Journal of Cellular Biochemistry 89:556–562 (2003)

ATP Modulates Load-Inducible IL-1β, COX 2, and MMP-3 Gene Expression in Human Tendon Cells

M. Tsuzaki,[1]* D. Bynum,[1] L. Almekinders,[1] X. Yang,[1] J. Faber,[2] and A.J. Banes[1,3,4,5]

[1]Department of Orthopaedics, University of North Carolina, Chapel Hill, North Carolina 27599
[2]Department of Cell and Molecular Physiology, University of North Carolina, Chapel Hill, North Carolina 27599
[3]Department of Biomedical Engineering, University of North Carolina, Chapel Hill, North Carolina 27599
[4]Curriculum in Applied and Materials Sciences, University of North Carolina, Chapel Hill, North Carolina 27599
[5]Flexcell International Corp., Hillsborough, North Carolina 27278

Abstract Tendon cells receive mechanical signals from the load bearing matrices. The response to mechanical stimulation is crucial for tendon function. However, overloading tendon cells may deteriorate extracellular matrix integrity by activating intrinsic factors such as matrix metalloproteinases (MMPs) that trigger matrix destruction. We hypothesized that mechanical loading might induce interleukin-1beta (IL-1β) in tendon cells, which can induce MMPs, and that extracellular ATP might inhibit the load-inducible gene expression. Human tendon cells isolated from flexor digitorum profundus tendons (FDPs) of four patients were made quiescent and treated with ATP (10 or 100 μM) for 5 min, then stretched equibiaxially (1 Hz, 3.5% elongation) for 2 h followed by an 18-h-rest period. Stretching induced IL-1β, cyclooxygenase 2 (COX 2), and MMP-3 genes but not MMP-1. ATP reduced the load-inducible gene expression but had no effect alone. A medium change caused tendon cells to secrete ATP into the medium, as did exogenous UTP. The data demonstrate that mechanical loading induces ATP release in tendon cells and stimulates expression of IL-1β, COX 2, and MMP-3. Load-induced endogenous IL-1β may trigger matrix remodeling or a more destructive pathway(s) involving IL-1β, COX 2, and MMP-3. Concomitant autocrine and paracrine release of ATP may serve as a negative feedback mechanism to limit activation of such an injurious pathway. Attenuation or failure of this negative feedback mechanism may result in the progression to tendinosis. J. Cell. Biochem. 89: 556–562, 2003.

Key words: cyclic loading; tendon cells; IL-1β; MMPs; ATP

Tendinosis is a common disorder characterized by acute or chronic pain and degenerative change in the matrix [Jozsa and Kannus, 1997; Smith, 2000]. The process is a progressive loss of biomechanical integrity followed by rupture. Histologic examination of ruptured tendons reveals a degenerative disorganization of collagen fibers and necrotic lesions, without white cell infiltration, acute inflammation, or matrix remodeling [Jozsa and Kannus, 1997]. However, matrix degeneration may have been preceded by more subtle changes involving proinflammatory cytokine release by fibroblasts. Although excessive exercise and occupational repetitive motion have been implicated as causes [Carpenter et al., 1998, 1999], the etiology of the disorder and underlying molecular mechanism(s) are unknown. Non-steroidal anti-inflammatory drugs have been used to treat the painful symptoms.

Activated matrix metalloproteinases (MMPs) such as MMP-3 (stromelysin 1), which degrades aggrecan, and MMP-1 (collagenase 1) and MMP-2 (gelatinase A), which degrade collagens, are thought to be responsible for matrix degeneration [Van der Zee et al., 1996; Saito et al., 1998; Riley et al., 2002]. MMPs are a

Grant sponsor: NIH; Grant numbers: AR 38121, AR 45833; Grant sponsor: NIH-NHLBI; Grant number: HL62584; Grant sponsor: Hunt Foundation, Merck, Inc.; Grant sponsor: Flexcell International Corp.

*Correspondence to: Dr. M. Tsuzaki, DDS, PhD, 277 Burnett-Womack Bldg. CB# 7055, Department of Orthospaedics, University of North Carolina, Chapel Hill, NC 27599-7055. E-mail: Mari_Tsuzaki@med.unc.edu

Received 11 December 2002; Accepted 3 March 2003
DOI 10.1002/jcb.10534

family of at least 16, zinc-dependent enzymes essential for matrix turnover in physiologic remodeling and pathological conditions [Birkedal-Hansen et al., 1993]. Inflammatory cells including macrophages and monocytes release MMPs, which can also be induced by cytokines such as interleukin 1-beta (IL-1β) [Ries and Petrides, 1995; Saito et al., 1998; Van der Zee et al., 1996]. We have reported that IL-1β induced MMP-1, -3, and -13 in tendon cells in vitro and ex vivo [Archambault et al., 2002a,b; Tsuzaki et al., 2003]. MMPs are commonly secreted into the extracellular matrix as inactive proenzymes where they are complexed with specific inhibitors (tissue inhibitors of metalloproteinases, TIMPs) [Gomez et al., 1997]. Activation of MMPs involves another class of enzymes including plasminogen and plasminogen activator [Saito et al., 1998; Van der Zee et al., 1996].

