ATP reduces gel compaction in osteoblast-populated collagen gels

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1Flexcell International Corporation, Hillsborough; 2Joint Department of Biomedical Engineering of North Carolina at Chapel Hill and North Carolina State University, Raleigh; and Departments of 3Cell and Molecular Physiology, 4Genetics, and 5Curriculum in Applied and Materials Science, University of North Carolina, Chapel Hill, North Carolina

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Qi J, Chi L, Faber J, Koller B, Banes AJ. ATP reduces gel compaction in osteoblast-populated collagen gels. J Appl Physiol 102: 1152–1160, 2007. First published November 22, 2006; doi:10.1152/japplphysiol.00535.2006.—Bone remodeling is a localized process, but regulated by systemic signals such as hormones, cytokines, and mechanical loading. The mechanism by which bone cells convert these systemic signals into local signals is not completely understood. It is broadly accepted that the “prestress” in cytoskeleton of cells affects the magnitude of cellular responses to mechanical stimuli. Prestress derives from stiff cytoskeletal proteins and their connections within the cell and from cell contractility upon attaching to matrix. In an in vitro model of three-dimensional gel compaction, the relative cellular prestress levels in the same matrix environment were determined by matrix compaction rate: a greater compaction rate resulted in a higher level of prestress. In the present study, the effects of ATP on the prestress of osteoblasts were studied using mouse MC3T3-E1 cells grown in three-dimensional bioartificial tissues (BATs). ATP (≥100 μM) reduced the compaction rate of BATs in a dose-dependent manner. ADP, 2′-(or 3′)-O-(4-benzoylbenzoyl) ATP, and UTP, but not α,β-methylene ATP, also reduced the compaction rate but to a lesser extent. Pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid tetrasodium did not block the effect of ATP on BAT compaction rate. These results indicate that both P2X and P2Y receptors are involved in ATP-induced reduction of BAT compaction rate. Steady fluid flow and RT-PCR results showed that ATP reduced cell attachment on type I collagen by downregulating the expression of integrin α1. These results suggest a potential role for P2 receptors in matrix remodeling and repair and as a potential drug target in treatment of bone diseases.

adenosine triphosphate; three-dimensional culture; purinoceptor; osteoblast; extracellular matrix

The adaptive response of bone cells to mechanical strain is a primary determinant of skeletal architecture and bone mass (14, 16, 45). In vivo mechanical loading induces new bone formation and increases bone mineral density, whereas disuse, immobilization, and weightlessness induce bone loss (16). Although the mechanisms defining how bone cells convert mechanical loading into chemical signals are not completely understood, the prestress in the cytoskeleton (residual stress) of cells is well known to affect their response to mechanical loading (40, 50).

ATP release from mechanically stimulated cells (shear stress, stretch, osmotic swelling, and compression) is exhibited by many cell types (7, 33). Purinoceptor pathways intersect those that regulate mechanical load responses (50). Therefore, extracellular ATP could modulate cellular responses to mechanical loading (42). It was hypothesized that extracellular ATP may act as a regulator and/or transducer of mechanosignal transduction by regulating a cell’s set point for prestress. Since bone remodeling is a localized process (3), the local extracellular ATP may play a critical role in bone remodeling by converting the broad, systemic mechanical stimuli into local signals. Extracellular ATP, released from cells by lytic or nonlytic mechanisms, plays a number of diverse roles in vivo and in vitro, such as membrane permeability (27), induction of apoptosis (44), organic anion transport (such as bilirubin) (5), calcium mobilization (12), cell proliferation (38), contractility (34), wound healing (8), excitation of sympathetic neurons (25), inhibition of tumors (28), and bone remodeling (3). ATP interacts with P2 receptors that have been divided into two subfamilies: P2X (ligand-gated cation channels) and P2Y (G protein-coupled receptors) (23). To date, seven P2X and eight P2Y receptors have been cloned and characterized (11, 23). P2X receptors are activated exclusively by adenine nucleotides, whereas P2Y receptors are responsive to adenosine nucleotides, whereas P2Y receptors are responses to adenosine (P2Y1, P2Y11, P2Y12, and P2Y13) or uridine nucleotides (P2Y6 and P2Y14) or to both (P2Y2 and P2Y4). P2 receptors are expressed ubiquitously, but specific tissue responses are achieved by cell-specific expression profiles (3). P2 receptors are expressed on osteoblasts, and nucleotides are important local signaling molecules in bone (3, 9). However, few studies have investigated the potential roles of extracellular ATP in the regulation of bone remodeling (26).

