β2-Adrenergic receptors inhibit the expression of collagen type II in growth plate chondrocytes by stimulating the AP-1 factor Jun-B

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Mitchell J, Lai LP, Peralta F, Xu Y, Sugamori K. β2-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 300: E633–E639, 2011. First published December 21, 2010; doi:10.1152/ajpendo.00515.2010.—The sympathetic nervous system can regulate both osteoblast and chondrocyte growth and activity through β2-adrenergic receptors (β2-AR). We have shown previously that β2-AR activate both adenyl cyclase and mitogen-activated protein kinases ERK1/2 in growth plate chondrocytes prepared from ribs of embryonic E18.5 mice. Here we examined β2-AR inhibition of collagen type II (Col II) expression in growth plate chondrocytes and the molecular pathways involved. Stimulation of β2-AR by isoproterenol inhibited Col II mRNA and protein levels by ~50% beginning at 2 h, with both remaining suppressed over 24 h. This inhibition was blocked by propranolol and inhibitors of either MEK1 or PKA. Isoproterenol stimulated an AP-1-luciferase reporter and increased the expression of AP-1 factors c-Fos, Fra-1, Fra-2, c-Jun, and Jun-B but had no effect on Jun-D. Stimulation of AP-1 activity was blocked by inhibitors of MEK1 or PKA. siRNA inhibition of AP-1 factors showed that depletion of only Jun-B attenuated isoproterenol-mediated inhibition of Col II. Transfection with jun-B or c-fos showed selective inhibition of Col II mRNA and a Col II expression through ERK1/2 and PKA stimulation of the AP-1 factor Jun-B that inhibits the expression of Sox-6 and Col II.

chondrocytes; growth plate; activator protein-1 factor; Sox-6

THE SYMPATHETIC NERVOUS SYSTEM mediates a number of effects on components of the skeletal system. The best characterized of these is the anti-osteogenic effect of leptin on bone remodeling that is mediated by sympathetic stimulation of β2-adrenergic receptors (β2-ARs) expressed on osteoblasts (3). Leptin, a peptide hormone produced by adipocytes, signals the amount of adipose energy stores to the brain. Leptin receptors in the hypothalamus regulate a number of signals that converge to increase sympathetic activity and release of norepinephrine from peripheral neurons. Mice deficient in β2-AR or normal mice treated with β-blockers have high bone mass, and studies have shown that β2-ARs on osteoblasts directly inhibit osteoblast proliferation and indirectly increase osteoclast differentiation (4). These two effects are mediated by different pathways. β2-AR stimulates PKA phosphorylation of CREB, activating both activator protein-1 (AP-1) factors and the clock gene, which together coordinate the rate of osteoblast proliferation (5). Osteoclast differentiation is stimulated by receptor activator of NF-κB ligand (RANKL) secreted by osteoblasts, and this is mediated by β2-AR stimulation of activating transcription factor 4, another member of the CREB family of transcription factors that upregulates RANKL transcription (4, 27). The final outcome of β2-AR activation on osteoblasts is a significant increase in bone turnover with loss of bone mineral density.

The sympathetic nervous system also has effects on bone development, at least in rodents, since mice deficient in leptin have reduced long bone growth that can be rescued by leptin administration (24). This effect is mediated by both direct and indirect effects on chondrocytes. Leptin itself stimulates the growth and differentiation of chondrocytes in cultures of mouse condyles (17); however, leptin stimulation of the sympathetic nervous system appeared to have the opposite effect since rats treated for 4 wk with a selective β2-AR agonist had shortened bone length in addition to decreased bone mineral density and mineral content of the femur and tibia (11). The effects of β2-AR activation on bone development could be mediated by an effect on chondrocytes since we and others have demonstrated that mouse chondrocytes express β2-AR (15, 26). β2-ARs on chondrocytes are functionally coupled to stimulation of both adenyl cyclase and the ERK1/2 mitogen-activated protein kinases, and agonist activation resulted in stimulation of growth and inhibition of chondrocyte differentiation marker genes collagen type X and Indian hedgehog (15). A recent study has shown β2-AR expression in both proliferating and hypertrophic chondrocytes using in situ hybridization of neonatal mouse tibia (26).

In this study, we have examined the pathways by which β2-ARs regulate the expression of the major collagen type expressed in cartilage, collagen type II (Col II), by examining the effect of β2-AR agonist on Col II expression in growth plate chondrocytes. We demonstrate that adrenergic stimulation of ERK1/2 and PKA is required for Col II inhibition. We also show that β2-AR stimulation of the AP-1 factor Jun-B mediates Col II and Sox-6 inhibition.

