleotides like ATP, UTP, ADP, and UDP are important in a range of processes in the human organism including energy metabolism, nucleic acid synthesis, and in many enzymatic reactions. Most organs and tissues are regulated by extracellular nucleotides [6], including bone. The concentration of nucleotides in the surrounding environment should be sufficiently high in order to activate P2 receptors and subsequently modulating cellular activities [7]. The intracellular ATP concentration is usually 3-5 mM while the extracellular ATP concentration is kept very low due to two mechanisms:

(i) the plasma membrane is almost completely impermeable to the ATP molecule, and (ii) the presence of ectonucleotidases and phosphatases on the outside of the cells, that rapidly degrade ATP to ADP, AMP, adenosine, and inorganic phosphate [8].

However, under specific physiological and/or pathological circumstances the extracellular ATP concentration may increase for a short period of time due to release from the cells [8]. This release can happen: (i) as a consequence of the death of cells where the plasma membrane is no longer intact and thus permeable to ATP, and (ii) by vesicular release from nerve terminals or from activated platelets during an inflammatory process, and (iii) by release via specific transmembrane channel proteins as a response to certain stimuli [6].

P2 AND OSTEOBLAST BIOLOGY

Several studies have shown that ATP and other nucleotides induce IP3 formation and increases in intracellular calcium concentrations in osteoblasts [9,10]. A range of P2 receptors are expressed in human osteoblasts both of the P2Y subtypes such as P2Y1, P2Y2, P2Y4, and P2Y6 [11], as well as of the P2X subtypes such as P2X1, P2X4-P2X7 [12]. However, a change in P2 receptor expression is taking place during osteoblasts differentiation [13], and not all receptors can be found in an osteoblast at a given time point. Further, some of the receptors are only expressed as mRNA but not as functional proteins on the cell surface [14].

In order to regulate bone turnover, the nucleotides must be present in the bone micro environment [7]. Nucleotides are shown to act in an autocrine or paracrine manner on bone cells [15] presumably as a result of release from osteoblasts in response to mechanical stimuli [15]. Other *in vitro* studies suggest that ADP is converted to ATP by the membrane-bound enzyme ecto-nucleoside diphosphokinase and that this process is taking place in the close proximity of the osteoblasts [16]. It has also been suggested that a continuous release of ATP from osteoblasts is taking place through specific membrane proteins [6]. Functional *in vitro*

