

Norepinephrine Modulates IL-1 β -induced Catabolic Response of Human Chondrocytes

Hyun Sook Hwang

Hallym University Sacred Heart Hospital

Mi Hyun Lee

Hallym University Sacred Heart Hospital

Dong Jin Go

Hallym University Kangnam Heart Hospital

Hyun Ah Kim (✉ kimha@hallym.ac.kr)

Hallym University Sacred Heart Hospital

Research Article

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Abstract

Background

The influence of the sympathetic nervous system (SNS) on metabolism of bone and cartilage expressing β -adrenergic receptors (AR) was suggested. Here, we investigated the relation between SNS and interleukin-1beta (IL-1 β)-induced cartilage metabolism.

Methods

Human articular chondrocytes and articular cartilage were collected from patients with osteoarthritis (OA). Chondrocyte monolayer and cartilage explant culture were stimulated with IL-1 β . The activity of β -ARs was modulated by an agonist, norepinephrine (NE), and antagonists, including propranolol, atenolol, nebivolol, and nadolol.

Results

The levels of β_1 -, β_2 -, and β_3 -AR in OA cartilage and IL-1 β -treated chondrocytes were lower than normal cartilage and untreated cells. Treatment of chondrocytes with IL-1 β and β -blockers, including propranolol, atenolol, nebivolol, and nadolol, for 6 h significantly upregulated IL-1 β -induced expression of MMP-1, -3, and -13, compared to chondrocytes treated with IL-1 β alone, indicating that antagonism of β -AR confers catabolic signals. On the other hand, NE antagonized IL-1 β -induced catabolic response. In addition, NE significantly inhibited IL-1 β -induced release of glycosaminoglycan (GAG) from cartilage explant culture. In addition, β -AR activity significantly affected IL-1 β -stimulated phosphorylation of JNK and ERK. These results indicate that β -AR signal is associated with cartilage metabolism.

Conclusions

Our findings showed that β -ARs is a regulator of cartilage catabolism induced with IL-1 β .

Background

Osteoarthritis (OA) is a prevalent degenerative joint disease, which shows features such as cartilage loss, synovitis, and accompanying joint pain [1]. Synthesis of extracellular matrix (ECM) in chondrocytes is maintained by balance of anabolic factors and catabolic factors, such as matrix metalloproteinases (MMPs), and are also influenced by a variety of molecules, including pro-inflammatory mediators and cytokines [1, 2].

Stimuli from inflammation and mechanical stress are detected by sensory nerve fibers and is transmitted to the central nervous system, which subsequently activates the sympathetic nervous system (SNS). A series of processes, including release of neurotransmitter such as norepinephrine (NE), lymphocyte recruitment, and increase of blood and lymph flow, are induced at the site of inflammation [3]. In particular, the sympathetic nerve fibers expressing α - and β -adrenergic receptors (AR) are found in various

tissues, including synovium, bone, and chondrocytes. Previous studies reported results linking the SNS with the skeletal system. The endogenous catecholamine NE was detected at high concentration in synovial fluid from patients with joint trauma [4]. NE regulates bone development and chondrocyte metabolism via β -AR [5]. NE has also been shown to regulate apoptosis and proliferation of several cell types including chondrocytes via β -AR [6–8]. Of note, NE exhibits a distinct impact depending on the receptor it signals through such that inflammatory response of OA chondrocyte signals via β -AR while cell cycle and apoptosis signals via α -AR [9].

Furthermore, the influence of AR signaling on skeletal tissue homeostasis has been reported in animal and human studies. Intra-cerebroventricular administration of leptin reduced bone formation and bone mass in mice, with leptin acting on the hypothalamus to increase sympathetic outflow, leading to the activation of β -ARs on osteoblasts and resulting in decreases in osteoblast proliferation, differentiation, and bone formation [10, 11]. Treatment with the nonselective β -blocker propranolol increased bone mass in ovariectomized as well as ovary-intact female mice [11]. In addition, a study of postmenopausal women showed that patients treated with β 1-AR–selective blockers had decrease of bone resorption marker and increase of bone mineral density than did nonusers [12]. However, it is little known whether the SNS signaling affects cartilage metabolism in chondrocytes or OA patients.

In this study, we investigated whether the regulation of β -AR activity by NE and its antagonists has an influence on interleukin-1 β (IL-1 β)-induced catabolic and anabolic responses in articular chondrocytes.

Methods

Materials

Recombinant human interleukin-1 β (IL-1 β) was purchased from R&D Systems (Minneapolis, MN, USA). Propranolol (P), atenolol (A), nebivolol (B), and nadolol (N) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Antibodies against p-I κ B α , p-p38/p38, p-ERK/ERK, and p-JNK/JNK were purchased from Cell Signaling Technology (Danvers, MA, USA). Horseradish peroxidase (HRP)-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Primers used in qRT-PCR were obtained from Cosmogenetech (Seoul, Korea).

Cartilage collection and chondrocyte isolation from cartilage

OA cartilage samples were obtained from the knee joints of OA patients [$n = 8$, 74.25 ± 4.68 years] at the time of total knee replacement surgery. Patient diagnoses were determined using the criteria set forth by the American College of Rheumatology. Normal cartilage samples were obtained from the femoral head of patients [$n = 6$, 63.17 ± 12.81 years] with femoral neck fractures and no known history of OA or RA. Sample was obtained only from grossly normal-looking cartilage. The collection and use of human tissue samples was reviewed and approved by the Institutional Review Board of Hallym University Sacred Heart Hospital, Anyang, Korea (approval number 2018-05-040). All patients provided written informed consent.