As a tissue develops, its cells fabricate an extracellular matrix in a given geometry, according to developmental pathway cues (19). During this process, cells utilize a mechanical sensor(s) and feedback control to establish a set point for a basal prestress at the cell level. This strain set point is determined, in part, by the cell’s connectivity to the substratum and internal architecture that balance external and internal forces in a tensegrity structure (40). Cells respond to external tension by adjusting their shape, connections to matrix, and other cells and their prestress. Thus cells develop a prestress level for a given external tension and attempt to modulate their cell-matrix contacts, pseudopod lengths, degree and types of cytoskeletal organization, and modulus of elasticity based on the prestress values (1).

In the present study, mouse osteoblast-like cells were used to investigate the effect of extracellular ATP on bone cell prestress by monitoring the rate of collagen gel compaction. MC3T3-E1 cells were grown in a novel three-dimensional (3D) culture system in which cell-generated force and movement compact the gel matrix through connections between cells and matrix (17). Tension in the matrix increases due to
tractional structuring (39). This, in turn, causes cellular pre-
stress levels to increase during the compaction of collagen gels (39). It was believed that increase in cell prestress would make cells more sensitive to mechanical stimulation (6). We have reported that ATP blocked a cellular response to mechanical loading (calcium signaling) (42); therefore, it was hypothe-
sized that high-concentration ATP would reduce cellular pre-
stress and thus reduce collagen gel compaction rate and that P2 receptors play an important role in this process. Since bone repair and remodeling are regulated by both chemical and mechanical stimuli (14, 16, 45), the results in the present study will also be helpful in understanding bone repair and remod-
eling processes.

MATERIALS AND METHODS

Materials. All nucleotides except the stable analogs of ATP and ADP were obtained from Amersham Biosciences (Piscataway, NJ). Type II collagenase was purchased from Worthington (Lakewood, NJ). 2′-(or-3′)-O-(4-benzoylbenzoyl)ATP, tris(triethylammonium) salt [BzBzATP or BzATP, a P2X<sub>4</sub>-preferring agonist (10)] was purchased from Invitrogen (Carlsbad, CA). Adenosine 5′-(3-thio-
triphasphate) trilithium salt (ATPyS), adenosine-5′-O-(2-thiodiphos-
phate) trilithium salt (ADPβS), α,β-methylene adenosine 5′-triphos-
phate lithium salt (mATP, an agonist for P2X receptors), pyridoxal-
phosphate-6-azophenyl-2′,4′-disulfonic acid tetrasodium [PPADS,
a P2X-preferring inhibitor (30)], and all other chemicals, unless
indicated otherwise, were purchased from Sigma (St. Louis, MO).

Cell culture. The murine osteoblast-like cell line, MC3T3-E1, was
derived from calvariae from newborn mice (41). It is an established
cell line, but the cells maintain much of the tightly linked controls
between proliferation and differentiation that usually are seen only
in primary cells (35). MC3T3-E1 cells were maintained in DMEM
medium with 10% bovine calf serum (BCS) and 1% penicillin/
streptomycin (Gibco, Grand Island, NY). Human osteoblast-like cells,
SaOS-2, were maintained in MCCOY's 5A medium containing 10%
fetal bovine serum (FBS; Hyclone, Logan, UT) and 1% penicillin/
streptomycin (Gibco, Grand Island, NY). Primary, murine osteoblast
 cells were isolated from tibial bones of wild-type (wt) and P2Y<sub>1</sub>/P2Y<sub>2</sub>
double-knockout (DKO) mice, according to the method described in
Ref. 46. Cells were grown in DMEM medium with 10% FBS
(Hyclone) and 1% penicillin/streptomycin (Gibco). Cells from
passage 1 were used in this study.

