MECHANICALLY-INDUCED STRAIN UPREGULATES CONNEXIN-43 MRNA EXPRESSION IN TENDON CELLS

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INTRODUCTION:

Gap junctions are involved in multiple mechanotransduction signaling pathways [1,2]. In addition, mechanical load has been shown to regulate the expression of the primary gap junction protein, connexin-43 (cx-43) [3,4,5]. In avian tendon cells, cx-43 is the most prevalent species. Avian tendons cells subjected to 0.035 cyclic strain for 8 hours followed by 16 hours of rest, up-regulated cx-43 mRNA levels [6]. However, no earlier timepoints of mechanical load were investigated. We hypothesized that avian tendon cells would increase cx-43 mRNA expression to mechanically induced strain in a rapid, dose-dependent fashion. Furthermore, we hypothesized that stretch would increase intracellular calcium concentration ($[Ca^{2+}]_{ic}$) in avian tendon cells prior to a significant "load dose" that stimulates cx-43 gene expression.

METHODS:

Cells from flexor digitorum profundus tendons of 61-day-old Ross or 52-day-old White Leghorn chickens were isolated by sequential enzymatic and mechanical scraping techniques. To evaluate the changes in gene expression, internal fibroblasts (ATIF) were plated at 25k cells/cm^2 on Bioflex^{TM} 6-well plates and grown to quiescence. On the fifth day after culture, the ATIFs were placed in serum-free medium (DMEM-H with 1% penicillin, streptomycin, and amphotericin B, 0.1 mM ascorbate-2-phosphate, and 20 mM Hepes, pH 7.2). On day six, the Bioflex^{TM} plates were placed on the baseplate of the Flexercell Strain unit. ATIFs were subjected to 0.03 cyclic strain at 1 Hz for 0.5, 1, 2, 4, 6, 8, and 12 hours at 37 °C then cells were collected for total RNA immediately following stretching. Quantitative RT-PCR was used to analyze cx-43 mRNA levels relative to 18s-rRNA expression.

To evaluate the Ca^{2+} response, cells were spot cultured at 2k cells/10 µL on collagen-coated rubber membranes and grown to confluence and quiescence. On the sixth day after culture, the cells were rinsed with Earles' Balanced Salt Solution (EBSS) with 20 mM HEPES, pH 7.2, Ca^{2+} and Mg^{2+}, incubated at RT in 5 µM Fura-2AM for 1 h, then rinsed with EBSS. The membrane was transferred to a StageFlexor^{TM} stretch device, which applies an equibiaxial strain to the cells across a 25 mm loading post. The device was mounted on the stage of an Olympus upright fluorescence microscope to permit assessment of $[Ca^{2+}]_{ic}$ using a ratio dye method. Image I software was used to monitor $[Ca^{2+}]_{ic}$ in each cell. Baseline Ca^{2+} was quantified at no stretch conditions. Strain was applied cyclically at 3% or 5% elongation, 1 Hz, for 1, 5, 10, 30, 60, 120, or 180 seconds. All data are represented as mean ± SEM. Statistical significance was determined using a one way ANOVA.

RESULTS:

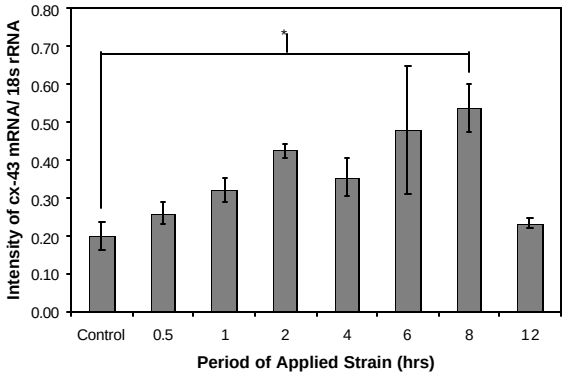

Figure 1. Connexin-43 mRNA expression increased in response to mechanically-induced substrate strain. mRNA levels increased as early as 30 minutes of loading, reached a peak around 8 h and returned to control levels by 12 h. *p<0.05 compared to control. n = 7 tendons isolated together, 3 replicates per time point.

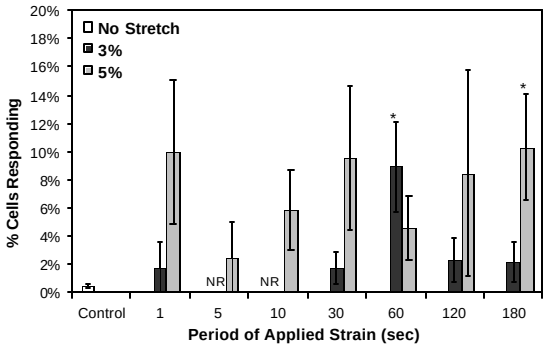

Figure 2. Mechanically-induced substrate strain did not induce a significant increase in $[Ca^{2+}]_{ic}$ compared to the no stretch control under most of the conditions analyzed. Values for 3% elongation for 1 min and 5% elongation for 3 min groups were significantly different than control. However, at these timepoints, only 10% of the cells responded. *p<0.05 compared to control. n = 3 isolations, 1-2 replicates/ timepoint.

DISCUSSION:

Gap junctions are present in most connective tissues and are essential for normal growth and development. In avian tendon explants, gap junctions regulate collagen synthesis [7]. We found that substrate strain quickly modulates cx-43 mRNA expression. However, strain failed to induce in tendon cells, a significant increase in $[Ca^{2+}]_{ic}$. A similar response occurs in avian tendon cells subjected to fluid flow [8]. In addition, rabbit tendon cells do no increase $[Ca^{2+}]_{ic}$ in response to flow but do upregulate MMPs and COX-2 [9]. Thus, mechanical loading may regulate gene expression, specifically connexin-43 in avian tendon cells, via a Ca^{2+}-independent pathway.

ACKNOWLEDGMENTS:

NIH AR38121

REFERENCES:

1. Banes et al., Curr Opin Orthop 2001; 12:389-396.
2. Sikavitsas et al., Biomaterials 2001; 22:2581-2593.
3. Cowan et al., Circ Res 1998; 82:786-793.
4. Zhuang et al., Circ Res 2000; 87:316-322.
5. Wang et al., Biochem Biophys Res Comm 2000; 267:551-557.
6. Banes et al., Trans Orthop Res Soc 1996.
7. Banes et al., Clin Orthop Rel Res 1999; 367S:S356-S370.
8. Elfervig et al., Trans Orthop Res Soc 2001.
9. Archambault et al., J Biomech 2002; 35:303-309.