IL-1β is a potent proinflammatory cytokine produced by macrophages and monocytes [Bankers-Fulbright et al., 1996], and has been detected at sites of acute and chronic inflammation in osteoarthritic cartilage [Moos et al., 1999]. IL-1β is capable of inducing diverse genes by acting on numerous signaling pathways [Guy et al., 1995; Bankers-Fulbright et al., 1996]. We have found that IL-1β mRNA is induced in tendon cells by exogenous IL-1β as well as mechanical loading, suggesting that endogenous IL-1β may establish a positive feedback loop in triggering fibroblast-mediated cytokine–MMP matrix destruction [Tsuzaki et al., 2003].

Adenosine 5′-triphosphate (ATP) is released from many cell types, including chondrocytes and disc annulus cells, by chemical or mechanical stimuli where it binds to specific cell surface receptors [El-Moatassim et al., 1992; Bodin and Burnstock, 1996; Graff et al., 2000; Yagutkin et al., 2000; Yamazaki et al., 2001]. Tendon cells express P2Y purinoceptor subtypes and respond to ATP with an increase in intracellular calcium ($[Ca^{2+}]_{ic}$) [Banes et al., 1995a, 2001]. Purinergic signaling, therefore, may be involved in mechanoreception and signal transduction in tendon cells.

We hypothesized that IL-1β may be induced in tendon cells after mechanical loading and precede the gross degenerative changes by inducing matrix degrading enzymes. In addition, motion may stimulate release of ATP from tendon cells to modulate or block a load response in a proposed signal-dampening pathway.

MATERIALS AND METHODS

Tendon Cell Isolation and Primary Culture

Flexor digitorum profundus tendons (FDPs) from four patients (26y ♂, 27y ♀, 37y ♂, 50y ♂, the UNC Memorial Hospitals) were obtained as discarded tissue after surgery. Cells were isolated by sequential enzymatic and mechanical treatment [Banes et al., 1988a,b]. Briefly, tissue was treated with trypsin (0.25%, 15 min) and collagenase (0.1%, 10 min), to release matrix rich in surface epitenon cells (TSC). After plating in complete medium below for 1 h, rapidly adhering TSC were separated from non-adherent cells. The remaining tissue was debrided of residual epitenon, minced, and incubated in collagenase (0.1%, 5 min) to release matrix rich in internal fibroblasts (TIF). After plating for 1 h, non-adhering cells were replated as the TIF population. TSC and TIF were grown in complete medium (DMEM with 10% fetal calf serum (FCS), 20 mM HEPES, pH 7.2, 0.1 mM ascorbate-2-PO_4, 100 U/ml penicillin and 100 μg/ml streptomycin) and used at passages 3 to 6.

ATP Pretreatment and Stretching Tendon Cells

A Flexercell® Strain Unit (Flexcell® International Co., McKeesport, PA) was used with 31 mm diameter cylindrical loading posts to apply an equibiaxial cyclic strain. TSC and TIF from each patient were seeded at 25k cells/cm^2 in collagen I-coated flexible-bottomed plates (BioFlex® culture plates, Flexcell International Co.) in complete medium, then growth-arrested by halving the medium with serum-free DMEM on days 3 and 5. On day 6, medium was changed to fresh serum-free medium, and the quiescent cells were incubated for 3 h at 37°C to allow ATP in the medium to reach basal levels. Cells then received ATP (2 μl) at final concentrations of 10 or 100 μM for 5 min before mechanical loading. Cells were stretched at 1 Hz with 3.5% elongation for 2 h, followed by an 18-h rest. Replicate cultures included groups of no-treatment/no-load as a negative control, ATP (100 μM) alone, mechanical load alone, and IL-1β alone (100 pM) as a positive control.

RT-PCR

Cell samples were collected (3 wells per sample) in 4 M guanidinium thiocyanate

denaturing buffer. Cell lysates were passed sequentially through shredder and RNA separation columns (Qiagen, Valencia, CA) to isolate total RNA. Total RNA was DNase-digested, phenol-chloroform purified, and the purity verified spectrophotometrically (A260/A280 ~1.9). Oligonucleotide primer sets specific for human sequences were synthesized for each gene of interest [Tsuzaki et al., 2003]. Total RNA (1 μg) was reverse-transcribed, amplified by PCR conditioned with a hot start, denatured at 94°C, extended at 72°C, and annealed at 52°C for 20, 25, 30, and 35 cycles (Perkin Elmer, Branchburg, NJ) to determine the linear range of the assay. Amplified cDNAs were separated in 1.8% agarose gels containing ethidium bromide. The cDNA band with expected size was digitized and quantified (Image I, Universal Imaging Co., West Chester, PA), and density normalized to that of β-actin.

ATP Luciferin–Luciferase Bioluminescence Assay

Quiescent tendon cells were washed with PBS, the medium replaced with serum-free, phenol red-free DMEM and incubated for 3 h in the baseplate of the strain unit without stretch to allow extracellular ATP to decrease to baseline levels. One hundred microliters of the supernatant fluid of each culture were collected at times, 0, 1, 2, 5, 10, 15, and 30 min after onset of cell stretching without stirring the culture or touching the cells. Samples from UTP (100 μM)-stimulated cells were collected at the same times. Cells were pretreated with $GdCl_3$ (20 μM) to block stretch-activated channels [Naruse et al., 1998] or suramin (100 μM) to block purinoceptors [Ostrom et al., 2000] for 30 min. Samples were placed immediately on ice, heated at 70°C for 5 min to inactivate ATPases, and stored at −20°C. ATP concentrations in the samples ([ATP]) were determined by a luciferin–luciferase bioluminescence assay in a 96-well, white microtiter plate using a series of ATP standards, plus medium and chemical reagent blanks. A luciferin–luciferase reagent was added to each well and 562 nm emission was read using a luminometer (Labsystems, Helsinki, Finland) and spectrophotometry program (Fluoroskan Ascent, FL).

Statistical Analysis

Statistical analyses were performed using SigmaStat software (SPSS Scientific, Chicago, IL). Significance ($P < 0.05$) was determined by one way repeated measures analysis of variance (ANOVA) with Dunnett's method or *t*-tests.

RESULTS

Normal quiescent human tendon cells, TSC or TIF, did not express detectable IL-1β, COX 2, MMP-1 or -3 mRNA by RT-PCR analysis, while 100 pM IL-1β stimulated robust expression (Fig. 1). ATP (100 μM) alone did not induce any of these genes (Fig. 1). Based on the initial result from determination of optimal PCR conditions by incrementing the cycle numbers and assessing cDNA band strength, up to 30 PCR cycles were used to detect each gene induction within the linear range of the amplification. In addition, we examined mRNA expression of TIMPs (Fig. 1). TIMP-1 and -2 were constitutively expressed in tendon cells, and IL-1β did not alter their expression.

Stretching tendon cells with 3.5% elongation at 1 Hz for 2 h induced expression of IL-1β, COX

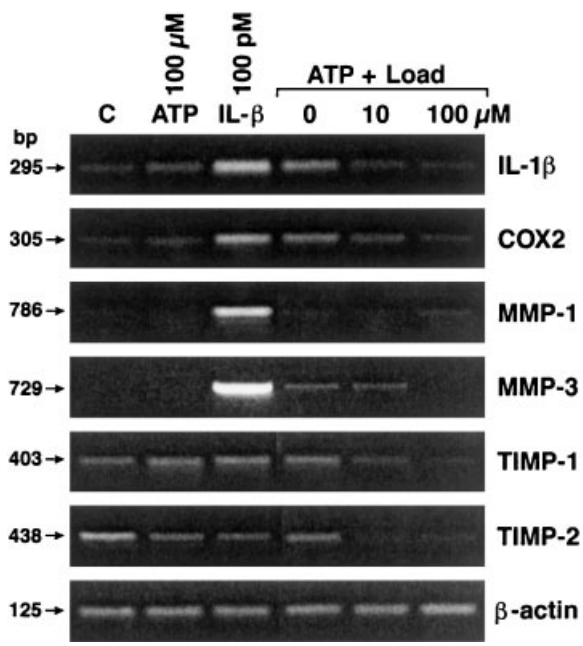

Fig. 1. Effect of mechanical load and ATP on inflammatory gene expression in human tendon cells. Representative mRNA expression of IL-1β, COX 2, MMP-1, -3, and TIMP-1, -2 in quiescent TSC. Mechanical loading for 2 h induced IL-1β, COX 2, and MMP-3 mRNA expression in human tendon cells. ATP alone had no effect, but reduced the subsequent load induction of these genes. C, no-treatment/no-load control; 100 pM IL-1β is the positive control; ATP + Load, mechanical loading for 2 h with 0, 10, or 100 μM ATP treatment. PCR cycle numbers used for each target mRNA were; 30 cycles for IL-1β, COX 2, MMP-1 and -3, 20 cycles for TIMP-1 and -2; and 25 cycles for β-actin.

2, and MMP-3 mRNA, but not MMP-1 mRNA (Fig. 1). Treatment of cells with ATP at 10 or 100 μM for 5 min reduced the subsequent load-induction of IL-1β, COX 2, and MMP-3 mRNA expression. Mechanical stretching did not increase TIMP-1 or -2 mRNA expression.

In ATP-treated and loaded cultures, IL-1β mRNA expression was decreased by 10 or 100 μM ATP treatment to approximately 50 and 30%, respectively, of the level induced by stretching alone (Fig. 2). Down-regulation of COX 2 and MMP-3 mRNA expression by ATP and load was similar to that for IL-1β (Fig. 2). Message levels for the $P2Y_2$ and IL-1 R1 receptors were not affected by ATP (1, 10, or 100 μM) (data not shown).

Both tendon surface-derived TSC and more internal fibroblasts (TIF) constitutively secreted ATP into the medium at a basal level (0.5–1 nM). Secretion increased in response to changing the medium, and this remained high at the initiation of the experiment (time 0) (Fig. 3). Stretching cells caused ATP concentration to decline with time. The values for the mechanical load effect on ATP release were significantly greater than the baseline at 30 min ($P < 0.05$), though they were not significantly so when compared to those at time 0 (Fig. 3). UTP (100 μM) induced a rapid and robust increase in ATP release in both cell types (Fig. 3). UTP-stimulated ATP secretion was suppressed by suramin ($P < 0.05$), an inhibitor of purinoceptors, and load-stimulated ATP secretion was totally blocked by Gd^{3+} ($P < 0.05$), a stretch-activated channel blocker (Fig. 4). Intracellular

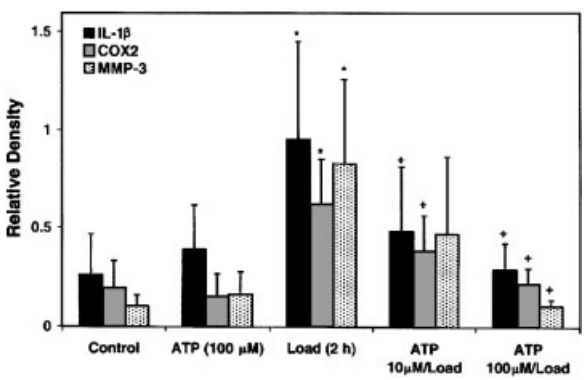

Fig. 2. ATP inhibits load-inducible IL-1β, COX2, and MMP-3 mRNA expression in human tendon cells. Data are expressed as relative densities of the cDNA PCR amplimer band to that of β-actin (n = 4 for four cultures, each from a different patient). Values are mean ± SD, *significantly different from control, + significantly different from load only ($P < 0.05$).

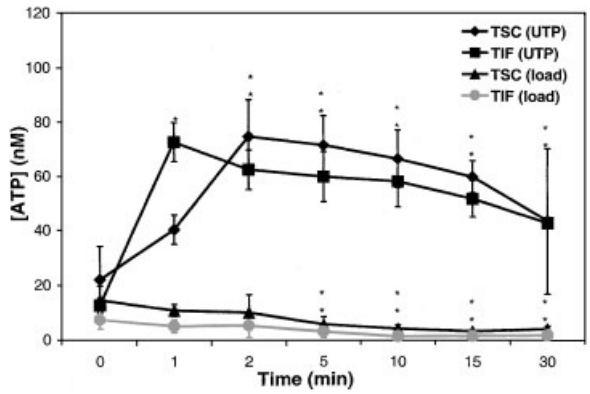

Fig. 3. ATP secretion in resting human tendon cells is decreased by load and by UTP. Data are concentration of ATP in the medium assayed at various times in cells (TSC, tendon surface cells; TIF, tendon internal fibroblasts) that have been stretched equibiaxially (Load) or following a treatment with 100 μM UTP. Values are mean ± SD (n = 3–6), *significantly different from time 0 control ($P < 0.05$).

[ATP] of resting human tendon cells was approximately 1 mM.

DISCUSSION

Cyclic loading induced expression of IL-1β, COX 2, and MMP-3 in human tendon cells, as has been reported for other connective tissues such as cartilage [Fujisawa et al., 1999] and periodontal ligament [Shimizu et al., 1994, 1998]. Recently, Archambault and coworkers have shown that fluid flow can induce MMP-3 in rabbit tendon cells in vitro [Archambault et al., 2002b]. These responses may be mediated by

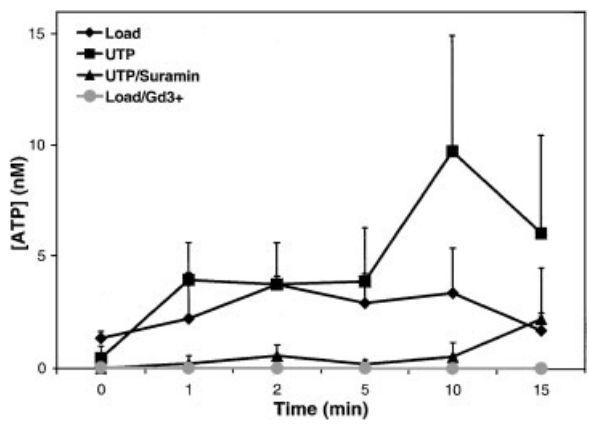

Fig. 4. ATP release from TSC in response to load or UTP. Inhibition by Gd^{3+} or suramin. Tendon cells (TSC) were incubated for 30 min with $GdCl_3$ (20 μM) or suramin (100 μM) in the medium before stretching or UTP (100 μM) treatment, respectively. Suramin inhibited ATP release in response to UTP ($P < 0.05$). Gadolinium completely blocked ATP secretion from stretched tendon cells ($P < 0.05$).

membrane receptors to autocrine/paracrine factors, ion channels, cell-matrix contacts, and other mechanisms [Banes et al., 1995a, 2001]. Extracellular ATP is a candidate molecule involved in load-induced responses since it is released by cells subjected to mechanical stimulation [Hamada et al., 1998; Yellowley et al., 1999; Ostrom et al., 2000].

IL-1β induction in tendon cells by mechanical strain suggests that endogenous IL-1β may play a role in tendon pathogenesis caused by repetitive loading. Tendon cells express IL-1 R1 receptor for IL-1β [Bankers-Fulbright et al., 1996; Tsuzaki et al., 2003]. The IL-1 R1 occupation activates numerous PKC-dependent signal transduction pathways, including p38 mitogen-activated protein kinases (MAPK) such as c-Jun N-terminal kinase (JNK) and stress-activated protein kinase (SAPK), in various cell types and tissues [Guy et al., 1995; Bankers-Fulbright et al., 1996]. IL-1β signaling induces mRNA and protein expression of COX 2, MMP-1, and -3. IL-1β induced PGE_2 release in tendon cells [Tsuzaki et al., 2003]. However, additional studies are required to test the hypothesis that load-induced IL-1β, COX 2, and PGE_2 are responsible for increased expression of MMPs resulting in tendon pathogenesis.

Tendinosis or tendon pain is often preceded by an episode of extensive exercise or injury [Carpenter et al., 1998]. Overloading in conjunction with injury results in more severe damage causing a loss of mechanical properties similar to that observed in the supraspinatus tendon in a rat overuse model [Carpenter et al., 1998; Soslowsky et al., 2000]. Mechanical induction of IL-1β may establish a positive feedback loop of local cytokine and MMP-3 expression by tendon cells. Moreover, MMP-3 can superactivate MMP-1 and accelerate the degradation of the collagen-rich matrix [Ries and Petrides, 1995]. Mechanical loading or IL-1β treatment did not change TIMP-1 or -2 expression, thus the imbalance between MMP and TIMP could favor MMP activity and matrix weakening [MacNaul et al., 1990; Tsuzaki et al., 2003]. With time, this may result in fibroblast-mediated matrix destruction. Microscopic injuries may also cause release of factors such as plasmin that can activate latent MMPs [Saito et al., 1998; Van der Zee et al., 1996].