MATERIALS AND METHODS

Reagents. Isoproterenol and propranolol were purchased from Sigma (Oakville, ON, Canada). Cell culture media and reagents were purchased from Gibco-BRL/Invitrogen (Burlington, ON, Canada). AP-1 luciferase reporter construct PathDetect AP-1 Cis-Reporting System was purchased from Stratagene (La Jolla, CA), and Col II 4×48-luciferase construct was a gift from Dr. Benoit de Cromrugghe, University of Texas, M. D. Anderson Cancer Center, Houston, TX. cDNAs encoding AP-1 factors in pcDNA3 vectors were a gift from Dr. S. Lye, Mount Sinai Hospital, Toronto, ON, Canada. pSG5-MKP-1 was a gift from Dr. N. Tonks, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, and pCMV5-dnMEK1 was a gift from Dr. K. L. Guan, University of Michigan, Ann Arbor, MI. The dn-PKA

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was a gift from Dr. G. S. McKnight, Washington University, Seattle, WA. siRNAs for c-Fos, Fra-1, Fra-2, c-Jun, and Jun-B as well as control siRNA were all purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal antibodies against c-Fos (sc-52), c-Jun (sc-44), and Jun-B (sc-73) were obtained from Santa Cruz Biotechnology, and antibodies against Fra-1, Fra-2, Col II, Sox-6, and actin were all purchased from Abcam (Cambridge, MA). Human chondrocyte nucleofector kit was purchased from Amaxa Biosystems (Co- logne, Germany). Power SYBR Green real-time PCR reagent was purchased from Applied Biosystems (Streetsville, ON, Canada).

**Primary chondrocyte preparation and cell culture.** Timed pregnant CD1 mice at E18.5 were purchased from Charles River Laboratories (St. Constant, QC, Canada). Animals were treated in accordance with the guidelines of the University of Toronto Animal Care Committee, which reviewed and approved our experiments. Primary chondrocytes isolated from the fetal mouse ribs as described previously (15) were plated at 150,000 cells/cm² in monolayer culture and maintained in DMEM (high glucose) containing 100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.25 mg/ml amphotericin B, and 10% fetal bovine serum. Cells were treated on day 2, and cell lysates and RNA were prepared on day 2 or day 3.

**RNA isolation and real-time PCR.** Total RNA was extracted using Trizol reagent (Gibco Invitrogen) as specified by the manufacturer and subsequently digested with DNase I, and 1-μg samples of RNA were reverse-transcribed by Moloney murine leukemia virus reverse transcriptase in a total volume of 20 μl. Real-time PCR amplification was performed using the 7500 Real-Time PCR System (Applied Biosystems), with the Power SYBR Green PCR master mix used according to the manufacturer’s standard cycling conditions. Primers were designed using Primer express software (version 3) (Applied Biosystems), with the sequences listed in Table 1; GAPDH was used as an internal control for the quantity of each cDNA. Analysis of the real-time PCR results was done by the 2-ΔΔCt method for the relative quantification of Col II, Sox 6, and AP-1 factors, where CT is the threshold cycle at which a significant increase in fluorescent signal is first detected.

**Transfection with cDNAs and siRNA.** Mouse primary chondrocytes were transfected with cDNAs encoding AP-1 factors using reagents from Amazia Nucleofector kit for human chondrocytes according to the manufacturer’s instructions. Briefly, 10⁶ freshly isolated growth plate chondrocytes were placed in six-well plates, and allowed to adhere overnight. The following day, cell medium was changed and cells were allowed to grow for a total of 36 h before being incubated for an additional 12 h in the presence and absence of 10 μM isoproterenol, followed by RNA or protein isolation. Control cells were examined for eGFP expression by primary growth plate chondrocytes. Ten-microgram samples were assessed for Col IIa1 expression by primary growth plate chondrocytes. Ten-microgram samples were assessed for Col IIa1 expression by primary growth plate chondrocytes. Ten-microgram samples were assessed for Col IIa1 expression by primary growth plate chondrocytes. Ten-microgram samples were assessed for Col IIa1 expression by primary growth plate chondrocytes.

