ATP RELEASE BY MECHANICALLY LOADED CHONDROCYTES IN PELLET CULTURE

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**Contribution:** The occurrence of baseline ATP concentrations despite the presence of active ATPpase suggests that ATP is continuously released by chondrocytes at rest and that the rate of ATP release is dramatically increased by mechanical loading. The mechanism of ATP appears to be a regulated process and does not require cell lysis. The accumulation of PPI following hydrolysis of ATP may be important to prevent mineralization of articular cartilage under normal conditions in vivo, but may stimulate CPPD crystal deposition in cases of excessive ATP release.

**References:**

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**Background:** Extracellular ATP in cartilage activates P2 purinoceptors and functions as a pharmacological mediator of matrix turnover. ATP is also a substrate for the generation of pyrophosphate (PPI) which can lead to CPPD crystal deposition in cases of excessive ATP release.

**Methods:** Chondrons were isolated from porcine articular cartilage by enzymatic digestion and maintained in pellet cultures after centrifugation in V-bottom 96-well culture plates. Culture media (Opti-MEM supplemented with 2% FBS, 25 µg/ml ascorbate-2-PO4) were refreshed every 48 hours for 6 days and after weaning to serum-free (SF) media, pellets were maintained in 6 ml culture tubes in SF media for an additional 1-2 weeks.

Dynamic compression was performed with 2-3 week-old pellets using a Compression Plus™ device (Flexcell Intl. Corp.). ATP accumulation in culture media was measured using a firefly luciferase assay. ATP hydrolysis and subsequent accumulation of 32Pi and 32P-PPI was measured by HPLC following the addition of exogenous 32P-[ATP] to resting cultures. Relative cell viability was determined using an MTT assay for mitochondrial enzyme activity (Sigma).

**Results:** Measurement of endogenous ATP in media of resting cultures indicated that ATP was continuously released to maintain a baseline concentration of approximately 2-4 nM. Cyclic compression resulted in a four-fold increase in ATP release that reached maximal at 5-15 minutes and declined thereafter to baseline within 60 minutes (Fig. 1). ATP release increased with applied pressure (0.15-0.6 MPa), but in all cases ATP fall to baseline concentrations within 60 minutes despite continued loading. Cultures cyclically compressed for 60 minutes at 0.3 MPa could again be induced to release ATP, but only with elevated pressure (0.6 MPa). This second peak of ATP release (8.0±1.1 nM at 0.6 MPa) was less than half the release observed in cultures loaded at 0.6 MPa for the first time (22.5±1.3 nM) suggesting chondrocyte desensitization to load. MTT assays showed no difference in cell viability between pellets at rest or up to 24 hours after cyclic compression.

A regulated membrane transport mechanism for nucleotide release rather than cell injury was indicated by modulation of ATP release by octanol and halothane. Octanol (0.5 nM) inhibited ATP release, resulting from fluid movement or cyclic compression, by approximately 50% (Fig. 2), whereas halothane (1 mM) increased the rate of ATP release in response to dynamic loading.

The decline in ATP concentration was due to the activity of one or more pyrophosphohydrolase enzymes, as evidenced by the accumulation of pyrophosphate (PPI) in resting cultures incubated with [32P]-ATP. The accumulated [32P]-PPI remained stable for up to seven hours in the culture media, indicating that PPI is a final breakdown product of ATP hydrolysis by chondrocytes.

**Discussion:** The occurrence of baseline ATP concentrations despite the presence of active ATPpase suggests that ATP is continuously released by chondrocytes at rest and that the rate of ATP release is dramatically increased by mechanical loading. The mechanism of ATP appears to be a regulated process and does not require cell lysis. The accumulation of PPI following hydrolysis of ATP may be important to prevent mineralization of articular cartilage under normal conditions in vivo, but may stimulate CPPD crystal deposition in cases of excessive ATP release.

**Conclusions:** Together these data indicate that extracellular ATP and PPI are generated by chondrocytes, both at rest and during mechanical loading, and suggest a role for ATP in normal chondrocyte cell signaling and in the pathogenesis of OA. (Supported by NIAMS AR43883 and a Glaxo Wellcome/UNC Collaborative Research Agreement.)