All methods were performed in accordance with the relevant guidelines and regulations of Hallym University and were approved by its ethical committee. Primary chondrocytes were isolated from articular cartilage as previously described [13].

Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from chondrocytes or cartilage tissues using TRIzol reagent as previously described [13]. cDNA was synthesized using Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI, USA). qRT-PCR was performed using a StepOnePlus real-time PCR system with the primers in Table S1.

Western blot analysis

Protein from chondrocytes was prepared with RIPA lysis buffer (Biosesang, Kyunggi, South Korea) and protein concentrations were determined using bicinchoninic acid protein assay (Thermo Fisher Scientific, Rockford, IL, USA). Equal amounts of proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted to a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, CA, USA). The membrane was blocked with 5% (w/v) nonfat milk in TBST and incubated with primary and secondary antibodies. The antibody complex was detected using an enhanced chemiluminescence detection kit (Santa Cruz Biotechnology).

Explant culture

For explant culture, articular cartilage was taken from normal donors and incubated in DMEM containing IL-1 β with or without NE and antagonists of β -adrenergic receptor for 1, 7, and 14 days. The media was changed every 2 days during the culture period. Media were collected at desired time points.

Safranin O staining

For measurement of proteoglycan content in the cartilage, Safranin O staining was performed as previously described. Briefly, paraffin-embedded cartilage sections were deparaffinized in xylene, hydrated through gradient 100%, 95%, 90, and 70% ethyl alcohol, and stained in hematoxylin solution for 1 min. Then, the slides were counterstained with 0.05% Fast green solution for 5 min, 1% acetic acid solution, and 0.1% Safranin O staining for 5 min.

Measurement of GAG released in culture medium

GAG content in sample was measured using Sulfated Glycosaminoglycan kit according to the manufacturer's instructions. Briefly, Blyscan dye reagent (1.0 ml) was added to the same volume of medium samples and glycosaminoglycan standard solution for a calibration curve followed by mixing by inverting and gentle shaking for 30 min. A sulfated GAG dye complex formed in each reaction tube was obtained by centrifugation at 12,000 rpm for 10 min. The pellets were dissolved in dissociation reagent (0.5 ml) and optical density was measured at 656 nm. GAG contents were calculated from the standard curve.

Statistical analysis

Data are expressed as the mean \pm standard deviation (SD). Statistical analysis was performed using a Mann-Whitney test between two groups and Kruskal-Wallis test with post hoc Dunn's test for multiple comparison (GraphPad Prism 6; GraphPad Software, La Jolla, CA, USA). A value of $P < 0.05$ was considered statistically significant.

Results

The level of beta-adrenergic receptors (β -AR) is significantly decreased in OA cartilage and IL-1 β -treated chondrocytes

To investigate whether the level of subtypes of β -AR was different in normal and OA cartilage, mRNA levels of β -ARs were measured in normal and OA cartilages. The expression level of β_1 , β_2 , and β_3 -AR in normal cartilages varied depending on the donor, however, it was significantly lower in OA cartilage (Fig. 1A). In addition, IL-1 β treatment led to significantly decreased expression of three β -AR subtypes, which lasted for 72 hours in cultured chondrocytes (Fig. 1B-D). These results demonstrate that the expression of β -ARs is downregulated in chondrocytes in response to a catabolic mediator as well as in OA.

β -adrenergic blockers and NE modulate IL-1 β -induced expression of catabolic and anabolic factors

Sympathetic β -adrenergic blockers positively affect IL-1 β -induced bone metabolism [12]. To examine whether β -adrenergic blockers modulate expressions of anabolic and catabolic factors, chondrocytes were treated for 6 h with IL-1 β with or without β -adrenergic blockers (0.1, 1.0, and 10 μ g/ml). Non-selective β -adrenergic blocker (propranolol) and β_1 -blocker (atenolol) used in our experiments significantly increased IL-1 β -induced expression of MMP-1, -3, and -13 especially at the highest concentration (Fig. 2A). In contrast, they reversed IL-1 β -induced suppression of anabolic factors aggrecan (ACAN), while only atenolol had such influence on collagen II (Col II) (Fig. 2B). Our data revealed that blockade of β -adrenergic receptors influenced chondrocyte response to IL-1 β , such that it enhances both catabolic and anabolic markers.

To next analyze the effect of NE, a β -adrenergic agonist, on cartilage matrix metabolism, chondrocytes were treated with IL-1 β with or without various concentrations (0.1, 1.0, and 10 μ g/ml) of NE for 6, 24, and 48 h. IL-1 β treatment increased MMPs expression in a time-dependent manner (Fig. 3A). Co-treatment with NE significantly inhibited IL-1 β -induced expression of MMPs dose-dependently. Contrary to 6 hour treatment (Fig. 2), non-selective β -adrenergic blocker (propranolol and nadolol) and β_1 -blocker (atenolol and nebivolol) did not increase MMP expression compared to treatment with IL-1 β alone after 48 hour

culture (Fig. 3A). NE led to reversal of IL-1 β -induced down-regulation of ACAN and Col II after 24 hours, which did not last after 48 hours. β -adrenergic blockers did not affect ACAN and Col II regulation after 48 hours of IL-1 β treatment (Fig. 3B), suggesting that β -adrenergic blockers are effective only for the regulation of short-term response to IL-1 β . These results demonstrate that activation of β -AR antagonizes IL-1 β -induced catabolism of articular chondrocytes.