### Table 1. Primers used for real-time PCR analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<tbody>
<tr>
<td>c-Fos</td>
<td>CAAGCCAGAGACAGATCAACTT</td>
<td>TTTCCCTCTCTTTCAGAGATG</td>
</tr>
<tr>
<td>c-Jun</td>
<td>CGCGGCGCTCCGCGAGTTG</td>
<td>GGATCCAAAGCAGGTTGAGAGA</td>
</tr>
<tr>
<td>Fra-2</td>
<td>GGAGGGCCGAGCTACAGATCGA</td>
<td>GGCATTCTGGTTCCTTCAAG</td>
</tr>
<tr>
<td>Jun-B</td>
<td>GACGGCCGCTTCTTGTGCAA</td>
<td>GCCTCATGTTGCTATCTT</td>
</tr>
<tr>
<td>Jun-D</td>
<td>CGATTCCGCGCTTACTTTATGTTT</td>
<td>ACAACACTGAAACGCAACCA</td>
</tr>
<tr>
<td>Col II</td>
<td>CGAGGCTGCCATGATGTTAGA</td>
<td>TGTTTGTCGACGACTCCT</td>
</tr>
<tr>
<td>GAPDH</td>
<td>CAGCGCCCCTGCGGTTCCTA</td>
<td>GGCGAGAGACGTAATC</td>
</tr>
<tr>
<td>Sox-6</td>
<td>TGGAACAACTCCGCTCCAAAGAA</td>
<td>TGCAAGGCGTTGCTTGTCG</td>
</tr>
<tr>
<td>Sox-9</td>
<td>AGGGCGGCGCTCCTGCAAAADC</td>
<td>GCCTCGTCGCTTCTT</td>
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Col II, collagen type II.
expression using an inverted fluorescence microscope prior to RNA and protein preparation, and the transfection rate was found to be ~80% as assessed by the number of GFP-expressing cells attached to the dishes.

Luciferase reporter constructs were transfected into cells plated at a density of 100,000 cells/cm² in 24-well plates grown overnight before transfection, using Lipofectamine and Plus reagents (Gibco-BRL/Invitrogen) as described previously (14). For assays using siRNA to block the rise in mRNAs encoding AP-1 factors, chondrocytes were plated in six-well plates and maintained overnight in DMEM with 10% FBS. The following day the cells were transfected with 1 µg of siRNA using 100 µl siRNA transfection reagent from Santa Cruz Biotechnology according to the manufacturer’s instructions. Control transfections were performed in the presence of control siRNA, also from Santa Cruz Biotechnology. Twenty-four hours after transfection cells were treated with either vehicle or 10 µM isoproterenol, followed by extraction to assess mRNA and protein levels of AP-1 factors. Cells were stimulated for 8 h with vehicle or 10 µM isoproterenol to assess the levels of Col II protein.

Cell lysate preparation and Western blotting. Following treatment, chondrocytes were rinsed in ice-cold PBS and lysed in buffer containing 50 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EGTA, 1% Nonidet P-40, and protease inhibitor cocktail (Roche Diagnostics, Laval, QC, Canada) on ice for 30 min. Cleared lysates were assessed for protein concentrations by an amido black protein assay, using bovine serum albumin as a standard (22). Proteins were separated and Western blots performed as outlined previously (14) using a secondary antibody from Cell Signaling Technology (Beverly, MA) and visualized by ECL enhanced chemiluminesence solution (GE Healthcare). Protein band intensities were quantified by scanning densitometry using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

RESULTS

β₂-AR regulation of col II mRNA and protein is mediated by stimulation of mitogen-activated protein kinase ERK1/2 and PKA in growth plate chondrocytes. Col II is expressed in proliferating and prehypertrophic chondrocytes. To examine the time course of Col II inhibition in primary chondrocytes, RNA and protein were extracted from cells at various times following addition of the β-adrenergic receptor agonist isoproterenol. Col II mRNA levels were significantly inhibited after 2 h of isoproterenol stimulation and were maximally inhibited by 64 ± 5% in 8 h and remained inhibited <24 h (Fig. 1A). The inhibition of Col II mRNA was dose dependent with significant inhibition with doses of isoproterenol between 0.1 and 10 µM (Fig. 1B). Inhibition of Col II protein was most evident after 8 h of stimulation by isoproterenol and also remained suppressed over 24 h (Fig. 1C).