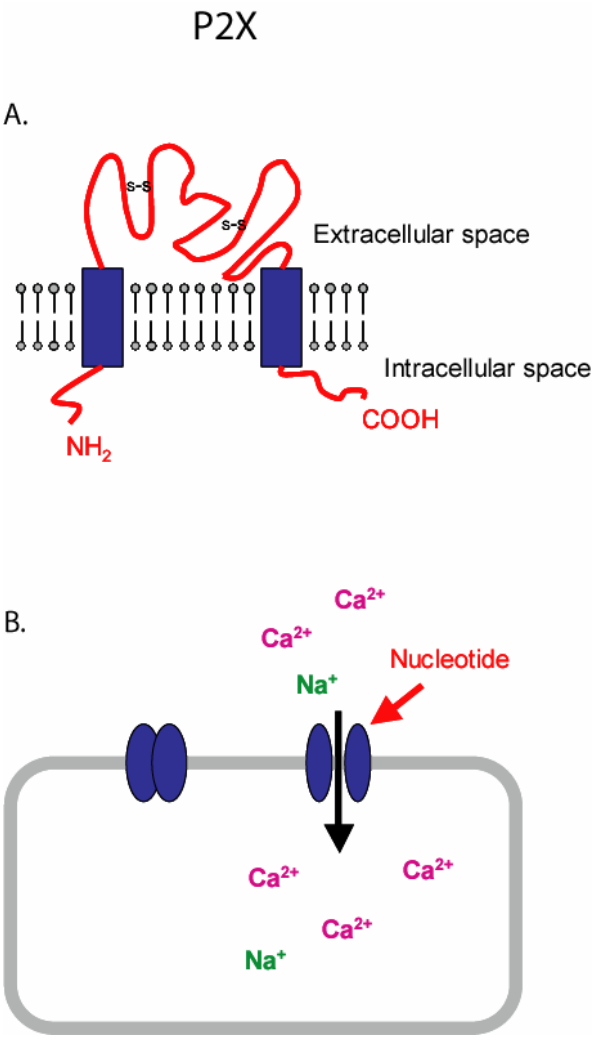

FIGURE 1. A: Schematic presentation of the structure of P2X receptors. P2X receptors are ligand-gated ion channels consisting of two transmembrane domains with the NH and COOH terminals intracellularly. B: Ligand binding induces opening of the channel resulting in influx of sodium, resulting in membrane depolarization and subsequent influx of calcium via voltage dependent calcium channels.

studies have shown that extracellular nucleotides such as ATP and UTP are able to reduce the formation of bone related proteins from osteoblasts [17,18] with the P2Y2 receptor subtype responsible for this effect [19]. Furthermore, some nucleotides exert an indirectly stimulating effect on osteoblast proliferation, which is most likely mediated via prostaglandin E [8].

Some of the above mentioned effects can be assigned to the ability of the nucleotides to increase the intracellular calcium concentration upon binding to P2 receptors. Thereby they activate or regulate ion channels and the transcription of genes. *In vitro* studies have shown that nucleotides exert an additive effect on the resorptive actions of parathyroid hormone (PTH), at least partially because the nucleotides potentiate the effect on the intracellular calcium concentration [20] which in turn may induce release of different mediators from the osteoblasts that subsequently stimulate osteoclastic bone resorption. Thus PTH may initiate bone resorption and thereby bone remodelling through nucleotides in the bone micro environment. More interesting are a couple of reports that actually document, that osteoblasts' response to PTH actually is dependent on the sensitization of the cells with nucleotides through P2 receptors. Nucleotides are present in the bone micro environment due to release from damaged cells or even as a result of physical activity and thus mechanically induced release from cells [21]. This way P2 receptor activation and nucleotides are able to localize systemic signals such as PTH, on bone. In addition there is probably an interaction of locally produced nucleotides and growth factors/prostaglandins released due to damage of tissue and cells, leading to proliferation and differentiation of osteoblasts.