NE suppresses IL-1 β -induced release of glycosaminoglycan (GAG) in cartilage explants culture

To investigate the effect of β -adrenergic agonist and blockers on ECM metabolism, GAG release was measured in medium from cartilage explants culture exposed to IL-1 β with or without NE or β -adrenergic blockers for 1, 4 and 7 days. Untreated cartilage control showed time-dependent increase in cumulative GAG release (Fig. 3C). IL-1 β treatment further increased GAG release into the medium compared with untreated control. NE significantly suppressed GAG release induced by IL-1 β . GAG release in combinational treatment with IL-1 β and β -adrenergic blockers (P, A, B, and N) for 4 and 7 days did not increase further compared to IL-1 β -treated cartilage (Fig. 3C). In addition, we analyzed the level of ECM in cartilage tissue 7 days after explants culture using safranin O staining. IL-1 β significantly decreased ECM level, whereas NE suppressed the reduction of ECM induced by IL-1 β (Fig. 3D). These results demonstrate that activation of β -AR prevents loss of ECM in cartilage.

NE suppresses IL-1 β -mediated signaling pathways

We investigated the effect of NE and β -adrenergic blockers on IL-1 β -activated signaling pathways that contribute to MMPs, Col II, and ACAN expression. Chondrocytes were pre-incubated with NE and β -adrenergic blockers for 2 h and then treated with IL-1 β for 15, 30, and 60 min. IL-1 β induced phosphorylation of p38, JNK, and ERK MAPKs and I κ -B α (Fig. 4A – B; Fig. S1A-B). β -adrenergic blockers enhanced IL-1 β -induced activation of JNK, ERK and I κ -B α (Fig. 4A; Fig. S1A), whereas NE significantly inhibited their activation by IL-1 β (Fig. 4B; Fig. S1B). p38 activation was not affected. These results demonstrate that IL-1 β -dependent activation of JNK and ERK MAPK and NF- κ B is influenced by NE and β -adrenergic blockers in the opposite way.

Discussion

β -ARs, target receptors of catecholamine involved in the SNS signaling, were down-regulated in both OA cartilage and IL-1 β -treated chondrocytes compared with normal cartilage and untreated chondrocytes, respectively. In the present study we investigated the role of β -AR signaling in cartilage metabolism using β -adrenergic agonist and antagonists. Stimulation of β -AR facilitated overall anabolic responses by suppressing catabolic mediator expression and increasing cartilage matrix proteins in IL-1 β -treated chondrocytes accompanied by down-regulation of ERK/JNK MAPK and NF- κ B signaling pathway. On the contrary, blocking of β -AR led to short-term increase of IL-1 β -mediated catabolic responses only. The decreased effect of β -blockers on IL-1 β -induced GAG release in long-term treatment is likely attributed to

alteration in signaling pathways associated with IL-1 β and β -AR. These results show that SNS signaling via β -AR is closely related with regulation of cartilage metabolism.

The autonomic nervous system is composed of SNS and parasympathetic nervous system (PNS) that perform opposite actions. The SNS is responsible for the maintenance of homeostasis in response to harmful events leading to fight-or-flight responses [3]. During heart failure (HF), long-term increase in endogenous catecholamines, including NE and epinephrine, due to activation of the SNS plays a role in the progression of HF [14]. This is mediated via β -AR, and β -AR antagonists reduce morbidity and mortality in congestive HF. On the other hand, activation of β -ARs relaxes airway smooth muscle and inhaled β -AR agonists are considered as essential bronchodilator drugs in the treatment of bronchial asthma [15].

Several clinical and animal model studies have investigated the influence of the SNS on the severity of arthritis. In antigen-induced arthritis mouse model, chemical sympathectomy and pharmacological blockade of AR reduced arthritis severity such as joint inflammation and arthritis score [16]. Depletion of catecholamines by sympathectomy and elimination of sensory afferents by administration of capsaicin decreased joint injury in arthritic rats [17]. Patients with RA exhibits milder pain by sympathetic blockade using guanethidine [18]. In murine chondrocyte monolayer culture, stimulation of β_2 -AR by isoproterenol inhibits Col X and Indian Hedgehog (Ihh) mRNA level through activation of ERK1/2 MAPK [19]. Sox-6 and Col II expression was inhibited by stimulation of β_2 -AR and this inhibitory effect was suppressed by propranolol, β_2 -AR antagonist [20], indicating that activation of β -AR signaling has negative effects on matrix protein synthesis in chondrocytes. By contrast, we found that β_1 -, β_2 -, and β_3 -AR expressions were reduced in OA cartilage relative to normal cartilage and pro-inflammatory cytokine IL-1 β down-regulated three subtypes of β -AR compared to untreated control. In addition, our data showed that β -AR agonist prevented loss of ECM through decreased expression of pro-catabolic factors and increased expression of anabolic factors. These contradictory results are likely to be due to difference in types and concentrations of AR agonists and antagonists, AR subtypes interacting with them, and subsequent downstream signaling pathways.

