TRPC3 activates K_{Ca} to modulate chondrocyte cell volume regulation

R. Lewis[1] and R. Barrett-Jolley[1]

[1]Institute of Ageing and Chronic Disease, University of Liverpool, UK

Healthy chondrocytes exist with depolarised resting membrane potentials (*Vm*); critical for volume regulation[1]. Chondrocytes express several cell volume regulation channels, including Transient Receptor Potential (TRP) V4, V5 and V6 and epithelial sodium channels[1-3]. We investigated mechanisms of cell volume regulation in healthy chondrocytes.

Patch clamp electrophysiology was used to measure canine chondrocyte *Vm* upon application of hypotonic saline (*Vm*=-12±3mV; n=21). Hypotonic challenge caused a significant RMP hyperpolarisation of 11±1mV (n=9; $p<0.05$). We hypothesised this was due to Ca^{2+} influx activating a Ca^{2+}-activated potassium channel (K_{Ca}).

Studies propose TRPV4 as a possible component of Ca^{2+} influx so we investigated this channel. However, the TRPV4 inhibitor, RN1734, failed to prevent hypotonicity-induced hyperpolarisation (ΔVm=-19±4mV, n=4; $p<0.05$). Furthermore, this hyperpolarisation was significantly greater than hyperpolarisation with hypotonic solution alone ($p<0.05$). Application of TRPV4 agonist, 4αPDD, caused no significant change in *Vm* (*n*=12), whole cell current (*n*=7) or cell volume (*n*=8). Inhibiting the large-K_{Ca} (BK) and TRPC channels did abolish hypotonicity-induced hyperpolarisation (ΔVm=6±2mV; n=6 and ΔVm=1±2mV; n=4, respectively). TRPC3 was identified as the channel contributing to calcium influx; inhibition of this channel prevented hypotonicity-induced hyperpolarisation (XXXmV; *n*=3).

This leads us to conclude that TRPV4 may not be the central mediator of cell volume regulation as previously proposed. We propose a coupling of the TRPC3 and BK channels contributes to control of chondrocyte volume regulation.

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