Hdud, Ismail M. and Mobasheri, Ali and Loughna, Paul (2014) Effects of cyclic equibiaxial mechanical stretch on α-BK and TRPV4 expression in equine chondrocytes. SpringerPlus, 3 . 59/1-59/3. ISSN 2193-1801

SpringerPlus
a SpringerOpen Journal

SHORT REPORT Open Access

Effects of cyclic equibiaxial mechanical stretch on α-BK and TRPV4 expression in equine chondrocytes

Ismail M Hdud[1], Ali Mobasheri[1,2] and Paul T Loughna[1,2*]

Abstract

Background: Chondrocytes are regularly exposed to load-induced stimuli and have the capability to sense and respond to applied mechanical stress. However, the mechanisms involved in chondrocyte mechanotransduction are not clearly understood. The purpose of this study was to explore the effects of cyclic equibiaxial mechanical stretch on the expression of α-BK and TRPV4 channels.

Findings: Freshly isolated equine articular chondrocytes were subjected to mechanical stress (8% elongation at frequency of 0.5 Hz for 8 h). Western blotting was used to investigate the expression of BK_{Ca} and TRPV4 channel proteins. Mechanical stretch increased the expression of BK_{Ca} channels by 1.8 fold but TRPV4 expression was not affected.

Conclusions: Upregulation of BK_{Ca} channel may be the result of direct membrane stretch or elevated intracellular Ca^{2+}.

Keywords: Chondrocyte; Mechanical stretch; TRPV4; BK channel; Mechanotransduction

Introduction

Chondrocytes are the only resident cells within the extracellular matrix (ECM) of articular cartilage (Archer and Francis-West, 2003). They are highly sensitive to mechanical load and are routinely exposed to a diverse variety of mechanical stimuli (Urban, 1994). Although biomechanical factors are important for articular chondrocyte metabolism and the synthesis, maintenance and degradation of ECM (Inoue et al., 1990) excessive or inappropriate mechanical loads can lead to harmful effects on cartilage. This in turn can lead to the initiation and progression of joint diseases such as osteoarthritis (OA) (Guilak, 2011).

Stretch-induced deformation of the chondrocyte membrane is thought to be one of the key processes involved in the responses to mechanical stimulation (Martina et al., 1997). The ion channels involved in chondrocyte mechanotransduction pathways have not been unambiguously identified. However, there are several candidates including large conductance "big" potassium channels (BK) and transient receptor potential vanilloid (TRPV) channels. BK channels are activated by increases in $[Ca^{2+}]_i$, and membrane potential (Huang et al., 2011). BK channels play an important role in various physiological functions such as regulation of vascular smooth muscle tone, endocrine cell secretion and neuronal firing (Xiang et al., 2011). Recent studies have identified BK channels in articular chondrocytes (Mobasheri et al., 2010). TRPV channels, especially isoforms 1–4 are moderately Ca^{2+} permeable channels (Phan et al., 2009; Nilius and Owsianik 2011) that play diverse roles in cellular function and TRPV4 seems to have a mechanosensory role (Wang et al., 2011, Liedtke et al., 2000). The aim of this study was to determine whether the expression levels of these purported mechanosensory ion channels are themselves modulated by mechanical signals.

Methods

Cartilage shavings (including superficial and middle zones) were dissected from metacarpophalangeal joints of healthy mature horses euthanized for unrelated clinical reasons. Ar-

* Correspondence: paul.loughna@nottingham.ac.uk
[1]School of Veterinary Medicine and Science, Faculty of Medicine and Health Sciences, The University of Nottingham, Sutton Bonington Campus, Leicestershire LE12 5RD, UK
[2]Medical Research Council-Arthritis Research UK Centre for Musculoskeletal Ageing Research, Leicestershire, UK

ticular chondrocytes were isolated as previously described (Mobasheri et al., 2010). Chondrocytes were cultivated in monolayer in DMEM supplemented with 10% fetal calve serum (FCS) and 2% antibiotics. The chondrocyte phenotype was maintained by not passaging the cells beyond passage 2.

Equine articular chondrocytes were cultivated in Bioflex® tissue culture plates pre-coated with collage type I (Flexcell International Corporation USA) at 2×10^{-5} per well in DMEM medium and cultured at 37°C, 95% air and 5% CO_2 until sub-confluent. Equibiaxial mechanical stretching was conducted using an *in vitro* system (Flexercell Strain Unit, FX-4000, Flexcell International Corporation USA) as we have described previously (Atherton et al., 2009). Medium was refreshed every other day. In the current study, chondrocytes were subjected to 8% elongation at 0.5 Hz frequency continuously for 8 hours inside the cell culture incubator. The control cells were grown on the same type of plates and kept at the same conditions without exposing to mechanical stress. Western blotting was carried out as previously described (Atherton et al., 2009) using rabbit polyclonal antibodies against TRPV4 (Abcam) and α-BK (Sigma Aldrich).