In line with our results showing the suppression of IL-1 β -induced catabolic responses by NE in monolayer and explant culture of human chondrocyte, several reports have demonstrated that catecholamines, including NE and dopamine, a precursor of NE, had anti-inflammatory effects in a variety of cells and *in vivo* models. NE reversed cartilage catabolism and inflammatory responses stimulated by IL-1 β [9]. Adoptive transfer of tyrosine hydroxylase-positive neuronal cells generated from mesenchymal stem cells which exhibit a typical catecholaminergic phenotype led to markedly reduced severity of collagen induced arthritis in mice [21]. Dopamine prevented cartilage degradation in a DMM-induced OA mouse model and reduced MMPs expression and elevated Col II expression in IL-1 β -treated chondrocytes via NF- κ B and JAK2/STAT3 signaling pathway [22]. In lipopolysaccharide-stimulated microglia cells, dopamine suppressed nitric oxide production [23].

Bone remodeling is also under sympathetic control, such that β -blocker treatment enhanced bone mass in wild type and ovariectomized mice [11]. Clenbuterol, a β_2 -AR agonist, suppressed longitudinal growth of bones in young rats together with muscular hypertrophy [24]. On the contrary, another study showed that clenbuterol relieved sciatic nerve injury-induced loss of bone mineralization [25]. Regulation of the SNS by leptin via β_2 -AR decreases osteoblast number and increases osteoclast differentiation leading to reduction of bone mass in mice [26, 27]. β_2 -AR-deficient mice exhibited greater bone mass in response to mechanical loading compared to wild type, but it was not shown in β_1 -knockout and $\beta_{1,2}$ -AR double knockout mice. In addition, administration of isoproterenol a non-selective β -AR agonist to wildtype mice increased bone resorption, indicating the possibility that β_1 - and β_2 -AR could play opposite role in bone metabolism [28].

It is also noted that the SNS is closely related with regulation of inflammatory responses, e.g. proinflammatory cytokine production. In a study of postmenopausal women with hypertension, central blockade of the SNS with moxonidine reduced serum TNF- α level [29]. TNF production is regulated through stimulation of α -AR or β -AR by catecholamines such as NE [30, 31].

NF- κ B pathway is an inducible transcription factor involved in inflammation and cellular differentiation, and in ECM homeostasis [32]. In particular, the activation of NF- κ B leads to increase in catabolic gene expression such as MMPs, ADAMTS5, and proinflammatory mediators, including cyclooxygenase-2 and inducible nitric oxide synthase. We found that regulation of β -AR activity with NE and four antagonists of AR had opposite influence on activation of JNK and ERK MAPK and NF- κ B compared to IL-1 β -treated chondrocytes, subsequently leading to alteration in expression of anabolic and catabolic factors. Thus, the SNS modulated pro-catabolic and anti-anabolic responses induced by IL-1 β through regulation of JNK and ERK MAPK and NF- κ B in chondrocytes. In addition, it is likely that β -ARs induce different biological responses through activating diverse downstream signaling molecules, including adenylate cyclase, depending on types, concentration, and treatment time of agonists and antagonists.

Conclusions

In conclusion, our findings demonstrate that β -AR agonist NE converted IL-1 β -driven catabolic responses to cartilage anabolism in articular chondrocytes. Cartilage homeostasis is likely to be closely related with the SNS level and activity. Whether modulators of the SNS signaling may be beneficial for prevention of OA progression should be studied in further animal experiments.

Declarations

Ethics approval and consent to participate

This study was reviewed and approved by the Institutional Review Board of Hallym University Sacred Heart Hospital, Anyang, Korea (approval number 2018-05-040). Written informed consent was obtained

from all participants in this study. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Availability of data and materials

Correspondence and requests for all data required to evaluate the work should be addressed to H.A.K.

Competing interests

The authors have no conflicting interests.

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Authors contributions

HSH and MHL were involved in the conception and design of the study and acquisition and analysis of data. DJG and HAK conceived and designed the experiments and wrote the paper. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

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Not applicable.

References

1. Goldring MB. The role of the chondrocyte in osteoarthritis. *Arthritis Rheum.* 2000;43(9):1916–26.
2. Hwang HS, Kim HA. Chondrocyte Apoptosis in the Pathogenesis of Osteoarthritis. *Int J Mol Sci.* 2015;16(11):26035–54.
3. Pongratz G, Straub RH. The sympathetic nervous response in inflammation. *Arthritis Res Ther.* 2014;16(6):504.
4. Jenei-Lanzl Z, Grassel S, Pongratz G, Kees F, Miosge N, Angele P, Straub RH. Norepinephrine inhibition of mesenchymal stem cell and chondrogenic progenitor cell chondrogenesis and acceleration of chondrogenic hypertrophy. *Arthritis Rheumatol.* 2014;66(9):2472–81.

