

**UPREGULATION OF STRESS-ACTIVATED PROTEIN KINASES (SAPKs)
IN RESPONSE TO CYCLIC STRAIN OF TENDON CELLS: A potential cellular
mechanism for repetitive stress injuries in tendons**

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INTRODUCTION

The strain-induced signaling of cells through mechanotransduction pathways has been shown to play a significant role in maintaining the normal homeostasis of connective tissues. While cyclic loading has been shown to be beneficial to tendon health, repetitive tissue strain has also been implicated in the etiology of repetitive stress (overuse) injuries of tendons. Although the precise mechanism(s) by which repetitive strain initiates tissue injury is still unknown, it is likely that this is a cell-mediated event.

Previous studies in our laboratory have demonstrated that cells respond to physical stress by an up-regulation of stress-activated protein kinases (SAPKs) such as c-Jun N-terminal kinase (JNK). This appears to be mediated through a calcium dependent mechanotransduction pathway. SAPKs are a family of signal transduction proteins that are activated under a diverse set of environmental cellular stresses. The prolonged activation of JNK and its subsequent phosphorylation of various transcription factors have been implicated in the initiation of the apoptosis cascade in some cell lines. Induction of apoptosis and subsequent localized cell death may be an initiating factor in the pathogenesis of overuse injuries. Our lab has investigated the effects of cyclic strain as well as other environmental factors (i.e. heat, osmotic stress, etc) which have been implicated in the etiology of repetitive stress injuries on the upregulation of JNK in both tendon cells in monolayer as well as tendon cells *in situ*.

METHODS

Canine patellar tendon fibroblasts (TFBs) as well as rat tail tendons (RTTs) from adult Sprague-Dawley rats were used to evaluate the effect of cyclic strain as well as environmental conditions on the *in vitro* upregulation of SAPKs. The TFBs were cyclically strained in monolayer using a Flexercell® strain unit while the RTTs were cyclically strained using a custom designed computer driven cyclic strain device. The TFBs and the RTTs were cyclically loaded for 2hrs and phosphorylated JNK (p-JNK) expression was evaluated on a Western blot using a polyclonal antibody to p-JNK. The effect of strain amplitude as well as strain frequency on p-JNK was also examined. In addition, the effect of environmental stresses (hyperthermia [44°C], osmotic stress, and hypoxia), alone and in combination, on p-JNK expression were also evaluated.

RESULTS AND DISCUSSION

Cyclic strain resulted in an immediate up-regulation of p-JNK at 15 minutes. This activity peaked at 30 minutes and returned to near resting levels at 120 minutes. While the reason for this decrease in p-JNK expression is not clear it could be due to an intracellular phosphatase system that is activated to regulate p-JNK expression.

The magnitude of cyclic strain was found to have a dose-dependent effect on the activation of phosphorylated JNK in both monolayer TFBs and tendon cells *in situ* (RTTs). However, the activation of p-JNK

was not affected by the frequency of the cyclic strain in either system.

Environmental stresses such as hyperthermia and hyperosmolality each result in an upregulation of p-JNK. When combined with cyclic strain both hyperthermia and hyperosmolality result in a persistent increase in p-JNK expression when compared to cyclic strain under normal environmental conditions. Thus, it appears that the upregulation of p-JNK expression can be cumulative. It is possible that under certain environmental stresses (which have been implicated in the pathogenesis of overuse injury) p-JNK expression could be prolonged enough to initiate localized cell death. This “intrinsic” stimulus may contribute to the pathogenesis of repetitive stress injuries in tendons.

The effect of hypoxia, alone and in conjunction with cyclic strain, as well as the effect of various combinations of environmental alterations on the expression of p-JNK is currently being evaluated.

SUMMARY

Cyclic straining of tendon cells in monolayer or *in situ* result in an upregulation of SAPKs. This occurs in a dose-dependent manner, which is amplitude regulated and not frequency regulated. Environmental stresses (hyperthermia, osmotic stress) have also been shown to upregulate p-JNK expression. We propose a cellular mechanism by which cyclic strain and local environmental conditions could produce an intrinsic stimulus, which may contribute to the pathogenesis of repetitive stress injuries.