Fabrication of 3D bioartificial tissue cultures in Tissue Train
culture plates. Three days before bioartificial tissues (BATs) were
made, cells were transferred onto SurfFlex II culture plates coated
with type I collagen (Flexcell International, Hillsborough, NC) at 5,000
cells/cm<sup>2</sup> and grown for 72 h to confluence. Osteoblast-populated 3D
collagen gels were formed as linear, tethered constructs in six-well,
35-mm-diameter Tissue Train culture plates (Flexcell International)
(15). Four plates were situated in one gasketed baseplate that fits on
a CO<sub>2</sub> incubator shelf. The surface of each Tissue Train culture plate
well has a pair of flexible but inelastic nonwoven nylon mesh anchors
bonded at east and west poles (Fig. 1). Anchor stems at opposing
poles were treated with type I collagen that mechanically bonded to
the gel and allowed cell attachment. In preparation for casting a
collagen gel, nylon disks with a planar face and a 25 × 3 × 3 mm
central trough (loading trough) were placed beneath each well. The
central trough aligned with each pair of anchor stems in the Tissue
Train culture plate. Holes in the loading trough communicated with a
vacuum port, allowing the flexible membranes to be drawn into the
trough, creating a space for the cell-populated gel. Cells were grown
to confluence on SurfFlexII culture plates, trypsinized, and counted
(model Z1, Beckman Coulter). The type I collagen gel consisted of
70% vitrogen (3.2 mg/ml; Cohesion, Palo Alto, CA), 20% 5 × MEM,
and 10% FBS. The gel solution was mixed well and adjusted to pH 7.2
using 1 N sodium hydroxide. The cells at 2 × 10<sup>5</sup> cells/100 µl were
suspended in the gel solution, and 100-µl cell-gel mixtures were
transferred by pipette into each loading trough of the Tissue Train
culture plates. Plates were incubated at 37°C, 5% CO<sub>2</sub>, for 1 h to allow
gelation. Culture plates were then removed from the baseplate, and
2 ml of DMEM medium with 2% serum (in the case of SaOS-2 cells,
10% FBS was used) were added to each well, and the cultures were
incubated at 37°C, 5% CO<sub>2</sub>, for the indicated times.

Compaction kinetics of BATs. The 3D BATs were scanned each
day using a ScanFlex device, an automated image collection system
(Flexcell International). The gel areas were measured using Sigma-
Scan Pro 5.0 (SPS3D, Chicago, IL). The compaction curves were
plotted as culture area vs. time. The compaction rate was calculated as
follows: \( \nu = (S_0 - S_t)/S_0 \times 100\%/t \), where \( S_0 \) is the gel area at
time \( 0 \), \( S_t \) is the gel area at time \( t \), and \( t \) is culture time (day). At the end of
each experiment, cells were released from the BATs by incubating with 2 mg/ml type II collagenase dissolved in serum-free DMEM medium at 37°C, 5% CO₂, for 30 min. Cells were sedimented and stained with Trypan blue, and viable cells were counted.

**Effects of nucleotides and analogs on the compaction rate of BATs.** Nucleotides, their stable analogs, BzBzATP or mAATP, were added at the indicated concentrations to each well in the presence or absence of 100 μM PPADS after gelation, and the gel compaction rates were recorded as described above.

**Measurement of intact ATP in the culture medium.** Media were removed at indicated time points, and the ATP concentrations were measured using a luciferin-luciferase assay (42).

**Assay of cell attachment on type I collagen.** To determine the strength of cell attachment on type I collagen, MC3T3-E1 cells from the BATs treated with or without ATP were released from the hydrogels, as described above, plated on type I collagen-coated slides (Flexcell International) at 10,000 cells/cm², and incubated at 37°C, 5% CO₂, for 1 h. Then the cells were subjected to a steady fluid flow of 50 dyn/cm² with a computer-controlled FlexFlow device (Flexcell International). Cell images were recorded each minute using a phase-contrast microscope; the number of cells remaining after application of shear stress was counted. The percentage of adherent cells was measured using a luciferin-luciferase assay (42).