5. Opolka A, Straub RH, Pasoldt A, Grifka J, Grassel S. Substance P and norepinephrine modulate murine chondrocyte proliferation and apoptosis. *Arthritis Rheum.* 2012;64(3):729–39.
6. Communal C, Singh K, Pimentel DR, Colucci WS. Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of the beta-adrenergic pathway. *Circulation.* 1998;98(13):1329–34.
7. Lai KB, Sanderson JE, Yu CM. High dose norepinephrine-induced apoptosis in cultured rat cardiac fibroblast. *Int J Cardiol.* 2009;136(1):33–9.
8. Suzuki A, Palmer G, Bonjour JP, Caverzasio J. Catecholamines stimulate the proliferation and alkaline phosphatase activity of MC3T3-E1 osteoblast-like cells. *Bone.* 1998;23(3):197–203.
9. Lorenz J, Schafer N, Bauer R, Jenei-Lanzl Z, Springorum RH, Grassel S. Norepinephrine modulates osteoarthritic chondrocyte metabolism and inflammatory responses. *Osteoarthritis Cartilage.* 2016;24(2):325–34.
10. Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, Shen J, Vinson C, Rueger JM, Karsenty G. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell.* 2000;100(2):197–207.
11. Takeda S, Eleftheriou F, Levasseur R, Liu X, Zhao L, Parker KL, Armstrong D, Ducy P, Karsenty G. Leptin regulates bone formation via the sympathetic nervous system. *Cell.* 2002;111(3):305–17.
12. Khosla S, Drake MT, Volkman TL, Thicke BS, Achenbach SJ, Atkinson EJ, Joyner MJ, Rosen CJ, Monroe DG, Farr JN. Sympathetic beta1-adrenergic signaling contributes to regulation of human bone metabolism. *J Clin Invest.* 2018;128(11):4832–42.
13. Hwang HS, Park SJ, Lee MH, Kim HA. MicroRNA-365 regulates IL-1beta-induced catabolic factor expression by targeting HIF-2alpha in primary chondrocytes. *Sci Rep.* 2017;7(1):17889.
14. Mahata SK, Zheng H, Mahata S, Liu X, Patel KP. Effect of heart failure on catecholamine granule morphology and storage in chromaffin cells. *J Endocrinol.* 2016;230(3):309–23.
15. Barisione G, Baroffio M, Crimi E, Brusasco V. Beta-Adrenergic Agonists. *Pharmaceuticals (Basel).* 2010;3(4):1016–44.
16. Ebbinghaus M, Gajda M, Boettger MK, Schaible HG, Brauer R. The anti-inflammatory effects of sympathectomy in murine antigen-induced arthritis are associated with a reduction of Th1 and Th17 responses. *Ann Rheum Dis.* 2012;71(2):253–61.
17. Levine JD, Dardick SJ, Roizen MF, Helms C, Basbaum AI. Contribution of sensory afferents and sympathetic efferents to joint injury in experimental arthritis. *J Neurosci.* 1986;6(12):3423–9.
18. Levine JD, Fye K, Heller P, Basbaum AI, Whiting-O'Keefe Q. Clinical response to regional intravenous guanethidine in patients with rheumatoid arthritis. *J Rheumatol.* 1986;13(6):1040–3.
19. Lai LP, Mitchell J. Beta2-adrenergic receptors expressed on murine chondrocytes stimulate cellular growth and inhibit the expression of Indian hedgehog and collagen type X. *J Cell Biochem.* 2008;104(2):545–53.

20. Mitchell J, Lai LP, Peralta F, Xu Y, Sugamori K. beta2-adrenergic receptors inhibit the expression of collagen type II in growth plate chondrocytes by stimulating the AP-1 factor Jun-B. *Am J Physiol Endocrinol Metab.* 2011;300(4):E633-9.
21. Jenei-Lanzl Z, Capellino S, Kees F, Fleck M, Lowin T, Straub RH. Anti-inflammatory effects of cell-based therapy with tyrosine hydroxylase-positive catecholaminergic cells in experimental arthritis. *Ann Rheum Dis.* 2015;74(2):444–51.
22. Lu W, Ding Z, Liu F, Shan W, Cheng C, Xu J, He W, Huang W, Ma J, Yin Z. Dopamine delays articular cartilage degradation in osteoarthritis by negative regulation of the NF-kappaB and JAK2/STAT3 signaling pathways. *Biomed Pharmacother.* 2019;119:109419.
23. Wang B, Chen T, Li G, Jia Y, Wang J, Xue L, Chen Y. Dopamine Alters Lipopolysaccharide-Induced Nitric Oxide Production in Microglial Cells via Activation of D1-Like Receptors. *Neurochem Res.* 2019;44(4):947–58.
24. Kitaura T, Tsunekawa N, Kraemer WJ. Inhibited longitudinal growth of bones in young male rats by clenbuterol. *Med Sci Sports Exerc.* 2002;34(2):267–73.
25. Zeman RJ, Hirschman A, Hirschman ML, Guo G, Etlinger JD. Clenbuterol, a beta 2-receptor agonist, reduces net bone loss in denervated hindlimbs. *Am J Physiol.* 1991;261(2 Pt 1):E285-9.
26. Elefteriou F, Ahn JD, Takeda S, Starbuck M, Yang X, Liu X, Kondo H, Richards WG, Bannon TW, Noda M *et al.* Leptin regulation of bone resorption by the sympathetic nervous system and CART. *Nature.* 2005;434(7032):514–20.
27. Kajimura D, Hinoi E, Ferron M, Kode A, Riley KJ, Zhou B, Guo XE, Karsenty G. Genetic determination of the cellular basis of the sympathetic regulation of bone mass accrual. *J Exp Med.* 2011;208(4):841–51.
28. Pierroz DD, Bonnet N, Bianchi EN, Boussein ML, Baldock PA, Rizzoli R, Ferrari SL. Deletion of beta-adrenergic receptor 1, 2, or both leads to different bone phenotypes and response to mechanical stimulation. *J Bone Miner Res.* 2012;27(6):1252–62.
29. Poyhonen-Alho MK, Manhem K, Katzman P, Kibarskis A, Antikainen RL, Erkkola RU, Tuomilehto JO, Ebeling PE, Kaaja RJ. Central sympatholytic therapy has anti-inflammatory properties in hypertensive postmenopausal women. *J Hypertens.* 2008;26(12):2445–9.
30. Spengler RN, Allen RM, Remick DG, Strieter RM, Kunkel SL. Stimulation of alpha-adrenergic receptor augments the production of macrophage-derived tumor necrosis factor. *J Immunol.* 1990;145(5):1430–4.
31. Severn A, Rapson NT, Hunter CA, Liew FY. Regulation of tumor necrosis factor production by adrenaline and beta-adrenergic agonists. *J Immunol.* 1992;148(11):3441–5.
32. Choi MC, Jo J, Park J, Kang HK, Park Y. NF-kappaB Signaling Pathways in Osteoarthritic Cartilage Destruction. *Cells.* 2019;8(7).