Statistical analysis: Average data are presented as a mean ± S.E.M, Student's t-test or ANOVA was used to determine the statistical significance, followed by Bonferroni's test for multiple comparisons. $P \leq 0.05$ considered statistically significant.

Results and discussion

Cyclic stretching significantly increased the expression of the α-BK channel (by 1.8 fold) but in contrast the expression of the TRPV4 channel was not effected by mechanical stretch (Figure 1).

The response of the α-BK channel to mechanical stretch suggests that membrane stretching may upregulate this channel directly. The α-BK channel present in articular chondrocytes may function as a mechanosensor and elevation in tensile stress could lead to activation of the channel. In the current study the increased expression of this channel was observed in response to stretch suggests that upregulated expression may be linked to

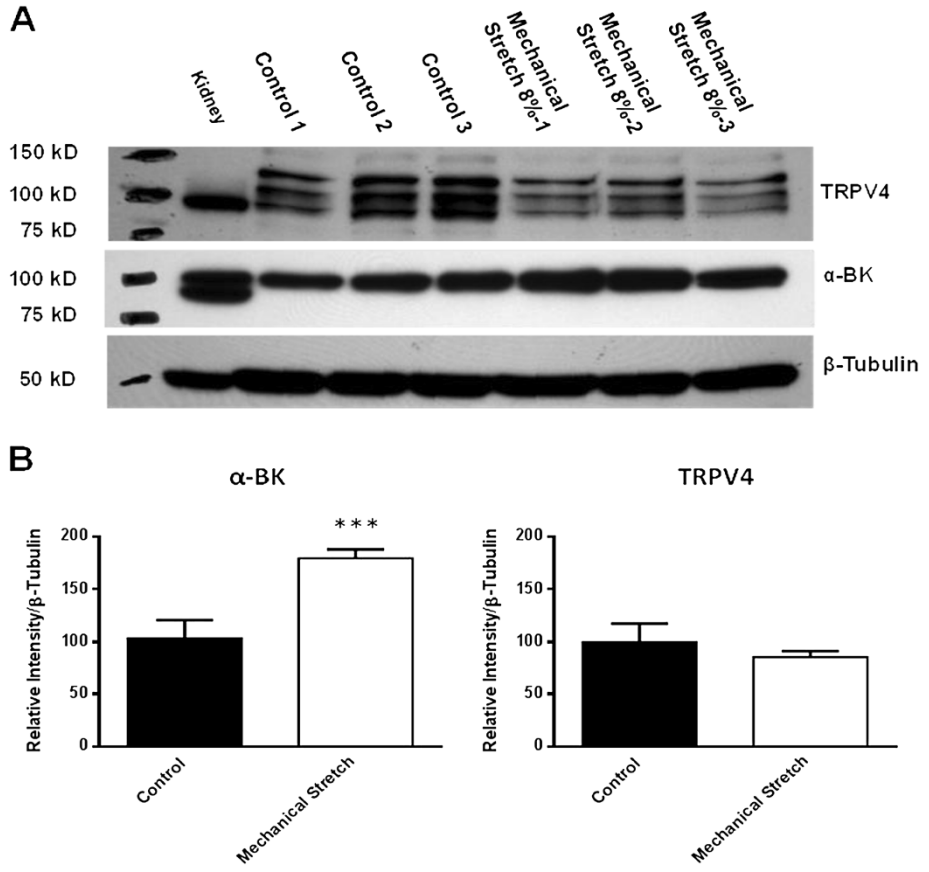

Figure 1 The effects of mechanical stretch on α-BK and TRPV4 protein levels in chondrocytes. (A) Expression of α-BK and TRPV4 channels in equine articular chondrocytes following exposure to mechanical stretch for 8 h. **(B)** The fold increase in the expression of α-BK and TRPV4 channels based on densitometric analysis of western blot by comparing to a housekeeping protein (β-tubulin). Data presented the mean ± S.E. and statistical significance is indicated by *** = $P < 0.001$ compared to unstretched controls.

the activation of the channel. Application of cyclic tensile stretch to rat growth plate chondrocytes was shown to enhance the synthesis of ECM proteins such as of collagen and aggrecan (Mouw et al., 2007).

In load bearing joints chondrocytes are exposed to mechanical forces that lead to membrane deformation, which in turn, may significantly alter cartilage matrix production (Urban, 1994). Chondrocyte membrane stretch could occur by application of mechanical compression leading to cartilage deformation and consequently, deforming the chondrocyte to an approximate elliptical shape (Liappis et al., 2011; Urban, 1994). In this study, the stretching of the membrane occurs through the cell deformation applied by direct mechanical stretch which could lead to activation of several intracellular signaling cascades. Increased expression of the BK channel through mechanical stretching could indicate enhanced responsiveness of a mechano-sensing channel (Ca^{2+}-independent). An alternative hypothesis for BK channel upregulation requires the elevation of intracellular Ca^{2+} concentration (Ca^{2+}-dependent) that could occur through intracellular Ca^{2+} release (Grandolfo et al., 1998) or Ca^{2+} entry through Ca^{2+} channels such as L-type voltage gated Ca^{2+} channel (VGCC) and/or members of TRP channel family. The level of TRPV4 protein was not significantly effected following exposure to mechanical stress which is interesting as it has been previously shown that the overall activity of this channel is directly related to its expression level (Veys et al., 2012).