We found that human tendon cells release ATP in vitro. Moderate stretching did not significantly increase [ATP] in the medium. However, mechanical stimulation by medium changing caused ATP release prior to stretching as reported by others [Watt et al., 1998; Ostrom et al., 2000]. Recent data from our group have shown that human tendon cells express purinoceptors ($P2Y_2$) and respond to extracellular ATP or UTP in vitro with an increase in $[Ca^{2+}]_{ic}$ [Francke et al., 1998]. We also observed that prolonged ATP stimulation caused tendon cells and intervertebral disc annulus cells to become transiently unresponsive to mechanical stimulation failing to propagate a calcium wave [Francke et al., 1998; Minchew et al., 1999]. The mechanism underlying this phenomenon is not clear. ATP-catalyzed extracellular phosphorylation of the receptor does not contribute to the desensitization. Therefore, it may involve a certain intracellular pathway(s) [Nobles and Abbott, 1998]. ATP could also alter the phosphorylation status of proteins by activating protein kinase cascades such as MAPK pathway [El-Moatassim et al., 1992]. Purinergic signaling and receptor desensitization may intersect with mechanotransduction at pathways such as JNK/SAPK [Hamada et al., 1998]. Nevertheless, ATP modulated the subsequent load signal and load-inducible gene expression in tendon cells, resulting in the reduction of IL-1β, COX 2, and MMP-3 expression. We observed that UTP stimulated tendon cells to release ATP robustly. ATP may stimulate more ATP release in a positive feedback loop, since UTP and ATP share certain P2Y class purinoceptors including $P2Y_2$ [Ralevic and Burnstock, 1998]. Although the concentrations of extracellular UTP and pyrimidine nucleotides are not established, UTP may act as a second effector by inducing or amplifying ATP release to transduce signals. Blocking purinoceptors with suramin greatly reduced UTP-stimulated ATP release, implicating a positive feedback loop may be part of the mechanism. Moreover, the stretch-activated channel blocker, Gd^{3+}, completely abrogated load-stimulated release of ATP, implicating stretch-activated channels in the response [Yang and Sachs, 1989; Naruse et al., 1998].

Response to mechanical stimulation is important for cell survival and growth as well as various tissue-specific functions, especially in tissues subjected to continuous mechanical forces such as in muscle, bone, cartilage, tendon, and ligament [Banes et al., 1995b, 2001]. Tendon healing is significantly improved by

motion therapy both biochemically and biomechanically [Koob et al., 1992]. Loss of mechanical stimuli causes atrophy [Hannafin et al., 1995], but overloading may lead to repetitive motion disorder including tendinosis or tendinopathy [Almekinders et al., 1995; Carpenter et al., 1999]. A mechanical signal transduced via ion channels and receptor signaling may activate either catabolic or anabolic pathways, or both at the same time [Banes et al., 1995a,b, 2001; Hannafin et al., 1995]. Therefore, the quality and quantity of mechanical loading may affect the microenvironment of tendon cells to stimulate healing or drive tissue destruction. Alternatively, motion may stimulate ATP release from tendon cells, and the secreted ATP may act in an autocrine/paracrine fashion as a compensatory load-blockade mechanism. Chondrocytes secrete a basal level of ATP (0.2–0.4 nM) and ATP release is increased by cyclic compression [Graff et al., 2000]. Rabbit and human annulus fibrosus cells secrete a basal level of ATP (~1 nM) and release more ATP by vibratory loading at 6 Hz [Yamazaki et al., 2001]. Extracellular ATP can retard collagen gel contraction by MC3T3 E1 osteoblast-like cells in a 3D culture model (Qi and Banes, unpublished).

Ecto-NTPase-catalyzed hydrolysis results in the inactivation of ATP [Zimmermann, 2000]. However, the breakdown products of purine nucleotides, ADP, AMP, and adenosine, are pharmacologically active. Therefore, the action of extracellular ATP in connective tissues may be even more complex. At present, knowledge regarding the receptor sites for the nucleotide ligands in tendon is limited. Our data indicate that connective tissue cells express purinoceptors and respond to ATP by increasing $[Ca^{2+}]_{ic}$, but then are refractory to a subsequent load stimulation and are blocked in gene expression. Taken together, these data indicate that ATP acts as a pulse dampening mechanism to modulate excess load stimuli. Use of ATP and regulation of the P2 class of purinoceptors may be an option in the treatment of tendon disorders.

REFERENCES

Almekinders LC, Banes AJ, Bracey LW. 1995. An in vitro investigation into the effects of repetitive motion and nonsteroidal antiinflammatory medication on human tendon fibroblasts. Am J Sports Med 23:119–123.

Archambault J, Tsuzaki M, Herzog W, Banes AJ. 2002a. Stretch and interleukin-1β induce matrix metalloproteinases in rabbit tendon cells in vitro. J Orthop Res 20:36–39.

Archambault JM, Elfervig MK, Tsuzaki M, Herzog W, Banes AJ. 2002b. Rabbit tendon cells produce MMP-3 in response to fluid flow without significant calcium transients. J Biomech 35:303–309.

Banes AJ, Link GW, Bevin AG, Peterson HD, Gillespie GY, Bynum D, Watts S, Dahners L. 1988a. Tendon synovial cells secrete fibronectin in vivo and in vitro. J Orthop Res 6:73–82.