We have demonstrated previously that stimulation of primary chondrocytes with isoproterenol results in stimulation of adenyl cyclase and ERK1/2 MAPK (15). To determine which of these two signal transduction pathways is involved in Col II inhibition, cells were stimulated by isoproterenol in the presence of 5 µM H-89, a selective PKA inhibitor, 5 µM U-0126, a selective...
A number of studies have demonstrated that signaling pathways are required for effects of the adrenergic receptor antagonist propranolol completely blocked the effect of isoproterenol on Col II mRNA (Fig. 2A). The presence of the PKA inhibitor H-89 partially inhibited isoproterenol stimulation of this reporter. mRNAs encoding a number of AP-1 factors of the Fos and Jun families were examined in chondrocytes treated for 1 or 2 h with isoproterenol (Fig. 3B). β2-AR stimulation increased the levels of mRNAs encoding c-Fos, Fra-1, Fra-2, c-Jun, and Jun-B but had no effect on the level of Jun-D. Corresponding increases in the various AP-1 proteins were seen 2 h after isoproterenol stimulation of the cells, with c-Fos and Jun-B showing the most robust responses (Fig. 3C). The stimulation of the various AP-1 factors by isoproterenol was partially inhibited in the presence of MEK1 and PKA inhibitors and completely blocked when both inhibitors were added together to the cells (Fig. 3D).

Isoproterenol stimulation increases AP-1 factors and stimulates AP-1 activity. A number of studies have demonstrated important roles for AP-1 factors in the regulation of chondrocyte differentiation (7, 8). Since the expression of AP-1 proteins is regulated by PKA and MAPK pathways (1), we examined whether isoproterenol could regulate AP-1 activity in the chondrocytes in culture. As shown in Fig. 3A, isoproterenol treatment for 16 h increased the luciferase activity of a reporter containing six AP-1 binding sites threefold. This stimulation was inhibited by the addition of propranolol or the MEK1 inhibitor U-0126, whereas the PKA inhibitor H-89 partially inhibited isoproterenol stimulation of this reporter. mRNAs encoding a number of AP-1 factors of the Fos and Jun families were examined in chondrocytes treated for 1 or 2 h with isoproterenol (Fig. 3B). β2-AR stimulation increased the levels of mRNAs encoding c-Fos, Fra-1, Fra-2, c-Jun, and Jun-B but had no effect on the level of Jun-D. Corresponding increases in the various AP-1 proteins were seen 2 h after isoproterenol stimulation of the cells, with c-Fos and Jun-B showing the most robust responses (Fig. 3C). The stimulation of the various AP-1 factors by isoproterenol was partially inhibited in the presence of MEK1 and PKA inhibitors and completely blocked when both inhibitors were added together to the cells (Fig. 3D).

β2-AR stimulation of Jun-B plays a role in inhibition of Col II. To determine whether the stimulation of any of the AP-1 factors by isoproterenol played a role in β2-AR-mediated inhibition of Col II, cells were treated with isoproterenol in the presence of siRNA for each of the stimulated AP-1 factors. As shown in Fig. 4A, inhibition of Jun-B significantly abrogated the effect of isoproterenol on Col II mRNA, whereas inhibition of c-Fos, Fra-1, Fra-2, or c-Jun was without effect.

We next assessed whether overexpression of Jun-B in chondrocytes had any effect on Col II expression. When chondrocytes were transfected with cDNA encoding Jun-B the level of Col II mRNA was inhibited, whereas overexpression of c-Fos was without effect (Fig. 5A). Both AP-1 factors were similarly regulated by PKA and MAPK pathways (1), we examined whether isoproterenol could regulate AP-1 activity in the chondrocytes in culture. As shown in Fig. 3A, isoproterenol treatment for 16 h increased the luciferase activity of a reporter containing six AP-1 binding sites threefold. This stimulation was inhibited by the addition of propranolol or the MEK1 inhibitor U-0126, whereas the PKA inhibitor H-89 partially inhibited isoproterenol stimulation of this reporter. mRNAs encoding a number of AP-1 factors of the Fos and Jun families were examined in chondrocytes treated for 1 or 2 h with isoproterenol (Fig. 3B). β2-AR stimulation increased the levels of mRNAs encoding c-Fos, Fra-1, Fra-2, c-Jun, and Jun-B but had no effect on the level of Jun-D. Corresponding increases in the various AP-1 proteins were seen 2 h after isoproterenol stimulation of the cells, with c-Fos and Jun-B showing the most robust responses (Fig. 3C). The stimulation of the various AP-1 factors by isoproterenol was partially inhibited in the presence of MEK1 and PKA inhibitors and completely blocked when both inhibitors were added together to the cells (Fig. 3D).

