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TRPV1-dependent ERK1/2 activation in porcine lens epithelium

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ABSTRACT

Recently we determined that the Transient Receptor Potential Vanilloid 4 ion channel (TRPV4) has a crucial signaling role in a pathway that regulates various aspects of lens epithelium function. Here, we report on a different TRPV channel, TRPV1, in porcine lens. The presence of TRPV1 in the lens was evident from RT-PCR studies and Western blot analysis of MAPK signaling pathway activation caused by the TRPV1 agonist capsaicin. TRPV1 mRNA was detected in the epithelium of porcine as well as human lens. Transient ERK1/2 and p38 MAPK phosphorylation was detected within 1 min in the epithelium isolated from intact porcine lenses exposed to capsaicin (100 nM), a selective TRPV1 agonist, and the response was significantly inhibited by A889245 (1.0 μM), a TRPV1 antagonist. A similar ERK 1/2 and p38 response in the epithelium, also inhibitable by A889245, was evident in lenses treated with hyperosmotic solution (350 vs 300 mOsm). Lenses pre-treated with either the cytosolic Ca^{2+} chelator BAPTA-AM or the PKC inhibitor sotrastaurin (1.0 μM) had a diminished ERK1/2 activation response to capsaicin and hyperosmotic solution. Taken together the findings support the notion that TRPV1 functions as a plasma membrane ion channel that, when activated, permits the entry of extracellular calcium into the lens epithelium, leading to activation of PKC, ERK1/2 and p38 MAPK. It is significant that the findings confirm earlier proposals that hyperosmotic stress is linked to TRPV1 channel activation in the mouse lens. Further studies are ongoing to determine what functional changes are triggered by the TRPV1-linked signaling pathways and how they might relate to lens volume homeostasis.

1. Introduction

The lens has two cell types, densely packed fibers that make up most of the structure and a monolayer of epithelial cells that covers the anterior face. Mature fiber cells, which lack organelles, are differentiated to the extent that homeostasis of the fiber mass depends on the epithelium. Functional integration between the two cell types is made possible by the unique architecture of the lens and by efficient coupling between neighboring fibers (Goodenough et al., 1980; Rae and Kuszak, 1983; Lo and Harding, 1986; Bassnett et al., 1994; Kuszak et al., 1995; Mathias et al., 2010). Because mature fiber cells have scant Na,K-ATPase activity (Delamere and Dean, 1993), the lens must rely to a large extent on the epithelium for sodium-potassium homeostasis of the fiber mass (Gao et al., 2000). Epithelial cells have robust Na,K-ATPase activity (Tamiya et al., 2003) (Delamere and Dean, 1993).

Recent studies showed evidence for a TRPV4 channel-dependent signaling mechanism in the epithelium that responds in lenses treated with low osmotic strength solution (Shahidullah et al., 2012) or damage to the fiber mass (Shahidullah et al., 2015). TRPV4 activation initiates signaling pathway responses that lead to changes in epithelium function. TRPV4-dependent responses include hemichannel opening, ATP release and an increase in the activity of Na,K-ATPase in the epithelium (Shahidullah et al., 2012; Mandal et al., 2015). Consistent with TRPV4 activation having a role in the swelling response to hyposmotic solution, a study in mouse lens pointed to activation of TRPV4 by increased cellular hydrostatic pressure. Interestingly, the contrasting response of the mouse lens to hyperosmotic solution was insensitive to TRPV4 antagonists but sensitive to ruthenium red, leading to the proposal it is TRPV1-dependent. Moreover, the mouse lens was found to display a transient hydrostatic pressure response to the TRPV1 agonist capsaicin (Gao et al., 2015). These findings suggest that the lens might express functional TRPV1 channels in addition to TRPV4. Studies here were carried out to determine whether TRPV1 responses are detectable in the porcine lens.

2. Methods

2.1. Materials

A889425 was purchased from Alomone Labs (Hadassah Ein Kerem,

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Jerusalem BioPark, Israel). Capsaicin, BAPTA-AM and U0126 were purchased from Sigma (St. Louis, MO, USA). The following antibodies were sourced from Cell Signaling Technology (Danvers, MA, USA): mouse monoclonal anti phospho-P44/42 MAP kinase (Thr-202/Tyr-204); rabbit polyclonal anti P44/42 MAP kinase; rabbit polyclonal anti-phospho-P38 MAPK (Tyr-180/Tyr-182); rabbit polyclonal anti SAPK/JNK; mouse monoclonal anti phosphor-SAPK/JNK (Thr-183/Tyr-185). Other antibodies were: mouse monoclonal p38 (Thermo Fisher Scientific, Waltham, MA); mouse monoclonal anti-AKT (GenWay Biotechnology, San Diego, CA); rabbit polyclonal anti-phospho-AKT (Ser-473) (Santa Cruz Biotechnology, Dallas, TX). Secondary antibodies (LI-COR Biosciences Lincoln, NE, USA) were: goat anti-rabbit conjugated with IRDye 680 and goat anti-mouse conjugated with IR Dye 800. The Micro BCA Protein Assay Kit was from Thermo Fisher Scientific. Sense and anti-sense primers for human and porcine TRPV1 were obtained from Integrated DNA Technologies, Inc. (Coralville, Iowa).

2.2. