MECHANICAL STRETCHING INDUCES INCREASED INTRACELLULAR [Ca2+]ic IN HUMAN TENDON CELLS

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Introduction: Tendons are constantly subjected to mechanical load such as shear stress and strain during day-to-day activities. Human tendon surface cells (HTSC) respond to mechanical load through a variety of different pathways, which involve ion channels and second messengers. HTSCs respond to mechanical indentation with a micropipette, fluid-induced shear stress and ATP by increasing intracellular calcium concentration ($[Ca^{2+}]_{ic}$). The response to shear stress is gadolinium and pertussis toxin sensitive, implicating both stretch activated channels and G-proteins, and requires extracellular calcium. Previous results have shown that the response to a mechanical indentation with a micropipette is temporarily reduced after the addition ATP. We hypothesized that human tendon surface cells would respond to mechanical stretching by increasing $[Ca^{2+}]_{ic}$ through multiple pathways.

Methods: Cells from flexor digitorum profundus tendons, zone III, of 3 patients were isolated by sequential enzymatic, mechanical scraping and selective adherence techniques. The surface epitenon cells were plated at 25k cells/cm^2 in Medium 199 with 10% fetal calf serum (FCS), 0.5 mM ascorbate, 20 mM HEPES pH 7.2, and antibiotics. To evaluate the $[Ca^{2+}]_{ic}$ response, HTSCs were spot cultured at 2k cells/10 μL in the middle of a flexible silicone membrane and grown to quiescence. On the sixth day after culture, the cells were rinsed with Earles' Balanced Salt Solution (EBSS) with HEPES, pH 7.2, Ca^{2+} and Mg^{2+}, incubated at room temperature in 5 μM FURA-2AM for 90 minutes, then rinsed with EBSS. The membranes were transferred to a StageFLexor™ stretch device operated by a Flexercell™ strain unit, which applies an equibiaxial strain to the cells across a 25 mm loading post. The unit was mounted on the stage of an Olympus upright fluorescence microscope to permit assessment of intracellular calcium levels using a ratio dye method. Baseline Ca^{2+} was quantified at no stretch conditions then strain was applied either statically (1%, 2%, 4%, and 6% elongation, 1 min) or cyclically (0.1 Hz, 1%, 2%, 4%, and 6% elongation, 1 min). Image I MetaFluor software was used to monitor $[Ca^{2+}]_{ic}$ in each cell. To analyze the possible pathways involved in the signaling response, cells were pre-treated with either 10 μM gadolinium, 1 μM pertussis toxin, 5 μM indomethacin or 1 μM ATP. As a positive control, 1 μM ATP was added to the cells after stretch ± modulators. Cells were also stretched in the absence of exogenous Ca^{2+} to determine the source of Ca^{2+} in the signaling response.

Results: HTSCs responded to mechanically induced strain by increasing intracellular calcium. The response to static stretching was two-fold greater than to cyclic stretching for the 4% and 6% elongation ($p<0.05$) (Fig 1). However, there was not significant difference at the lower strains between the responses to static and cyclic stretching. Furthermore, this response to stretching was not dependent on exogenous calcium (Table 1). Gadolinium, a blocker of stretch-activated ion channels, completely inhibited the response to stretching; however, neither indomethacin, an inhibitor of cyclooxygenases, pertussis toxin, an inhibitor of G-protein dependent pathways, or ATP ablated the response (Table 1). All cells responded to a direct stimulation by a bolus of ATP (1 μM) by increasing $[Ca^{2+}]_{ic}$ (Table 1).

Conclusions: The response to mechanical load of HTSC involves a variety of different pathways and chemical mediators. Unlike a response to fluid-induced shear stress, the stretch response was not dependent upon extracellular Ca^{2+}. However, similar to the shear stress response, stretch-activated channels are involved, as indicated by the blocked Ca^{2+} response after pre-treatment with gadolinium. The stretch response does not involve a pertussis toxin sensitive pathway since the Ca^{2+} response was not ablated or decreased by pre-treatment with pertussis toxin; however, data indicate that HTSCs utilize a pertussis toxin sensitive pathway in response to fluid shear stress. Cyclooxygenases are not involved in the stretch response since pre-treatment with indomethacin did not inhibit the Ca^{2+} signal. The data also show that the ATP does not temporarily reduce the response to stretch as seen in HTSCs stimulated by micropipette indentation. In conclusion, tendon cells use a variety of mechanisms to respond to mechanical signals with surrounding cells. Furthermore, tendon cells respond to substrate stretching differently as seen by the greater increase in Ca^{2+} signaling with statically stretched cells as compared to cyclically stretched cells. These findings indicate that tendon cells detect and respond to stretch and shear stress in different ways.

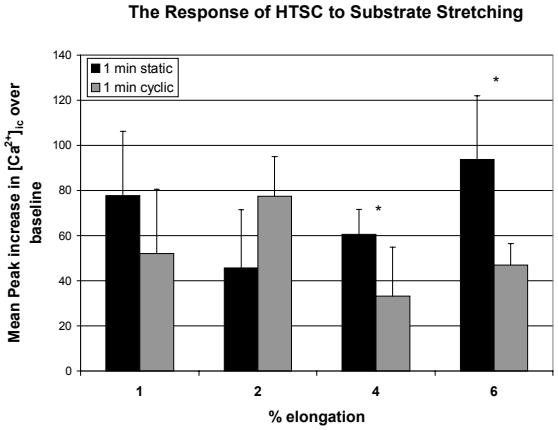

Figure 1. The response to substrate stretching was significantly greater for statically stretched cells at 4 and 6 % elongation as compared to cyclically stretched cells at the same strain ($p<0.05$).

Table 1. The response of HTSCs to static stretching and to ATP.

Static Stretch for 30 seconds at 6% elongation		
Modulators	Mean Peak Increase in $[Ca^{2+}]_{ic}$ over Baseline ± SD (nM) of all responding cells.	Ca^{2+} response to ATP
Control	92±12	+
Gadolinium (10 μM)	No Response	+
Pertussis Toxin (1 μM)	214±94*	+
Indomethacin (5 μM)	133±20	+
ATP (1 μM)	140±50	+
Ca^{2+}-Free Medium	111±13	+

*Statistically significant ($p<0.05$) response of responding cells as compared to control

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