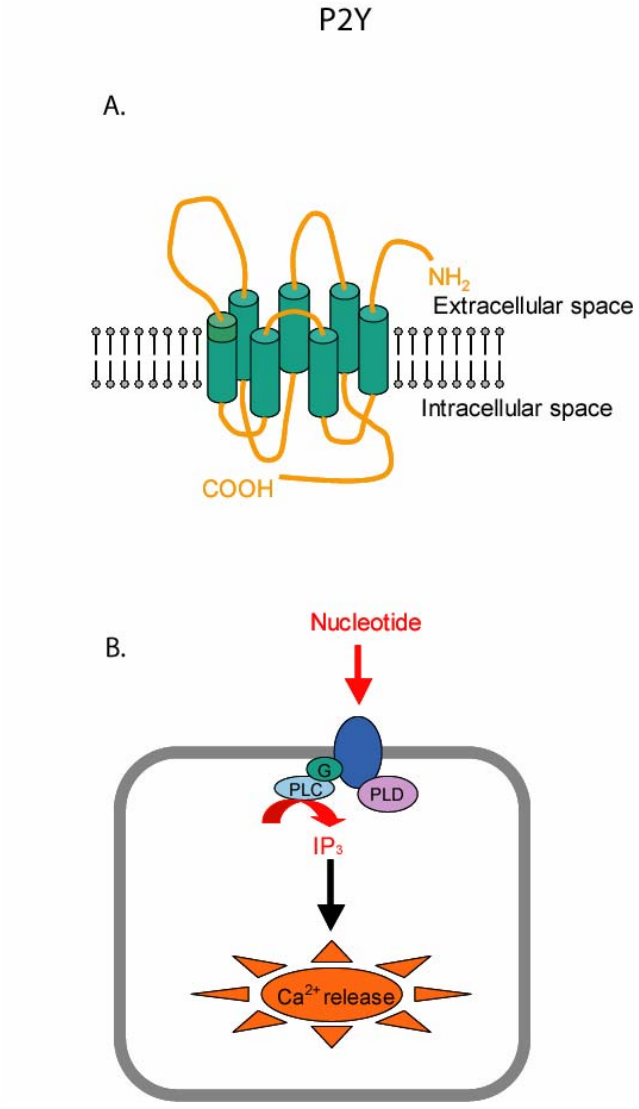

FIGURE 2. A: P2Y receptors are G protein-coupled 7 transmembrane domain receptors with the NH terminal and the nucleotide binding site extracellularly and with the COOH terminal intracellularly. B: Ligand binding induces inositol triphosphate (IP_3) production through phospholipase C (PLC) and subsequently release of calcium from intracellular stores. G: G-protein; PLD: phospholipase D.

P2 AND OSTEOCLAST BIOLOGY

As in osteoblasts, human osteoclasts express a number of P2 receptors, both of the P2Y and of the P2X subtypes. The osteoclast seems to express a large number of different P2 subtypes: P2Y1, P2Y2, P2Y4, P2Y6, and P2Y11, as well as P2X1, P2X4, P2X5, P2X6, and P2X7 [22]. Like in osteoblasts, not all receptor subtypes seem to be expressed as functional proteins on the plasma membrane as discrepancies are found between receptor expression determined by reverse transcriptase polymerase chain reaction and protein determinations using Western Blotting and immune histochemical techniques.

The effects in osteoclasts are varied. *In vitro*, ATP has a stimulating effect on osteoclastic bone resorption when given in low doses. This effect is potentiated in a milieu with low pH which indicates that the P2X2 receptor is involved, as the potency of ATP on this subtype is significantly increased when pH is decreased [23]. It has been proposed that the effect of ATP on bone resorption is indirect

via an up-regulation of receptor activator of nuclear factor-κB ligand (RANK ligand) in osteoblasts [22], but a recent report has documented that nucleotides acting through the P2Y6 receptor increase osteoclast survival directly by activating nuclear factor-κB [24]. Other nucleotides such as ADP are also key regulators of osteoclast biology. ADP acts via the ADP-selective P2Y1 receptor through which ADP induces bone resorption presumably via receptors on the mature osteoclast and indirectly via receptors on osteoblasts [25], as both cell types express P2Y1 receptors. Further, ADP seems to induce osteoclast formation from hematopoietic stem cells [26].

The P2X7 receptor seems to play a special role in the osteoclast. The receptor is distinct from the other P2X receptors as it upon normal ligand binding exerts its actions as an ion channel. However, upon prolonged activation of the receptor by ATP conformational changes occur and the protein transforms to a non-selective pore that is permeable to large hydrophilic molecules. This is important in the fusion of macrophage precursors and thereby in the formation of the multi-nucleated macrophage [27]. Interestingly, the ability to form a pore also seems to be involved in initiation of cell death as long exposure to high concentrations of ATP induces lysis of the cells. This is due to increased