Banes AJ, Donlon K, Link GW, Gillespie Y, Bevin AG, Peterson HD, Bynum D, Watts S, Dahners L. 1988b. Cell populations of tendon: A simplified method for isolation of synovial cells and internal fibroblasts: Confirmation of origin and biologic properties. J Orthop Res 6:83–94.

Banes AJ, Tsuzaki M, Yamamoto J, Fischer T, Brown T, Miller L. 1995a. Mechanoreception at the cellular level: The detection, interpretation, and diversity of response to mechanical signals. Biochem Cell Biol 73:349–365.

Banes AJ, Tsuzaki M, Hu P, Brigman BE, Brown T, Almekinders L, Lawrence WT, Fischer T. 1995b. PDGF-BB, IGF-I and mechanical load stimulate DNA synthesis in avian tendon fibroblasts in vitro. J Biomech 28:1505–1513.

Banes AJ, Lee G, Graff R, Otey C, Archambault J, Tsuzaki M, Elfervig M, Qi J. 2001. Mechanical forces and signaling in connective tissue cells: Cellular mechanisms of detection, transduction, and responses to mechanical deformation. Curr Opin Orthop 12:389–396.

Bankers-Fulbright JL, Kalli KR, McKean DJ. 1996. Interleukin-1 signal transduction. Life Sciences 59:61–83.

Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, Engler JA. 1993. Matrix metalloproteinases: A review. Crit Rev Oral Biol Med 4:197–250.

Bodin P, Burnstock G. 1996. ATP-stimulated release of ATP by human endotherial cells. J Cardiovasc Pharmacol 27:872–875.

Carpenter JE, Flanagan CL, Thomopoulos S, Yian EH, Soslowsky LJ. 1998. The effects of overuse combined with intrinsic and extrinsic alterations in an animal model of rotator cuff tendinosis. Am J Sport Med 26:801–807.

Carpenter JE, Thomopoulos S, Soslowsky LJ. 1999. Animal model of tendon and ligament injuries for tissue engineering applications. In: Section V, editor. Tendon/Ligament. 367: Clin Orthop Rel Res. S296–S311.

El-Moatassim C, Dornand J, Mani J-C. 1992. Extracellular ATP and cell signalling. Biochim Biophys Acta 1134:31–45.

Francke E, Stood A, Kenamond C, Faber J, Boitano S, Bynum D, Yang X, Sanderson M, Banes AJ. 1998. ATP stimulates an increase in cellular calcium in human tendon cells via purinergic receptors and temporally blocks gap junction signaling. 44th Annual Orthopaedic Research Society Meeting, (New Orleans, LA); # 91.

Fujisawa T, Hattori T, Takahashi K, Kuboki T, Yamashita A, Takigawa M. 1999. Cyclic mechanical stress induces extracellular matrix degradation in cultured chondrocytes via gene expression of matrix metalloproteinases and interleukin 1. J Biochem 125:966–975.

Gomez DE, Alonso DF, Yoshiji H, Thorgeirsson UP. 1997. Tissue inhibitors of metalloproteinases: Structure, regulation, and biological functions. Eur J Cell Biol 74: 111–122.

Graff RD, Lazarowski ER, Banes AB, Lee GM. 2000. ATP release by mechanically loaded porcine chondrons in pellet culture. Arthritis Rheum 43:1571–1579.

Guy GR, Philp R, Tan YH. 1995. Activation of protein kinases and the inactivation of protein phosphatase 2A in tumour necrosis factor and interleukin-1 signal-transduction pathways. Eur J Biochem 229:503–511.

Hamada K, Takuwa N, Yokoyama K, Takuwa Y. 1998. Stretch activates Jun N-terminal kinase/stress-activated protein kinase in vascular smooth muscle cells through mechanisms involving autocrine ATP stimulation of purinoceptors. J Biol Chem 273:6334–6340.

Hannafin JA, Arnoczky SP, Hoonjan A, Torzilli PA. 1995. Effect of stress deprivation and cyclic tensile loading on the material and morphologic properties canine flexor digitorum profundus tendon: An in vitro study. J Orthop Res 13:907–914.

Jozsa LG, Kannus P. 1997. Overuse injuries of tendons. In: Human tendons. Anatomy, physiology, and pathology, Part III. Champaign: Human Kinetics. pp 164–253.

Koob TJ, Clark PE, Hernandez DA, Thurmond FA, Vogel KG. 1992. Compression loading in vitro regulates proteiglycan synthesis by tendon fibrocartilage. Arch Biochem Biophys 298:303–312.

MacNaul KL, Chartrain N, Lark M, Tocci MJ, Hutchinson NI. 1990. Discoordinate expression of stromelysin, collagenase, and tissue inhibitor of metalloproteinases-1 in rheumatoid human synovial fibroblasts. Synergistic effects of interleukin-1 and tumor necrosis factor-alpha on stromelysin expression. J Biol Chem 265:17238–17245.

