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Kinin B1 and B2 receptor expression in osteoblasts and fibroblasts is enhanced by interleukin-1 and tumour necrosis factor-α. Effects dependent on activation of NF-κB and MAP kinases ☆

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ABSTRACT

Pro-inflammatory mediators formed by the kallikrein–kinin system can stimulate bone resorption and synergistically potentiate bone resorption induced by IL-1 and TNF-α. We have shown that the effect is associated with synergistically enhanced RANKL expression and enhanced prostaglandin biosynthesis, due to increased cyclooxygenase-2 expression. In the present study, the effects of osteotropic cytokines and different kinins on the expression of receptor subtypes for bradykinin (BK), des-Arg^{10}-Lys-BK (DALBK), IL-1β and TNF-α have been investigated. IL-1β and TNF-α enhanced kinin B1 and B2 receptor binding in the human osteoblastic cell line MG-63 and the mRNA expression of B1 and B2 receptors in MG-63 cells, human gingival fibroblasts and intact mouse calvarial bones. Kinins did not affect mRNA expression of IL-1 or TNF receptors. EMSA showed that IL-1β and TNF-α activated NF-κB and AP-1 in MG-63 cells. IL-1β stimulated NF-κB via a non-canonical pathway (p52/p65) and TNF-α via the canonical pathway (p50/p65). Activation of AP-1 involved c-Jun in both IL-1β and TNF-α stimulated cells, but c-Fos only in TNF-α stimulated cells. Phospho-ELISA and Western blots showed that IL-1β activated JNK and p38, but not ERK 1/2 MAP kinase. Pharmacological inhibitors showed that NF-κB, p38 and JNK were important for IL-1β induced stimulation of B1 receptors, and NF-κB and p38 for B2 receptors. p38 and JNK were important for TNF-α induced stimulation of B1 receptors, whereas NF-κB, p38 and JNK were involved in TNF-α induced expression of B2 receptors. These data show that IL-1β and TNF-α upregulate B1 and B2 receptor expression by mechanisms involving activation of both NF-κB and MAP kinase pathways, but that signal transduction pathways are different for IL-1β and TNF-α. The enhanced kinin receptor expression induced by the pro-inflammatory cytokines IL-1β and TNF-α might be one important mechanism involved in the synergistic enhancement of prostaglandin formation caused by co-treatment with kinins and one of the two cytokines. These mechanisms might help to explain the enhanced bone resorption associated with inflammatory disorders, including periodontitis and rheumatoid arthritis.

Introduction

Inflammatory processes are well known to interact with remodelling of bone tissue in the vicinity, causing both enhanced rate of bone resorption and new bone formation, with the former usually being more dominant [1–3]. Bone loss due to increased number of actively resorbing osteoclasts observed in periodontitis and rheumatoid arthritis is usually considered being caused by excess of cytokines stimulating osteoclastogenesis compared to those with inhibitory effects. Stimulatory cytokines include interleukin-1 (IL-1), IL-6, IL-11, IL-17, leukaemia inhibitory factor (LIF), oncostatin M (OSM), tumour necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β)

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Abbreviations: AP-1, activator protein-1; BCA, bicinchoninic acid; BK, bradykinin; BSA, essentially fatty acid free fetal bovine serum albumin; CASE, Cellular Activation of Signalling ELISA; COX-2, cyclooxygenase-2; CRE, cyclic AMP responsive element; DALBK, des-Arg^{10}-Lys-BK; D3, 1,25(OH)$_2$-vitamin D3; DTT, dithiothreitol; EMSA, electrophoretic mobility shift assay; ER, estrogen receptor; ERK, extracellular signal-regulated kinase; FCS, fetal calf serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GR, glucocorticoid receptor; IκBα, inhibitory κBα; IKK, IκB kinases; IFN, interferon; IL, interleukin-1; IL-1R, interleukin-1 receptor; JNK, c-Jun amino-terminal kinase; LIF, leukaemia inhibitory factor; MAPK, mitogen-activated protein kinase; α-MEM, α-modification of minimal essential medium; NF-κB, nuclear factor-κB; OSM, oncostatin M; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PDTC, pyrrolidine dithiocarbamate; PMSF, phenylmethylsulfonyl fluoride; PGE$_2$, prostaglandin E$_2$; q-RT-PCR, quantitative real-time RT-PCR; RANKL, receptor activator of NF-κB ligand; RT, reversed transcribed; RPL13A, ribosomal protein L13A; TBS, Tris buffered saline; TGF-β, transforming growth factor-β; TTBS, TBS with 0.05% Tween-20; TNF-α, tumour necrosis factor-α; TNFR, TNF-α receptor.

