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The Role of $P2Y_1$ and $P2Y_2$ Purinoceptors in Determining Mechanosensitivity in Connective Tissue Cells

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1 Introduction

Cells from diverse tissues differ in strain sensitivity. Osteoblasts respond to 0.5% strain whereas tenocytes respond to 1% strain. Moreover, specific response pathways have different activation thresholds. Experiments with tenocytes from purinoceptor knockout mice lacking an ATP receptor, fail to detect a mechanical input signal. We hypothesized that ATP was stimulatory and ADP was inhibitory to mechanical input stimuli to connective tissue cells.

2 Materials and Methods

Tenocytes from whole tail tendons or cells isolated from Achilles tendons of wt, P2Y1 and P2Y2 KO mice were used in calcium signaling experiments. Continuous osteoblast cell lines were also used (MC3T3E1). Tenocytes in whole tendons or cells plated in microspots (3K cells/10µlspot) were incubated with fura 2AM, washed and subjected to ratio imaging at 340/380 excitation and 510 and above emission in response to mechanical deformation or ATP or ADP ligand addition. In the matrix contraction experiment, the contraction rates of MC3T3-E1 cell-populated three-dimensional type I collagen gels were recorded daily in the absence or presence of 500 µM ATP and the agonists or antagonists of P2 receptors.

3 Results

Applied strain failed to elicit a Ca2+ signaling response in P2Y2-/- tenocytes lacking the ATP receptor whereas tenocytes from P2Y1-/- mice were hyper-responsive to strain. Likewise, tenocytes in whole tail tendons from wt mice were responsive to 1, 2 and 3 % strain in a dose response manner, cells in P2Y1-/- tendons were 2 fold more sensitive at 1% strain and 30% more responsive at 3% strain, whereas cells in P2Y2-/- or the double knockout tendons were far less responsive. ATP at $\geq$ 100 µM reduced the contraction rate of MC3T3-E1 cell-populated collagen gels by down-regulating the expression of integrin α1. Further studies showed that down-regulation of integrin α1 decreased cell attachment on type I collagen. By using P2 receptor specific agonists and antagonists, it was shown that both P2X and P2Y receptors are responsible for the regulation of three dimensional matrix contraction. The P2X4 receptor may be the major receptor involved in this process.

4 Discussion

These are the first results suggesting an interplay of ATP and ADP in regulating the degree of mechanosensitivity in connective tissue cells. ATP appears to be a co-stimulatory signal with strain to increase signal transduction to load. ADP is an antagonist that dampens the response. We postulate that this nucleotide/nucleoside pair represents a feedback control system to modulate a load response and negatively control the magnitude of mechanical signaling.

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