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![Fig. 3. β2-AR stimulation of activator protein 1 (AP-1) proteins and activity. A: primary chondrocytes were transfected with a luciferase reporter containing 6 consensus AP-1 binding sites and cDNA encoding β-galactosidase (β-Gal). Twenty-four hours later the cells were treated with vehicle (Con), with 1 μM Iso, or with Iso and 1 μM Pro, Iso, and 5 μM PKA inhibitor (H-89). Iso and 5 μM MEK1 inhibitor (U-0126), or Iso and H-89 plus U-0126 for 16 h before extraction and assay of luciferase activity. Relative light unit values were corrected for β-Gal activity in each sample. B: cells were stimulated with Con or 1 μM Iso for either 1 or 2 h before extraction and assay of mRNA levels of the indicated AP-1 factors by RT-PCR. C: cells treated with either Con or 1 μM Iso for 2 h were extracted and the levels of the indicated AP-1 proteins assessed in 30-μg samples of total cell extract by Western blot using specific antibodies. D: cells were stimulated with 1 μM Iso for 1 h or with Iso and 5 μM H-89, Iso and 5 μM U-0126, or Iso and H-89 plus U-0126 before extraction and assay of mRNA levels of the indicated AP-1 factors by RT-PCR. RQ values are calculated relative to values from control samples treated without Iso. Values in A and B represent means ± SD; *significant difference from the control sample (P < 0.01), #significant difference from cells treated with Iso alone (P < 0.05). Values in D represent means ± SD; **significant difference from the Iso alone sample (P < 0.01).](http://ajpendo.physiology.org/Download?uri=10.1152/ajpendo.00757.2010)
overexpressed (Fig. 5B) and able to stimulate an AP-1 luciferase reporter cotransfected into the cells (Fig. 5C). Together these data suggest a specific role for Jun-B in mediating β2-AR inhibition on Col II in chondrocytes.

Jun-B inhibits Sox-6 expression and inhibits Col II promoter activity. Stimulation of chondrocytic ATDC5 cells by adrenaline has been reported to inhibit expression of the sex-determining region of Y high-mobility group-1 (HMG) box transcription factor Sox-6 (26). Since Sox proteins are important regulators of collagen II expression in growth plate chondrocytes (16), we examined whether isoproterenol regulated Sox-9 or Sox-6 expression in our chondrocyte cultures. As shown in Fig. 6, A and B, Sox-6 mRNA and protein levels were inhibited after isoproterenol treatment, and this was attenuated by siRNA for Jun-B but unaffected by siRNA for c-Fos. Sox-9 mRNA levels were not affected by any of these treatments (data not shown).

Transfection of the chondrocytes with a Col II promoter construct containing four copies of a 48-base pair Col 2a1 intron I sequence reported to respond to Sox proteins (31) was inhibited following overexpression of Jun-B, whereas cotransfection with c-Fos did not affect the activity of this reporter construct (Fig. 6C). Similarly, Jun-B but not c-Fos overexpression significantly inhibited the level of Sox-6 mRNA in the cells (Fig. 6D).