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and to the group of inhibitory cytokines belong IL-4, IL-10, IL-12, IL-13, IL-18, interferon-γ (IFN-γ) and IFN-β [1,4]. We have shown that kinins also can stimulate bone resorption *in vitro* [5,6] and, maybe even more importantly, synergistically potentiate the bone resorbing effect of IL-1, an effect linked to synergistic enhancement of prostaglandin biosynthesis [7].

Kinins are small peptides derived by an endogenous metabolic cascade from high and low molecular weight kininogens by either tissue- or plasma-kallikreins. The peptides are well known for their effects on cardiovascular homeostasis, inflammation and for causing hyperalgesia [8–10]. In humans, four different biologically active kinins have been demonstrated; the nonapeptide bradykinin (BK; Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), the decapeptide Lys-BK and their carboxy-terminal des-Arg metabolites. Kinin receptors are expressed by a variety of cell types and the activation of these receptors plays important roles in many physiological and pathological processes.

Two different, seven-transmembrane G protein-coupled, kinin receptors have been pharmacologically characterized and molecularly cloned [11,12]. The kinin B2 receptors recognize BK and Lys-BK, whereas the B1 receptors have higher affinity for des-Arg^9-BK and des-Arg^{10}-Lys-BK (DALBK). The B2 receptor is constitutively expressed in a variety of different cell types including endothelial cells, synovial cells, sensory fibers, dermal and gingival fibroblasts, smooth muscle cells and epithelial cells. The expression of the B2 receptors can be enhanced by cytokines, cyclic AMP, estrogen and glucocorticoids [13–17] and the promoter contain binding elements for activator protein-1 (AP-1), cyclic AMP responsive element (CRE), nuclear factor-κB (NF-κB), estrogen receptor (ER) and glucocorticoid receptor (GR), as well as a silencer element [18]. The B1 receptors are generally more weakly expressed but are up-regulated during inflammation and by lipopolysaccharides, cytokines and during *in vitro* conditions [19]. The B1 promoter has numerous putative binding sequences for several transcription factors including NF-κB and AP-1 [20], and c-Jun has been shown to be an important mediator of B1 receptor regulation [21]. It has also been shown that pharmacological inhibitors of different mitogen-activated protein kinases (MAPKs) and of NF-κB can reduce increased expression of B1 receptors [22–25].

Kinins are often considered either proinflammatory or cardioprotective. We have shown that bone resorption in neonatal mouse calvariae can be stimulated by agonists for either B1 or B2 receptors and suggested that kinins in their role as proinflammatory peptides might be important in inflammation induced bone resorption in diseases such as periodontitis and rheumatoid arthritis [5,6]. In line with the view that osteoblasts are key cells for initiation of osteoclast formation, we have demonstrated that murine periosteal osteoblasts express both B1 and B2 receptors [26,27] linked to increased formation of inositiol phosphates and cytoplasmic Ca^{2+} [28]. We have also shown that the human osteoblastic cell line MG-63 express B1 and B2 receptors [29] and that both receptors can interact with IL-1 and TNF-α causing synergistic stimulation of prostaglandin formation [30]. The effect on prostaglandin formation is associated with B1 and B2 receptor potentiation of IL-1β and TNF-α induced stimulation of cyclooxygenase-2 (COX-2) mRNA expression and protein synthesis in the MG-63 cells [30]. The synergistic interaction by kinins and cytokines on prostaglandin formation and COX-2 mRNA expression was also observed in intact mouse calvarial bones and was associated with synergistically enhanced expression of receptor activator of NF-κB ligand (RANKL) mRNA and protein. Since RANKL is a crucial cytokine for osteoclast differentiation and activation, this finding most likely explains the synergistic interaction on bone resorption.

In the present study, we have investigated the effect of several osteotropic cytokines on kinin receptor expression and, vice versa, the effect of kinins on cytokine receptor expression. Since IL-1β and TNF-α were found to enhance both B1 and B2 receptor expression we have examined the signal transduction pathways involved.