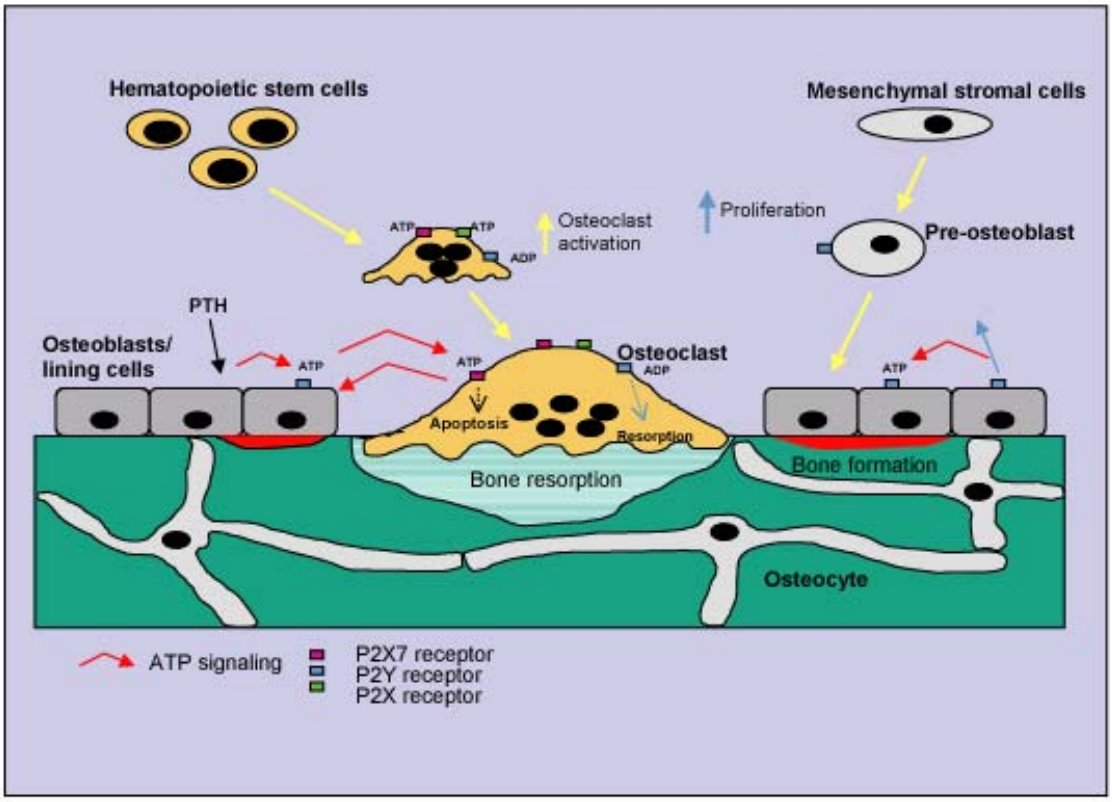

FIGURE 3. SCHEMATIC PRESENTATION OF DOCUMENTED EFFECTS OF NUCLEOTIDES ON PURINERGIC RECEPTORS IN BONE CELLS. Nucleotides act via P2Y and P2X receptors on osteoclast fusion and osteoclast apoptosis thereby regulating osteoclastic bone resorption. This effect can be modulated further by indirect action of parathyroid hormone on the osteoblasts. P2Y receptors on the osteoblasts are involved in the osteoblast proliferation and osteoblast activity and are thereby partially responsible for the regulation of bone formation.

permeability of the membrane with excessive transmembrane passage of ions whereby the cell looses important intracellular metabolites which in turn leads to cell death by necrosis or apoptosis [28]. The P2X7 receptor has most extensively been investigated in macrophages. However, osteoclasts are derived from the same hematopoietic precursors as the macrophages and it is likely that the receptor plays a similar role in the osteoclasts' development and activity. Several studies indicate this as it has been documented that osteoclasts upon ATP stimulation *in vitro* develop large permeable pores in the plasma membrane [14].

In support of the importance of the P2X7 receptor on osteoclast biology, an *in vivo* study has been performed, where bone status in a knock-out mouse model as examined. In the P2X7 knock-out mice the bone mass was reduced when compared to the wild type animals, histomorphometric analyses of the bones revealed an increased bone resorption in the knock-outs suggesting an increased osteoclast activity [29]. This indicates that under normal conditions the P2X7 receptor is a key regulator of osteoclast activity as it controls osteoclast cell death.