Figures

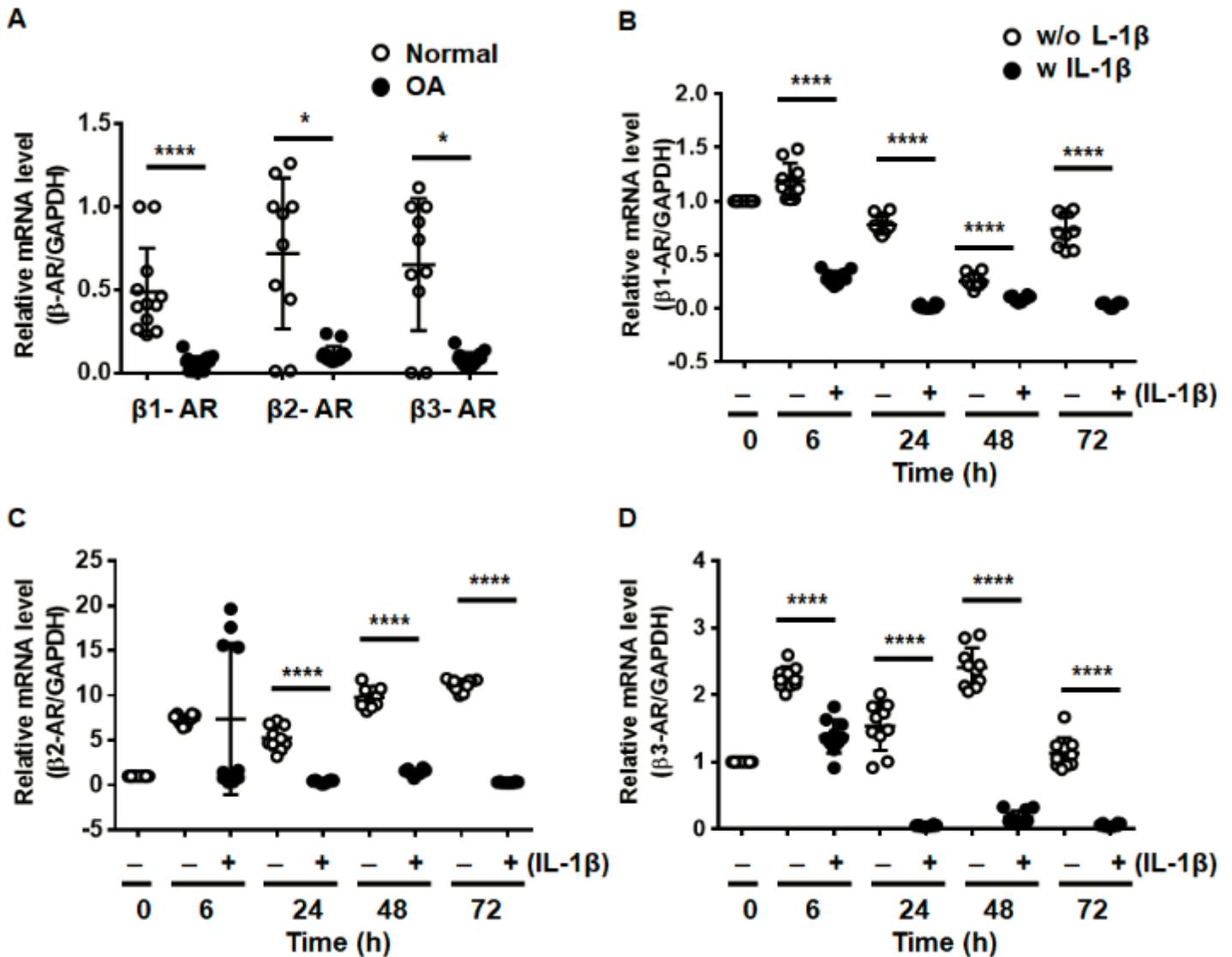


Figure 1

The levels of beta-adrenergic receptors are decreased in OA cartilage and IL-1 β -treated chondrocytes. (A) The relative expression of β 1-, β 2-, and β 3-adrenergic receptor (AR) in normal and OA cartilage. Data represent the mean \pm standard deviation (SD) for duplicate data from different donors. Normal cartilage (n = 6); OA cartilage (n = 8). (B)~(D) Expression of β 1-, β 2-, and β 3-adrenergic receptors in IL-1 β -treated chondrocytes. Chondrocytes were stimulated with IL-1 β (1 ng/ml) for 6, 24, 48, and 72 h. mRNA levels of β -ARs were quantified using Quanti Fast SYBR Green-based real-time PCR (RT-PCR). The expression ratios of β -adrenergic receptors relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH), the internal control, are shown. Data represent the mean \pm standard deviation (SD) for duplicate data from five different donors. *P < 0.05 and ****P < 0.0001 vs. normal cartilage tissue or untreated chondrocytes by Mann-Whitney test.