Conclusions

The results of this study suggest that in equine articular chondrocytes the α-BK but not the TRPV4 channel is upregulated by cyclic equibiaxial membrane stretch.

Competing interests

The authors declare no conflict of interests.

Authors' contributions

IMH carried out the Flexcell studies and western blotting, was involved in experimental design and drafting the manuscript; AM was involved in drafting the manuscript and PTL participated in the Flexcell studies, was involved in experimental design and drafting the manuscript. All authors read and approved the final manuscript.

Acknowledgments

I. M. Hdud was supported by a scholarship from the Libyan Ministry of Higher Education.

Received: 18 September 2013 Accepted: 21 January 2014
Published: 29 January 2014

References

Archer CW, Francis-West P (2003) The chondrocyte. Int J Biochem Cell Biol 35(4):401–404

Atherton PJ, Szewczyk NJ, Selby A, Rankin D, Hillier K, Smith K, Rennie MJ, Loughna PT (2009) Cyclic stretch reduces myofibrillar protein synthesis despite increases in FAK and anabolic signalling in L6 cells. The Journal of physiology 587(Pt 14):3719–3727

Grandolfo M, Calabrese A, D'Andrea P (1998) Mechanism of mechanically induced intercellular calcium waves in rabbit articular chondrocytes and in HIG-82 synovial cells. J Bone Miner Res 13(3):443–453

Guilak F (2011) Biomechanical factors in osteoarthritis. Best Pract Res Clin Rheumatol 25(6):815–823

Huang H, Pan Y, Ye Y, Gao M, Yin Z, Luo L (2011) Dipyrithione attenuates oleic acid-induced acute lung injury. Pulm Pharmacol Ther 24(1):74–80

Inoue H, Hiasa K, Samma Y, Nakamura O, Sakuda M, Iwamoto M, Suzuki F, Kato Y (1990) Stimulation of proteoglycan and DNA syntheses in chondrocytes by centrifugation. J Dent Res 69(9):1560–1563

Liappis AP, Gibbs KW, Nylen ES, Yoon B, Snider RH, Gao B, Becker KL (2011) Exogenous procalcitonin evokes a pro-inflammatory cytokine response. Inflamm Res 60(2):203–207

Liedtke W, Choe Y, Marti-Renom MA, Bell AM, Denis CS, Sali A, Hudspeth AJ, Friedman JM, Heller S (2000) Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. Cell 103:525–535

Martina M, Mozrzymas JW, Vittur F (1997) Membrane stretch activates a potassium channel in pig articular chondrocytes. Biochim Biophys Acta 1329 (2):205–210

Mobasheri A, Lewis R, Maxwell JE, Hill C, Womack M, Barrett-Jolley R (2010) Characterization of a stretch-activated potassium channel in chondrocytes. J Cell Physiol 223(2):511–518

Mouw JK, Imler SM, Levenston ME (2007) Ion-channel regulation of chondrocyte matrix synthesis in 3D culture under static and dynamic compression. Biomech Model Mechanobiol 6(1–2):33–41

Nilius B, Owsianik G (2011) The transient receptor potential family of ion channels. Genome Biol 12. doi:10.1186/gb-2011-12-3-218

Phan MN, Leddy HA, Votta BJ, Kumar S, Levy DS, Lipshutz DB, Lee SH, Liedtke W, Guilak F (2009) Functional characterization of TRPV4 as an osmotically sensitive ion channel in porcine articular chondrocytes. Arthritis Rheum 60(10):3028–3037

Urban JP (1994) The chondrocyte: a cell under pressure. Br J Rheumatol 33(10):901–908

Veys K, Labro AJ, De Schutter E, Snyders DJ (2012) Quantitative single-cell ion-channel gene expression profiling through an improved qRT-PCR technique combined with whole cell patch clamp. J Neurosci Methods 209:227–234

Wang IC, Ueng SW, Lin SS, Niu CC, Yuan LJ, Su CI, Chen CH, Chen WJ (2011) Effect of hyperbaric oxygenation on intervertebral disc degeneration: an in vitro study with human lumbar nucleus pulposus. Spine (Phila Pa 1976) 36(23):1925–1931

Xiang D, Guo Y, Zhang J, Gao J, Lu H, Zhu S, Wu M, Yu Y, Han W (2011) Interleukin-1 receptor antagonist attenuates cyclophosphamide-induced mucositis in a murine model. Cancer Chemother Pharmacol 67(6):1445–1453

doi:10.1186/2193-1801-3-59
Cite this article as: Hdud *et al.*: Effects of cyclic equibiaxial mechanical stretch on α-BK and TRPV4 expression in equine chondrocytes. *SpringerPlus* 2014 **3**:59.