Materials and methods

Materials

Spermidine, spermine, dithiothreitol (DTT), Bradford reagent and rabbit IgG were purchased from Sigma Chemical Co., St. Louis, MO, USA; fetal bovine serum (FBS) from ICN Pharmaceuticals Inc., Costal Mesa, CA, USA; T4 polynucleotide kinase from Invitrogen, Stockholm, Sweden; [γ-^{32}P]ATP and poly(dI-dC)·poly(dI-dC) from Amersham Biosciences/GE Healthcare, Uppsala, Sweden; pre-made TaqMan® Gene Expression Assays for mouse BK B1- and B2 receptors from Applied Biosystems, Foster City CA, USA; anti-actin (I-19), anti-p50 (C-19X), anti-p52 (C-5X), anti-p65 (C-20X), anti-c-Jun (NX), anti-c-Fos (4X), anti-IκBα (C-21), anti-ERK (extracellular signal-regulated kinase; C-16), anti-JNK (c-Jun amino-terminal kinase; FL), anti-p38 (C-20) and anti-phospho-ERK (E-4) primary antibodies from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA; anti-IgG-HRP secondary antibodies from Dakopatts, Glostrup, Denmark; Cellular Activation of Signalling ELISA, CASE™, kits for phosphorylated p38 and phosphorylated JNK from MedProbe (www.medprobe.com). All other chemicals and reagents used have been specified previously [30].

Bone cell culture

MG-63 cells are a human osteoblastic osteosarcoma cell line which expresses several osteoblastic phenotypes including biosynthesis of type I collagen and osteocalcin [31]. For experiments, cells were seeded at an initial density of 4–5 × 10^4 cells/cm^2 in either 2 cm^2 multiwell culture plates for prostaglandin analysis, or 9.5 cm^2 or 20 cm^2 culture dishes for gene expression analysis or 60 cm^2 for preparation of total cell lysates or nuclear extracts. To the dishes, α-MEM/10% FCS was added and cells were cultured for 1–2 days until 80–90% confluent monolayers were obtained. Then, the cells were washed 2 x in PBS and 1 x in serum free α-MEM, and subsequently incubated in α-MEM/1% FCS, with or without test substances for different periods of time.

Culture of calvarial bones

Calvarial bones from 5–7 days old CsA mice were dissected and cut into two halves. The bones were preincubated for 18–24 h in α-MEM containing 0.1% BSA and 1 μM indomethacin [32]. Following preincubation, the bones were extensively washed, incubated submerged for 24 h, in 24-wells culture plates, containing 1.0 ml indomethacin-free medium, with or without test substances and subsequently used for gene expression analysis. CsA mice were from our own inbred colony. Animal care and experiments were approved and conducted in accordance with accepted standards of humane animal care and use as considered appropriate by the Animal Care and Use Committee of Umeå University, Umeå, Sweden.

Isolation of human gingival fibroblasts

Human gingival fibroblasts were isolated from explants of human gingiva obtained by surgery of clinically healthy gingiva as previously described [33]. Cells growing out from the explants were subcultured in α-MEM/10% FCS and then seeded at a density of 80% in 6-wells plates (9.5 cm^2), with and without test substances. After 24 h of incubation, RNA was extracted for subsequent analysis of gene expression. The study was approved by the Human Studies Ethical Committee of Umeå University and informed consent was obtained by all patients.

Radioligand binding

MG-63 cells were cultured to 80% subconfluent monolayers in 2 cm^2 multiwell dishes. The cells were washed 1× in PBS/0.1% BSA and 1× in MEM/HEPES/0.1% BSA and then preincubated in MEM/HEPES/0.1% BSA with different cytokines (at different time points or different concentrations), kinins or in plain control medium. Subsequently, the cells were incubated in 250 μl MEM/HEPES/0.1% BSA containing 10 μmol/L phosphoramidone, 4 nM [^{3}H]-BK (~120,000 DPM/well) or 7 nM [^{3}H]-des-Arg^{10}-Lys-BK (~240,000 DPM/well), with or without different kinin receptor agonists. Cells were incubated at 4 °C for 150 min. At the end of the incubation, medium was aspirated and cells were washed 5× with PBS/0.1% BSA. Finally, 500 μl of Ca^{2+}- and Mg^{2+}-free phosphate buffer (137 mM NaCl, 2.7 mM KCl, 3 mM $NaH_2PO_4 \times H_2O$; pH = 7.2) containing EDTA (187 mg/L) and trypsin (100 mg/L) was added for 10 min and then the radioactivity in the suspension was analyzed using a liquid scintillation counter.

Analysis of prostaglandin production

Prostaglandin biosynthesis, in MG-63 cells, was assessed by analyzing the amounts of PGE_2 in the media at the end of the cultures [29]. PGE_2 was determined using a commercially available radio-immuno-assay kit, by following the instructions of the manufacturer.

RNA extraction

After incubation with or without test substances, the MG-63 cells or gingival fibroblasts were washed 2× in PBS and total RNA was isolated using Trizol LS Reagent or RNAqueous™-4PCR kit, by following the manufacturer's protocol. The calvarial bones were homogenized (Ultra-Turrax®, Jenke & Kunkel KG, Staufen, Germany) before RNA extraction using the RNAqueous™-4PCR kit. Extracted RNA was quantified