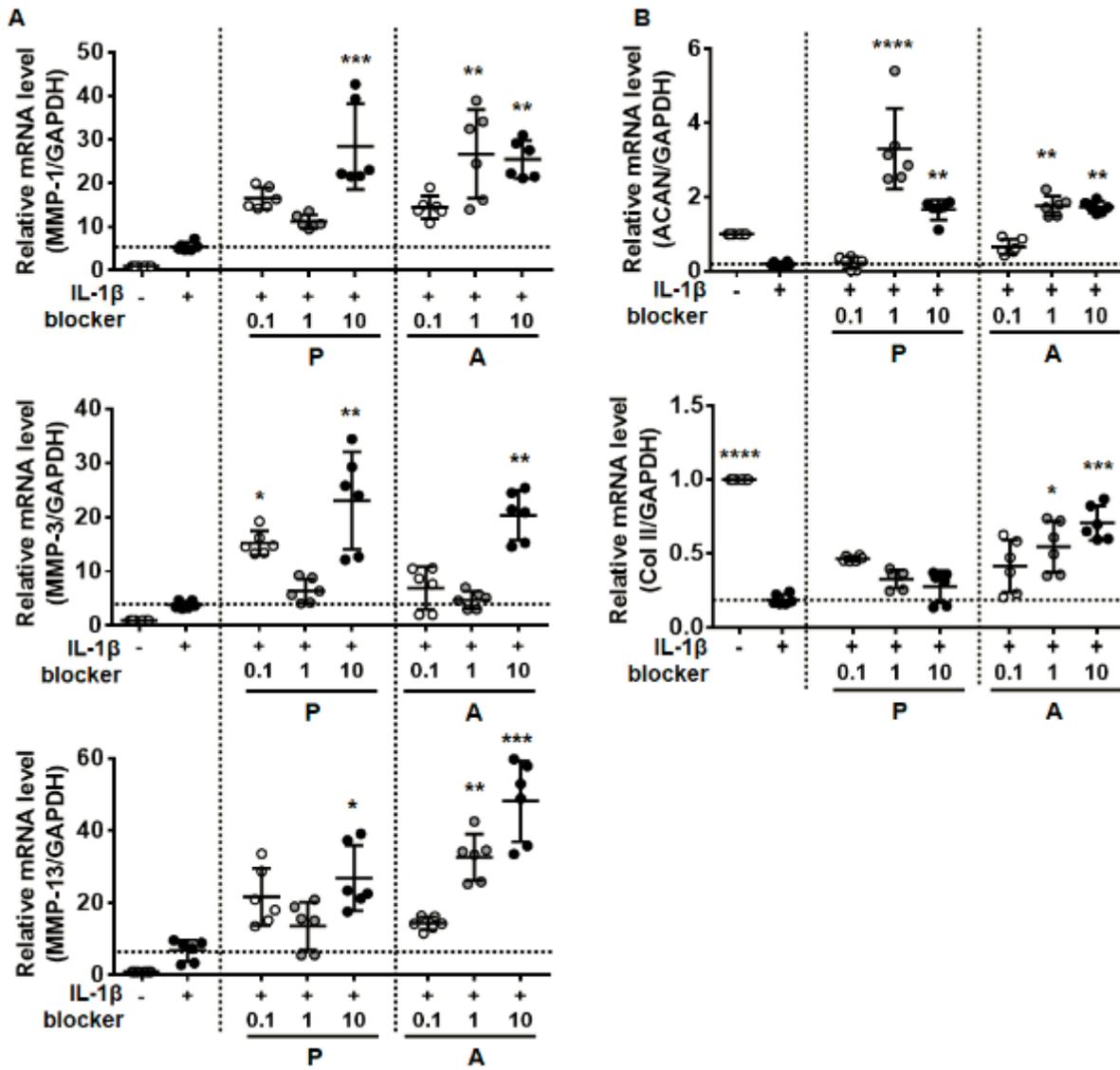


Figure 2

β -AR blockers affect IL-1 β -induced expression of catabolic and anabolic factors. (A), (B) The effect of β -AR blockers on IL-1 β -induced expression of (A) catabolic and (B) anabolic factors. Chondrocytes were pre-incubated with 0.1, 1.0, and 10 ng/ml of propranolol (P; non-selective β -adrenergic blocker) and atenolol (A; β 1-adrenergic blocker) for 2 h followed by treatment with IL-1 β (1 ng/ml) for 6 h. The mRNA levels of catabolic factors (MMP-1, -3, and -13) and anabolic factors (ACAN and Col II) were measured using RT-PCR. Data represent the mean \pm SD of duplicate data from three different donors. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ vs. IL-1 β -treated cells by Kruskal-Wallis test with post hoc Dunn's multiple comparison test.