**RESULTS**

**ATP reduced the compaction rate of MC3T3-E1-populated BATs.** Compaction of the gels proceeded over 5 days (Fig. 2), being greatest within the first 24 h. ATP (∼100 μM) reduced the compaction rate in a dose-dependent manner (Fig. 2B). The compaction rates of control and 10 μM ATP-treated gels were the same, with an overall 14 ± 1.0% per day compaction rate. This rate was reduced to 12 ± 0.4 μM (P < 0.05) and 8.4 ± 0.2% (P < 0.01) per day in the 100 and 500 μM ATP-treated groups, respectively.

**Kinetics of ATP hydrolysis.** Extracellular ATP is rapidly hydrolyzed by ecto-nucleotidases secreted by some cell lines.
To determine the hydrolysis rate of ATP in the culture medium of MC3T3-E1 cells, the concentrations of ATP in the medium were measured at different time points from 0 to 24 h, after addition of 500 μM ATP. Extracellular ATP was hydrolyzed more slowly in the medium from MC3T3-E1 cells compared with the rate reported for other cell lines (7). Nearly 20% of the ATP was not hydrolyzed after 24-h incubation with MC3T3-E1 cells (Fig. 3). These data indicate that MC3T3-E1 cells may express lower levels of ecto-nucleotidases.

Effects of nucleotides on the contraction rate of BATs. Nucleotides, in addition to ATP, are secreted by cells or arise as hydrolysis products (29). Therefore, the influence of other nucleotides on BAT compaction rate was also measured (Fig. 4). Both purine and pyrimidine nucleotides reduced the compaction rate [an overall compaction rate of 14 ± 1.0% per day for control, 11 ± 0.1% (P < 0.05) and 11 ± 1.4% (P < 0.05)] per day in the presence of 500 μM ADP or UTP, respectively, but ADP and UTP were less potent. To verify that the reduction of the compaction rate induced by ATP was not due to the hydrolysis of ATP or ADP, the effects of ATPγS and ADPβS, the nonhydrolysable analogs of ATP and ADP, respectively, were tested on the compaction rate. Both ATPγS and ADPβS reduced the gel compaction rate [11 ± 0.1% (P < 0.01) per day for ATPγS and 13 ± 0.3% (P < 0.05) per day for ADPβS], and ATPγS showed greater potency (Fig. 5A). To further confirm that the reduction in gel compaction was induced by ATP, the effects of ATP on the compaction of SaOS-2, a human osteoblast cell line, populated BATs were determined (Fig. 5B). The half-life for ATP with SaOS-2 cells is ~50 s (3). As expected, the compaction of SaOS-2-populated BATs was reduced by ATPγS but not by ATP.

Cell viability in BATs treated with ATP. Extracellular ATP reportedly induces cell apoptosis (44, 49). To determine whether the reduction in the compaction rate induced by ATP was due to cell death, the cell viability in the BATs treated with ATP was tested by Trypan blue exclusion (Fig. 6). The viable cell number declined from an initial value of 200,000 (200k) cells per BAT to 115k cells per BAT at day 1, then returned to initial values by day 3, and was stable from day 4 onward at ~170k cells per BAT. There were no dramatic differences between control and ATP-treated groups with respect to viable cell number within the time range in this study.

Recovery of cell contractility of ATP-treated MC3T3-E1 cells after washout of ATP. An ATP washout experiment was performed to determine whether the ability of cells to compact their matrix was reversible. Extracellular ATP was washed out at different time points after addition of 500 μM ATP from 24 to 96 h (the remaining ATP was refreshed daily) (Fig. 7). MC3T3-E1 cell-driven BAT compaction recovered rapidly and completely to control levels at day 5, if the ATP was washed out.
out within 48 h after addition. Incubation with ATP for 72 h delayed recovery. Cells incubated with ATP for 96 h did not show any recovery of the BAT compaction during the first 24 h post-ATP washout. These results indicate that ATP treatment for up to 48 h did not permanently damage the cells.