Minchew JT, Elfervig MK, Sood A, Tsuzaki M, Weinhold PS, Banes AJ. ATP transiently blocks mechanical load responsiveness in human annulus cells. Spine (accepted).

Moos V, Fickert S, Muller B, Weber U, Sieper J. 1999. Immunohistological analysis of cytokine expression in human osteoarthritic and healthy cartilage. J Rheumatol 26:870–879.

Naruse K, Yamada T, Sokabe M. 1998. Involvement of SA channels in orienting response of cultured endothelial cells to cyclic stretch. Am J Physiol 274:H1532–H1538.

Nobles M, Abbott J. 1998. Decline of the calcium response on successive stimulation of a rat brain endothelial cell P_{2U} purinoceptor. Acta Physiol Scand 162:69–76.

Ostrom RS, Gregorian C, Insel PA. 2000. Cellular release of and response to ATP as key determinants of the set-point of signal transduction pathways. J Biol Chem 275: 11735–11739.

Ralevic V, Burnstock G. 1998. Receptors for purines and pyrimidines. Pharmacol Rev 50:413–492.

Ries C, Petrides PE. 1995. Cytokine regulation of matrix metalloproteinase activity and its regulatory dysfunction in disease. Biol Chem Hoppe-Seyler 376:345–355.

Riley GP, Curry V, DeGroot J, Van El B, Verzijl N, Hazelman BL, Bank RA. 2002. Matrix metalloproteinase activities and their relationship with collagen remodelling in tendon pathology. Matrix Biol 21:185–195.

Saito S, Katoh M, Masumoto M, Matsumoto S, Masuho Y. 1998. Involvement of MMP-1 and MMP-3 in collagen degradation induced by IL-1 in rabbit cartilage explant culture. Life Sciences 62:PL 359–365.

Shimizu N, Yamaguchi M, Goseki T, Ozawa Y, Saito K, Takiguchi H, Iwasawa T, Abiko Y. 1994. Cyclic-tension force stimulates interleukin-1β production by human periodontal ligament cells. J Periodont Res 29:328–333.

Shimizu N, Ozawa Y, Yamaguchi M, Goseki T, Ohzeki K, Abiko Y. 1998. Induction of COX-2 expression by mechanical tension force in human periodontal ligament cells. J Periodontol 69:670–677.

Smith LL. 2000. Cytokine hypothesis of overtraining: A physiological adaptation to excessive stress? Med Sci Sports Exerc 32:317–331.

Soslowsky LJ, Thomopoulos S, Tun S, Flanagan CL, Keefer CC, Mastaw J, Carpenter JE. 2000. Overuse activity injures the supraspinatus tendon in an animal model: A histologic and biomechanical study. J Shoulder Elbow Surg 9:79–84.

Tsuzaki M, Guyton G, Garrett W, Archambault JM, Herzog W, Almekinders L, Buynum D, Yang X, Banes AJ. 2003. IL-1β induces COX 2 and MMP-1, -3 in human tendon cells. J Orthop Res 21:256–264.

Van der Zee E, Everts V, Beertsen W. 1996. Cytokine-induced endogenous procollagenase stored in the extracellular matrix of soft connective tissue results in a burst of collagen breakdown following its activation. J Periodont Res 31:483–488.

Watt WC, Lazarowski ER, Boucher RC. 1998. Cystic fibrosis transmembrane regulator-independent release of ATP. Its implications for the regulation of P2Y receptors in airway epithelia. J Biol Chem 273:14053–14058.

Yagutkin G, Bodin P, Burnstock G. 2000. Effect of shear stress on the release of soluble ecto-enzymes ATPase and 5′-nucleotidase along with endogenous ATP from vascular endotherial cells. British J Pharmacol 129:921–926.

Yamazaki S, Banes AJ, Weinhold PS, Graff RD, Tsuzaki M, Kawakami M, Minchew JT. 2001. Annulus cells release ATP in response to vibratory loading in vitro. J Cell Biochem (accepted).

Yang XC, Sachs F. 1989. Block of stretch-activated ion channels in Xenopus oocytes by gadolinium and calcium ions. Science 243:1068–1071.

Yellowley CE, Jacobs CR, Donahue HJ. 1999. Machanisms contributing to fluid-flow-induced Ca^{2+}mobilization in articular chondrocytes. J Cell Physiol 180:402–408.

Zimmermann H. 2000. Extracellulkar metabolism of ATP and other nucleotides. Naunyn-Schmiedeberg's Arch Pharmacol 362:299–309.