Data represent the mean \pm SD of duplicate data from three different donors. * $P < 0.05$, ** $P < 0.01$, and **** $P < 0.0001$ vs. IL-1 β -treated cartilage tissue by Kruskal-Wallis test followed with post hoc Dunn's multiple comparison test. (D) Safranin O staining of explant culture of human joint cartilage in the presence of IL-1 β and NE.

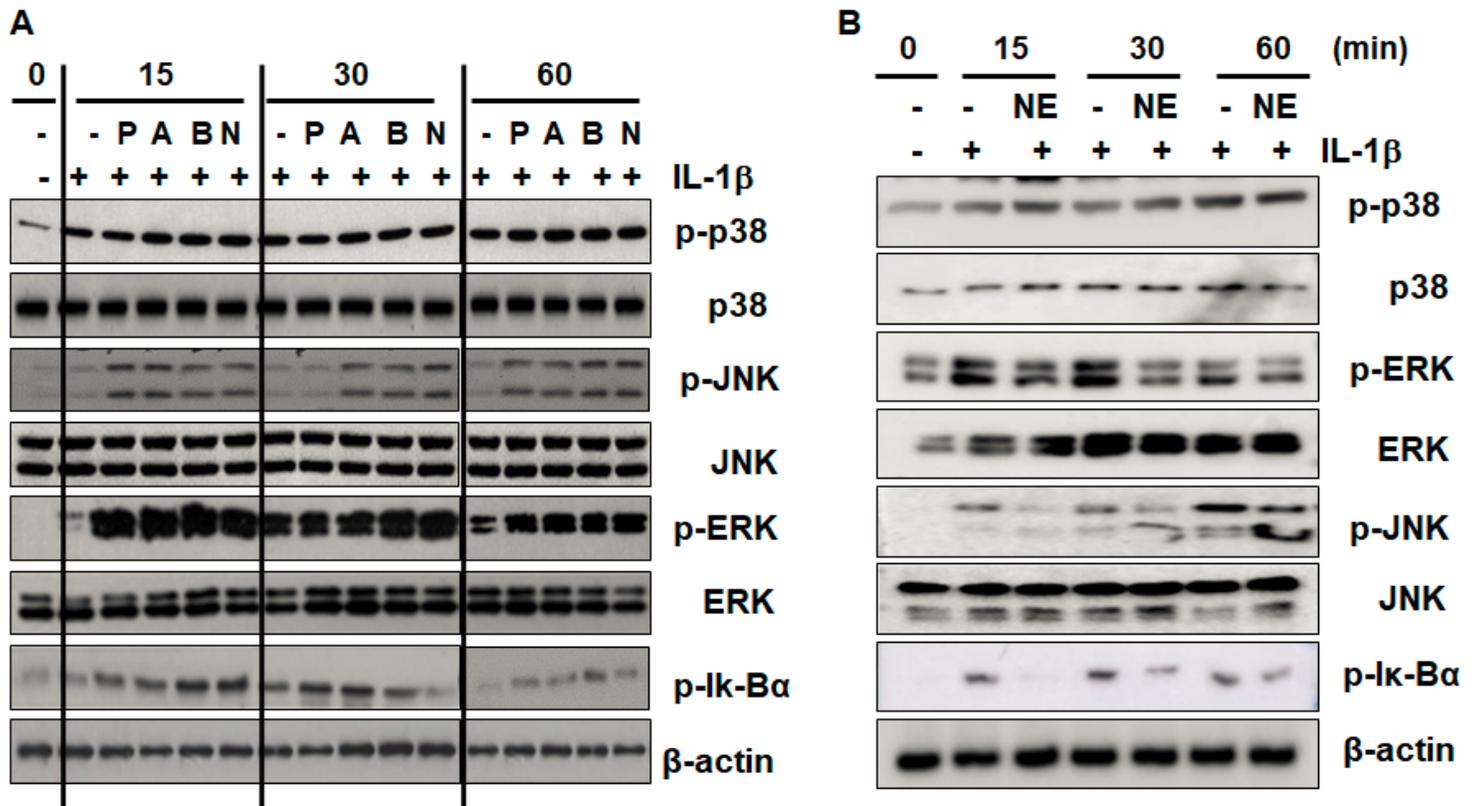


Figure 4

Effect of NE or β -AR blockers on IL-1 β -activated signaling pathways in human chondrocytes. Chondrocytes were pretreated with (A) β -AR blockers (P, A, B, and N; 1.0 ng/ml) or (B) NE (1.0 ng/ml) 2 h before stimulation with IL-1 β (1 ng/ml) for 15, 30, and 60 min. Phosphorylation of Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), p38 MAPK, and I κ -B α was analyzed by Western blot. Western blots shown are representative of four independent experiments. β -actin was used as a loading control. Propranolol (P), atenolol (A), nebivolol (B), and nadolol (N).

Supplementary Files

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