P2 RECEPTORS AS THERAPEUTIC TARGETS

A number of studies have now documented the role of P2 receptors in bone biology (FIG. 3), and especially in the regulation of bone formation and bone resorption. It is evident that this new information should be used to develop new therapeutic agents which may be used to modulate bone metabolism and thus prove useful in the treatment of metabolic bone disorders such as osteoporosis, inflammatory diseases (rheumatoid arthritis and periodontitis), as well as tumor-induced lysis. Furthermore, it could even be useful in the prevention of bone loss associated with loss of mechanical stimulation as a result of micro-gravity. Due to the range of receptors involved and the obvious effects on bone formation and resorption, they possess the potential to serve as targets for both anti-resorptive and anabolic agents. However, there are some limitations that should be by-passed when developing these agents. First, most of the known agonists are relatively non-specific and have effects on more than one receptor subtype. Next, the endogenous agonists are highly unstable due to enzymatic degradation by extracellular ecto-nucleotidases.

However, benzoylbenzoylATP (BzATP) is relatively specific for the P2X7 receptor and as it induces acute cytolysis in osteoclasts it could serve as a logical basis for the development of drugs that could reduce the osteoclast number *in vivo*. Other P2X receptors are involved in the regulation of bone resorption. Low doses of ATP stimulate osteoclast formation and activity [23]. These effects seem to be mediated via other P2 receptor subtypes than P2X7 and selective agonists against these P2X receptors may also be used as anti-resorptive drugs.

Smaller bisphosphonates such as clodronate and etidronate are already used for treatment of osteoporosis. In the body they are metabolized into ATP analogues that induce osteoclastic cell death. This effect might be mediated through interaction of the ATP-related metabolites with P2 receptors on the osteoclasts, inducing death of the osteoclasts. Since bisphosphonates are highly bone-selective it would be possible to design the analogues in such a way that they after being metabolized in osteoclasts are transformed into potent P2 agonists or antagonists exerting their effects directly on formation or resorption in the bone micro environment.

Only a few studies have so far documented the effect of antagonists on bone turnover. Suramin is a P2 antagonist primarily binding to P2X receptors and to a less extent to P2Y receptors. Suramin has been shown to inhibit bone resorption both *in vitro* and *in vivo* [23,30,31,32]. Suramin-like agents with increased specificity towards P2X receptors have been developed, showing only little binding to P2Y. In the future more specific antagonists to P2 receptors will be developed, and it will be very interesting to test their effects on bone formation and resorption.

CONCLUSION

P2 receptors are a large family of receptors that together with nucleotides play a major role in the regulation of bone metabolism with varying effects on bone turnover among receptors and among nu-

cleotides. These mechanisms are most probably involved in the bones' response to mechanical stimuli as well as under inflammatory conditions and are likely to serve as therapeutic targets for the development of new bone active agents. However, more research is needed in order to fully understand how nucleotides and P2 receptors are involved in the regulation of bone turnover. The evaluation of *in vivo* models of genetically modified mice is a powerful tool to explore the role of the P2 receptors in bone biology. Only the P2X7 model has so far been investigated from a bone point of view. The presence of different receptors as well as differences in receptor sensitivity between osteoblasts and osteoclasts could very well turn out to be a major advantage as it is possible to control the activity of the two cell types independently, and thereby form the basis of treating a range of metabolic bone diseases.

REFERENCES

[1] BURNSTOCK G, DUMSDAY B AND SMYTHE A [1972] Atropine resistant excitation of the urinary bladder: the possibility of transmission via nerves releasing a purine nucleotide. Br J Pharmacol 44: 451-461.

[2] RALEVIC V, AND BURNSTOCK G [1998] Receptors for purines and pyrimidines. Pharmacol Rev 50: 413-492.

[3] BUELL G, COLLO G AND RASSENDREN F [1996] P2X receptors: an emerging channel family. Eur J Neurosci 8: 2221-2228.

[4] JACOBSON KA, JARVIS MF AND WILLIAMS M [2002] Purine and pyrimidine (P2) receptors as drug targets. J Med Chem 45: 4057-4093.