Expression profile of P2 receptors in MC3T3-E1 cells. Due to the cross-reactivity of nucleotides with P2 receptors (23), the response of cells to extracellular nucleotides may be attributed to more than one P2 receptor or be determined by the P2 receptor expression profile. Osteoblasts reportedly express most of the 15 P2 receptors so far identified (3), but the P2 receptor profile varies between primary cells and established osteoblast cell lines, as well as between different nonosteoblast cell lines. Therefore, the P2 receptor expression profile was examined. Eleven of 14 tested P2 receptors were detected (the sequence of mouse P2Y11 is still not available in GenBank) (Fig. 8). Of the known P2X receptors, the P2X4 receptor showed the greatest expression level, P2X5 and P2X7 were at medium levels, while P2X3 and P2X1 were at very low levels, and P2X1 and P2X6 were not detected. Of the tested P2Y receptors assessed, all receptors were detected except P2Y12. The P2Y2 receptor showed the highest expression level in this class.

Multiple P2 receptors are involved in the regulation of BAT compaction by ATP. Both ATP and UTP reduced the compaction rate of MC3T3-E1 cell-populated BATs (Fig. 4), suggesting that P2Y receptors are involved in this process. To further investigate the involvement of P2X receptors in this process, specific agonists and antagonist of P2X receptors were used: mATP [an agonist for P2X receptors, but not for P2X4 (30)], BzBzATP [P2X4-preferring agonist at low concentration (10)], and PPADS [an antagonist for P2X receptors, but does not inhibit P2X4 (30)] (Fig. 9). mATP did not alter the compaction rate of BATs, while PPADS reduced the rate during the first 48 h. During the first 24 h, the compaction rate was reduced from 54 ± 2.4% (control) to 43 ± 1.2% (in the presence of PPADS, P < 0.01), 40 ± 1.0% (in the presence of ATPyS, P < 0.01), and 29 ± 1.4% (in the presence of ATPyS and PPADS; P < 0.01), respectively. The differences in compaction rates between control and ATPyS-treated, control and PPADS-treated, and between control and ATPyS/PPADS-treated BATs were 14, 11, and 25%, respectively. An additive effect was demonstrated between PPADS and ATP, which indicates that PPADS and ATP reduced the gel compaction rate through different pathways. It was reported that PPADS at 100 μM does not inhibit the P2X4 receptor (but blocks all of the other P2X receptors) (30); a more specific P2X4 agonist, BzBzATP, was used to determine the involvement of P2X4 receptor in this process (Fig. 9B). The results showed that BzBzATP at 100 μM reduced the compaction rate of MC3T3-E1 cell-populated BATs, indicating the potential involvement of BzBzATP-responsive P2X receptors, such as P2X4, in the ATP-induced regulation of BAT compaction rate. P2Y1 and P2Y2 receptors are not involved in the reduction of BAT compaction induced by extracellular ATP. Based on the selectivity of P2 receptors for nucleotides (11, 23), the most likely candidates of the P2Y receptors responsible for the regulation of BAT compaction by extracellular ATP are P2Y2 and P2Y4 receptors. To address the question of whether P2Y1 and P2Y2 receptors were involved in this process, an investigation was carried out to test the effects of nucleotides on the compaction rate of BATs populated with primary mouse osteoblastic cells from wt and P2Y1/P2Y2 DKO mice (Fig. 10).
Quantitative RT-PCR showed that ATP reduced the expression of integrin α1 by 60% on day 5; the expression of α2, α3, and β1 was not changed (Fig. 11B). The time course showed that the reduction in the expression of integrin α1 occurred from 24 h after addition of ATP and reached the lowest level on day 3 (Fig. 11C).

DISCUSSION

Bone is a dynamic, living tissue, whose structure and shape are maintained through a tightly coupled process of bone remodeling: bone resorption and bone formation (37). The timing and sequence of these two processes are tightly regulated by various factors (37). The detailed mechanisms are still not clear. However, bone remodeling is regulated by both cytokines and mechanical loading (3, 13). All of these regulators are systemic signals, but bone remodeling is a localized process. How bone tissue converts these systemic effectors to localized signals is not clear. Bowler et al. (3) have reported that extracellular nucleotides could sensitize the parathyroid hormone receptor by interacting with P2Y receptors and therefore augment local cellular responses to parathyroid stimulation in osteoblastic cells. Ostrom et al. (33) also reported that ATP could act as a key determinant in the set point of signal transduction pathways. These studies indicate a possible mechanism explaining how bone cells convert a systemic signal, such as parathyroid hormone stimulation, into a local cellular event.

