FLUID-INDUCED SHEAR STRESS ACTIVATES HUMAN TENDON CELLS TO SIGNAL THROUGH MULTIPLE CA2+ DEPENDENT PATHWAYS

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Introduction: Fluid-induced shear stress occurs when fluid is convected past cells adherent to a surface. Flexor tendons are subjected to shear stress during normal function as tendons undergo tensile strain with accompanying fluid movement. Tendon cells respond quickly with an increase in intracellular calcium content when subjected to cyclic substrate. The fluid-induced shear stress associated with 0.01 substrate strain is an average of 0.017 dynes/cm^2. Preliminary data has indicated tendon cells signal with a calcium transient when exposed to fluid flow. However, the signaling pathways involved in this process are unknown. We hypothesized that prostaglandins, cAMP, ion channels and G proteins are components of the Ca^{2+} signaling response to fluid-induced shear stress in human tendon cells.

Methods: Cells from tendons of 3 patients were isolated from flexor digitorum profundus tendons, zone III, 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, 0.5 mM ascorbate, 20 mM HEPES pH 7.2, and antibiotics. To evaluate the $[Ca^{2+}]_{ic}$ response, cells were spot cultured at 2k cells/10 µL and grown to quiescence. The spots placed across the middle of a rectangular slide to prevent the shear stress response from being caused by the release of mediators from upstream cells. On the sixth day after culture, the cells were rinsed with Earles' Balanced Salt Solution with HEPES, pH 7.2, Ca^{2+} and Mg^{2+}, incubated at RT in 5 µM FURA-2AM for 90 min, then rinsed with EBSS. Cultures were incubated for 30 minutes with compounds that block signaling pathways then rinsed again with EBSS. The cover slips were transferred to a FlexFlo^{TM} laminar flow chamber interfaced with a MasterFlex peristaltic pump. The device 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 flow conditions then shear stress was applied for 2 minutes at 1 dyne/cm^2. Image I Metamorph software was used to monitor $[Ca^{2+}]_{ic}$ in 25 to 30 cells per field segregated in quadrant.

Results: Surface epitenon cells from human tendons responded to 1 dyne/cm^2 fluid-induced shear stress with a 6-fold increase in intracellular calcium level over baseline (Figure 1; see Table 1 for group names, values are % Sheared control; * = $p<0.05$). About 80% of the cells responded to the shear stimulus: cells in every sector of the field signaled almost simultaneously. Pretreatment of the cells with 5 µM indomethacin reduced the response (74% of control) to shear stress at 1 dynes/cm^2 without delaying onset or duration (Table 2). Celicoxib-treated cells had a delayed onset and greater maximum response. Treatment with 5 µM verapamil, an inhibitor of L-type calcium channels, did not block the response. Treatment with 5 µM gadolinium, a stretch-activated channel blocker, did not block the response. Pertussis toxin (ADP-ribosylates Gα subunit) at 1 µg/ml delayed the shear response by 29 sec, reduced its level to 48% of control, but did not abolish signaling completely. Cholera toxin (activates adenylate cyclase) did not delay or reduce the response. Treatment of cells with all compounds simultaneously did not block the rise in $[Ca^{2+}]_{ic}$ in response to shear stress. Results were the same even when cells were challenged with a higher shear stress (3dynes/cm^2). All cells except the gadolinium treated ones responded significantly to addition of ATP post-flow (% No flow control, * $p<0.05$) as a positive control. Shear stress of up to 25 dynes/cm^2 in calcium-free medium did not result in an increase in intracellular calcium.

Conclusions: Shear stress can activate signaling pathways leading to secretion of mediators such as ATP, PGE_2 and cAMP and activate transcription factors leading to altered gene expression. Low magnitude (1 dyne/cm^2) fluid-induced shear stress can incite human tendon epitenon cells to increase their $[Ca^{2+}]_{ic}$ dramatically, within seconds after initiating flow. Therefore, fluid-induced shear stress and shear stress as a consequence of substrate strain act in different ways to cause cell signaling. Partial but not complete inhibition of the response by indomethacin (inhibits COX I and II) and Celicoxib (inhibits mainly COX II) indicate that cyclooxygenase activation and elaboration of prostaglandins is partly involved in the mechanism. Verapamil-sensitive calcium channels do not appear to be involved in the response to fluid-induced shear stress in tendon cells, however stretch-activated channels are involved. Partial inhibition of the response by Gα subunit blockade indicates that this G protein-dependent pathway may also be involved. Adenylate cyclase activation reduced the response, implicating cAMP as a response modifier. Cells treated simultaneously with the combination of all compounds responded to shear stress by increasing $[Ca^{2+}]_{ic}$, indicating an inhibitory pathway(s) that usually prevents a response to shear stress may have been blocked. These results indicate that the $[Ca^{2+}]_{ic}$ response to shear stress in tendon cells may be activated by several different signaling mechanisms. A potentially novel inhibitory pathway may be present, underscoring the diversity and redundancy of the shear stress detection systems in tendon and that these pathways are positively and negatively regulated.

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TABLE I Treatment	Pre-Flow Level	Shear Stress 1dyne/cm^2	ATP Post-Flow
1-No shear control	100	NA	500*
2-Sheared control	100	600*	400*
3-Indomethacin	100	74*	300*
4-Celicoxib	100	148	150*
5-Verapamil	150	137	200*
6-Gadolinium	150	113	150
7-Pertussis toxin	100	48*	350*
8-Cholera toxin	100	95	300*
9-Combination all	100	123	400*

[Ca 2+]ic in Human Tendon Cells Treated with Signaling Pathway Modulators

Figure 1

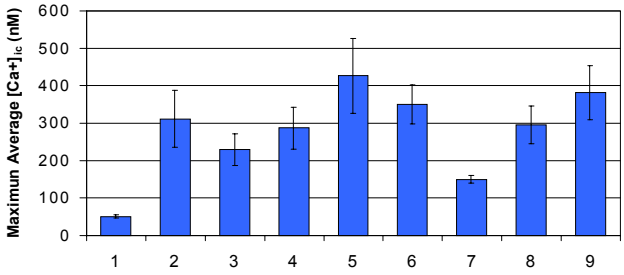

TABLE II Treatment	Delay from flow initiation to onset of signaling (s)	Duration of signaling (s)
1-No shear control	NA	NA
2-Sheared control	28	129
3-Indomethacin	28	123
4-Celicoxib	100	228
5-Verapamil	35	155
6-Gadolinium	30	149
7-Pertussis toxin	57	131
8-Cholera toxin	22	151
9-Combination all	26	160

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