[5] HARDEN TK, BOYER JL AND NICHOLAS RA [1995] P2-purinergic receptors: subtype-associated signaling responses and structure. Annu Rev Pharmacol Toxicol 35: 541-579.

[6] DUBYAK GR, AND EL MC [1993] Signal transduction via P2-purinergic receptors for extracellular ATP and other nucleotides. Am J Physiol 265: C577-C606.

[7] BOWLER WB, BUCKLEY KA, GARTLAND A, HIPSKIND RA, BILBE G AND GALLAGHER JA [2001] Extracellular nucleotide signaling: a mechanism for integrating local and systemic responses in the activation of bone remodeling. Bone 28: 507-512.

[8] DIXON SJ AND SIMS SM [2000] P2 purinergic receptors on osteoblasts and osteoclasts: Potential targets for drug development. Drug Development Research 49: 187-200.

[9] SCHOFL C, CUTHBERTSON KS, WALSH CA, MAYNE C, COBBOLD P, VON-ZUR MA, HESCH RD AND GALLAGHER JA [1992] Evidence for P2-purinoceptors on human osteoblast-like cells. J Bone Miner Res 7: 485-491.

[10] BOARDER MR, WEISMAN GA, TURNER JT AND WILKINSON GF [1995] G protein-coupled P2 purinoceptors: from molecular biology to functional responses. Trends Pharmacol Sci 16: 133-139.

[11] MAIER R, GLATZ A, MOSBACHER J AND BILBE G [1997] Cloning of P2Y6 cDNAs and identification of a pseudogene: comparison of P2Y receptor subtype expression in bone and brain tissues. Biochem Biophys Res Commun 237: 297-302.

[12] NAKAMURA E, UEZONO Y, NARUSAWA K, SHIBUYA I, OISHI Y, TANAKA M, YANAGIHARA N, NAKAMURA T AND IZUMI F [2000] ATP activates DNA synthesis by acting on P2X receptors in human osteoblast-like MG-63 cells. Am J Physiol Cell Physiol 279: C510-C519.

[13] DIXON CJ, BOWLER WB, WALSH CA AND GALLAGHER JA [1997] Effects of extracellular nucleotides on single cells and populations of human osteoblasts: contribution of cell heterogeneity to relative potencies. Br J Pharmacol 120: 777-780.

[14] JORGENSEN NR, HENRIKSEN Z, SORENSEN OH, ERIKSEN EF, CIVITELLI R AND STEINBERG TH [2002] Intercellular Calcium Signaling Occurs between Human Osteoblasts and Osteoclasts and Requires Activation of Osteoclast P2X7 Receptors. J Biol Chem 277: 7574-7580.

[15] JORGENSEN NR, GEIST ST, CIVITELLI R AND STEINBERG TH [1997] ATP- and gap junction-dependent intercellular calcium signaling in osteoblastic cells. J Cell Biol 139: 497-506.

[16] BUCKLEY KA, GOLDING SL, RICE JM, DILLON JP AND GALLAGHER JA [2003] Release and interconversion of P2 receptor agonists by human osteoblast-like cells. FASEB J 17: 1401-1410.

[17] JONES SJ, GRAY C, BOYDE A AND BURNSTOCK G [1997] Purinergic transmitters inhibit bone formation by cultured osteoblasts. Bone 21: 393-399.

[18] DOTY SB AND SCHOFIELD BH [1976] Enzyme histochemistry of bone and catilage cells. Prog Histochem Cytochem 8: 1-38.

[19] HOEBERTZ A, MAHENDRAN S, BURNSTOCK G AND ARNETT TR [2002] ATP and UTP at low concentrations strongly inhibit bone formation by osteoblasts: a novel role for the P2Y2 receptor in bone remodeling. J Cell Biochem 86: 413-419.