Both the wt and DKO cells showed similar compaction rates and similar sensitivities in the response to ATPγS and ADPβS. The overall compaction rates for wt, DKO, wt + ATPγS, wt + ADPβS, DKO + ATPγS, and DKO + ADPβS were 14 ± 1.4, 14 ± 0.8, 8.8 ± 0.8 (P < 0.01), 11 ± 0.3 (P < 0.05), 9.6 ± 0.8 (P < 0.01), and 11 ± 0.8% (P < 0.05) per day, respectively. ATPγS was more effective than ADPβS. These results were similar to those derived from MC3T3-E1 cells and confirmed that both P2Y1 and P2Y2 receptors are not involved in the reduction of BAT compaction rate induced by extracellular ATP.

Extracellular ATP reduced cell attachment on type I collagen. The steady flow results showed that ATP reduced cell attachment on type I collagen (Fig. 11A). After a 20-min flow, ~5% of ATP-treated MC3T3-E1 cells remained adherent on the surface, while 40% of control cells remained adherent. The overall compaction rates for wt, DKO, wt + ATPγS, wt + ADPβS, DKO + ATPγS, and DKO + ADPβS were 14 ± 1.4, 14 ± 0.8, 8.8 ± 0.8 (P < 0.01), 11 ± 0.3 (P < 0.05), 9.6 ± 0.8 (P < 0.01), and 11 ± 0.8% (P < 0.05) per day, respectively. ATPγS was more effective than ADPβS. These results were similar to those derived from MC3T3-E1 cells and confirmed that both P2Y1 and P2Y2 receptors are not involved in the reduction of BAT compaction rate induced by extracellular ATP.
response. In a previous study, our laboratory (42) has shown that mechanical loading, another systemic stimulus, may be converted into a local response through local elaboration of extracellular nucleotides. The results in the present study indicate that both P2X and P2Y receptors are responsible for modulating the prestress levels of osteoblasts.

ATP release by mechanical loading (fluid flow, tension, and compression) is a physiological phenomenon found in many cell types (7, 33). The local ATP concentration resulting from a nonlytic mechanism can reach the micromolar range in the vicinity of the cell membrane (2). All cells contain a high concentration of intracellular ATP (1–5 mM), as well as the capacity to release ATP (3). In addition, increased blood flow associated with a general inflammatory response could greatly increase the nucleotide concentration to the millimolar level upon platelet aggregation (3). Therefore, at the sites of tissue injury, wounding, or bone fracture, a high local ATP concentration could be present.

Osteoblasts express purinoceptors and respond to ATP (3, 21). Mechanical stimuli induce release of ATP from bone cells (3). Purines and pyrimidines are clearly involved in osteoblast responses to chemical and physical stimuli and share interacting pathways (3). However, it is difficult to clearly address the role of a specific P2 receptor in a given cellular function due to the overlap of reactivity of the ligands for P2 receptors and lack of specific inhibitors for each individual P2 receptor (4). PPADS is a P2X-preferring antagonist, but it does not inhibit P2X4 receptor (30); mATP is an agonist for P2X receptors, but not for P2X4 (30). The PPADS/mATP-insensitive, ATP-induced reduction in gel compaction indicates that P2X4 receptor may be involved. The sensitivity of gel compaction to BzBzATP at 100 μM further suggests the potential involvement of P2X4 receptor. However, most of the available data about P2 receptors were from the studies on heterologously expressed human or rat proteins (30), and it has been shown that P2 receptors from human and rat may respond differently to ATP, even though they shared high homology in amino acid sequences (24). Therefore, without further direct evidence, we cannot prove that P2X4 is the only P2X receptor that is involved in this process: some other P2X receptors, which are not sensitive to mATP or PPADS, but activated by BzBzATP, may also be involved. The sensitivity of gel compaction to ATP, ADP, and UTP indicates that P2Y receptor(s) is (are) also involved. We were not able to define which P2Y receptors may be involved due to the lack of specific P2Y inhibitors. Since the P2Y2 receptor was not involved, the other ATP/UTP-responsive receptor, P2Y4, may be the most likely candidate. However, it was reported that ATP is an antagonist of the human P2Y4 receptor (24). It was shown in the present study that ATP reduced the compaction rate of BATs populated not only with mouse osteoblasts but also with human osteoblasts. Therefore, it is unlikely that the P2Y4 receptor is involved in gel compaction. None of the known P2Y receptors, except P2Y2 and P2Y4, respond to both UTP and ATP (11, 23). Therefore, these results indicate that there may be a novel, unidentified P2Y receptor involved in this process. It was also observed that PPADS alone reduced the compaction rate during the first 48-h incubation. It has been reported that PPADS acts as an antagonist of inositol 1,4,5-trisphosphate (43) and inhibition of phosphatidylinositol 3-kinase blocked FBS-induced collagen gel compaction (48). Therefore, PPADS may reduce the gel compaction rate by blocking the functions of inositol 1,4,5-trisphosphate. However, PPADS did not affect the compaction rate after 48 h, which indicates that different