**DISCUSSION**

Adrenergic receptors expressed on growth plate chondrocytes have the potential to regulate endochondral bone formation. Stimulation of β2-ARs expressed in proliferative and prehypertrophic chondrocytes was shown here to inhibit Col II expression. Col II is the predominant matrix protein expressed by chondrocytes throughout the growth plate (13). Although downregulation of Col II is seen when chondrocytes dedifferentiate in monolayer culture, the effect of isoproterenol was sufficiently rapid to suggest more direct inhibition of Col II expression. To explore the mechanisms by which β2-AR may regulate Col II, we utilized pharmacological and molecular inhibitors of well-characterized targets of β2-AR stimulation, PKA and ERK1/2 (18, 19, 23). We have demonstrated previously that activation of β2-AR increases ERK1/2 phosphorylation and also elevates levels of cAMP (15). Inhibition of Col II expression through the β2-AR was partially mediated by both of these pathways. This apparently synergistic effect of PKA and ERK1/2 activations was consistent with previous studies showing inhibition of chondrocyte growth and differentiation by FGF receptor 3 activation through phosphorylation of ERK1/2 (12, 20, 30) and parathyroid hormone-related peptide inhibition of chondrocyte differentiation mediated through activation of adenylyl cyclase (2, 6, 21).
Downstream of PKA and ERK1/2 synthesis, a number of AP-1 factors were rapidly stimulated by the β2-ARs. The role of AP-1 factors in endochondral bone formation is not well understood. Deletion or overexpression of several AP-1 factors in mice results in skeletal phenotypes; however, these primarily relate to development or activity of osteoblasts and osteoclasts in bone (reviewed in Ref. 29). In osteoblasts, β2-ARs are reported to stimulate c-Fos expression, resulting in increased cyclin D1 and promotion of osteoblast proliferation (5). In the chondrocytic ATDC5 cell line, exogenous c-fos expression resulted in increased cell growth and downregulation of Col II (28). Similarly, human chondrosarcoma cells overexpressing c-fos showed decreased matrix synthesis (31). Expression of exogenous c-fos in our cells did not have any effect on Col II mRNA, although the level of c-Fos overexpression in our cells appeared to be much less than that seen in the two studies using chondrocytic cell lines, which may reflect differences in the level of transfection or ability to express exogenous proteins between primary chondrocytes and established chondrocytic cell lines. An additional difference between our study and that in ATDC5 cells was the induction of c-Jun in ATDC5 cells overexpressing c-fos; we did not see any increase in c-Jun or Jun-B mRNA in primary chondrocytes expressing exogenous c-fos. Because Fos proteins do not form functional homodimers, it is possible that the more discrete expression of c-Fos in our cells was not able to regulate Col II on its own. However, cotransfection of c-fos with the AP-1 reporter did show a robust increase in luciferase activity, suggesting that enough endogenous Jun proteins are expressed in these cells to stimulate a consensus AP-1 binding sequence. More relevant to the effects of β2-ARs were the studies in which isoproterenol stimulation of each AP-1 factor was attenuated by siRNA. These experiments indicated that Jun-B was a primary mediator of adrenergic inhibition of Col II in primary chondrocytes. Previous work in mice has shown a role for Jun-B in chondrocyte proliferation in that junB−/−/Ubi-junB mice, which have very low levels of Jun-B expression in bone, have shortened growth plates with reduced numbers of proliferative and prehypertrophic chondrocytes (7). We have reported previously that isoproterenol has a modest growth stimulatory effect on growth plate chondrocytes in culture, and it is possible that the stimulation of Jun-B contributed to that increase in growth. However, this remains to be determined.

Having established a role for Jun-B in adrenergic inhibition of Col II in primary chondrocytes, we wished to explore further a possible mediator of this regulation. The most well-characterized regulation of the Col2a1 gene is the stimulation of a 48-bp enhancer sequence from the first intron that is stimulated by the HMG box protein trio of Sox-9, Sox-6, and L-Sox-5 (16). Previous studies in ATDC5 cells suggested that Sox-6 was downregulated by adrenaline (26), and we also found similar decreases in
Sox-6 mRNA and protein in primary chondrocytes stimulated with isoproterenol, and this was significantly attenuated by knockdown of Jun-B. Expression of exogenous jun-B mimicked the effect of isoproterenol on Sox-6 mRNA and was also able to inhibit luciferase activity driven by four copies of the 48-bp enhancer sequence from the Col2a1 intron. There are no consensus AP-1 binding sites within the enhancer (32), and therefore, it seems unlikely that Jun-B directly regulates the activity of this reporter, but rather, inhibition was secondary to the downregulation of Sox-6 in the cells. Previous work in articular chondrocytes has shown a role for c-Jun as a downstream mediator of interleukin-1β repression of Col II expression that also involved inhibition of the Col 2a1 intron 1 enhancer sequence (9). Together with our work reported here, this suggests that several stimulators of Jun family proteins are capable of downregulating Col II in chondrocytes and that this repression may be mediated at the level of Sox protein regulation of the Col 2a1 enhancer.

In summary, our study has demonstrated that β2-ARs expressed by primary mouse chondrocytes can inhibit the expression of Col II. We have demonstrated that both PKA and ERK1/2 stimulation mediate this effect and that stimulation of the early response gene Jun-B plays a primary role in Col II inhibition. Evidence that Jun-B stimulation can inhibit expression of Sox-6 protein in chondrocytes suggests that this may constitute the primary pathway by which Col II expression is downregulated. Future studies are required to determine the link between Jun-B and Sox-6 gene regulation. Our studies add further evidence of a role for adrenergic receptors expressed in the growth plate in the regulation of chondrocyte matrix protein secretion.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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