[20] KAPLAN AD, REIMER WJ, FELDMAN RD AND DIXON SJ [1995] Extracellular nucleotides potentiate the cytosolic Ca^{2+}, but not cyclic adenosine 3',5'-monophosphate response to parathyroid hormone in rat osteoblastic cells. Endocrinology 136: 1674-1685.

[21] JORGENSEN NR, HENRIKSEN Z, BROT C, ERIKSEN EF, SORENSEN OH, CIVITELLI R AND STEINBERG TH [2000]

Human osteoblastic cells propagate intercellular calcium signals by two different mechanisms. J Bone Miner Res 15: 1024-1032.

[22] BUCKLEY KA, HIPSKIND RA, GARTLAND A, BOWLER WB AND GALLAGHER JA [2002] Adenosine triphosphate stimulates human osteoclast activity via upregulation of osteoblast-expressed receptor activator of nuclear factor-kappaB ligand. Bone 31: 582-590.

[23] MORRISON MS, TURIN L, KING BF, BURNSTOCK G AND ARNETT TR [1998] ATP is a potent stimulator of the activation and formation of rodent osteoclasts. J Physiol (Lond) 511(Pt 2): 495-500.

[24] KORCOK J, RAIMUNDO LN, DU X, SIMS SM AND DIXON SJ [2005] P2Y6 nucleotide receptors activate NF-kappaB and increase survival of osteoclasts. J Biol Chem 280: 16909-16915.

[25] STEINBERG TH, JORGENSEN NR, BONG JS, HENRIKSEN Z, ATAL N, LIN G, BENNETT BD, ERIKSEN EF, SORENSEN OH AND CIVITELLI R [2000] P2-mediated responses in osteoclasts and osteoclast-like cells. Drug Development Res 49: 5.

[26] RABADJIJA L, BROWN EM, SWARTZ SL, CHEN CJ AND GOLDHABER P [1990] H^+-stimulated release of prostaglandin E_2 and cyclic adenosine 3',5'-monophosphoric acid and their relationship to bone resorption in neonatal mouse calvaria cultures. Bone Miner 11: 295-304.

[27] CHIOZZI P, SANZ JM, FERRARI D, FALZONI S, ALEOTTI A, BUELL GN, COLLO G AND DI VIRGILIO F [1997] Spontaneous cell fusion in macrophage cultures expressing high levels of the P2Z/P2X7 receptor. J Cell Biol 138: 697-706.

[28] MURGIA M, PIZZO P, STEINBERG TH AND DI VF [1992] Characterization of the cytotoxic effect of extracellular ATP in J774 mouse macrophages. Biochem J 288: 897-901.

[29] KE HZ, QI H, WEIDEMA AF, ZHANG Q, PANUPINTHU N, CRAWFORD DT, GRASSER WA, PARALKAR VM, LI M, AUDOLY LP, GABEL CA, JEE WS, DIXON SJ, SIMS SM AND THOMPSON DD [2003] Deletion of the P2X7 nucleotide receptor reveals its regulatory roles in bone formation and resorption. Mol Endocrinol 17: 1356-1367.

[30] WALTHER MM, KRAGEL PJ, TRAHAN E, VENZON D, BLAIR HC, SCHLESINGER PH, JAMAI D, EWING MW, MYERS CE AND LINEHAN WM [1992] Suramin inhibits bone resorption and reduces osteoblast number in a neonatal mouse calvarial bone resorption assay. Endocrinology 131: 2263-2270.

[31] FARSOUDI KH, PIETSCHMANN P, CROSS HS AND PETERLIK M [1993] Suramin is a potent inhibitor of calcemic hormone- and growth factor-induced bone resorption *in vitro*. J Pharmacol Exp Ther 264: 579-583.

[32] YONEDA T, WILLIAMS P, RHINE C, BOYCE BF, DUNSTAN C AND MUNDY GR [1995] Suramin suppresses hypercalcemia and osteoclastic bone resorption in nude mice bearing a human squamous cancer. Cancer Res 55: 1989-1993.

RECEIVED ON 12-22-2005.
ACCEPTED ON 1-13-2006.