Fig. 11. ATP reduced the attachment of MC3T3-E1 cells on type I collagen by downregulating the expression of integrin α1. A: MC3T3-E1 cells were released with type II collagenase from BATs treated with or without 500 μM ATP on day 5 and plated on type I collagen-coated slides. One hour later, the cells were subjected to a steady flow of 50 dyn/cm² for 20 min. B: semiquantitative RT-PCR of integrins on RNAs from day 5. C: time course of ATP-induced downregulation of integrin α1. *P < 0.05.
signaling pathways may be involved at the initial stage vs. later stage of gel compaction. This switch may be due to the changes in cell shape and intracellular tension that occur during gel compaction (18).

It has been reported that a cell strain set point is established in cells during tissue development, depending on the local environment and changes during remodeling (1). The mechanism for regulating the compaction of collagen matrixes is complicated and not completely understood (17, 32). It has been reported that PDGF, transforming growth factor-β, EGF, and lysophosphatidic acid stimulate the compaction of collagen matrix by upregulating the expression of integrins (47). Therefore, regulation of integrin expression may be one of the key modulators for controlling matrix compaction. The results in this study indicate that ATP reduced BAT compaction rate by downregulating the expression of integrin α1. There are three different integrins that are responsible for cell attachment to type I collagen: α1β1, α2β1, and α2β1. Several studies have shown that both integrins α1 and α2 play an important role in collagen gel compaction (36, 47). However, in most of the cases, only one of these two integrin subunits was changed at gene expression levels, depending on the cell types used. The results in the present study indicate that integrin α1β1 may be the key regulator of matrix compaction in osteoblasts and may play an important role in bone metabolism. This was confirmed by the gel compaction rate of MG63 [a human osteoblast mainly expressing α2β1 integrin (20)] populated BATs, which was not affected by ATP or ATPγS (data not shown). By reducing cell attachment to matrix, ATP may also reduce cellular responses to mechanical stimuli.

In conclusion, it was shown here that ATP, ADP, and UTP reduced the compaction rate of 3D collagen gels populated with MC3T3-E1 cells. By using specific agonists and antagonist of P2 receptors and primary osteoblastic cells from P2Y1/P2Y2 DKO mice, we showed here that both P2X and P2Y receptors are responsible for the regulation of cell prestress by extracellular ATP in osteoblasts. ATP sensitizes cellular responses to stimuli at lower concentrations and may reduce cellular responses to stimuli at higher concentrations by reducing cell attachment to extracellular matrix. ATP may act as a protective/survival factor in osteoblasts subjected to excessive mechanical loads (33). Since matrix organization is an important step in bone repair/remodeling, the results in this study further confirmed the importance of P2 receptors in matrix organization and compaction during bone repair and remodeling (26). These receptors may represent a new class of drug targets that could be potentially useful in the treatment of bone diseases where remodeling exceeds bone deposition. Moreover, the reversibility of ATP treatment offers a useful means to modulate bone matrix formation in tissue engineering applications.

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DISCLOSURE

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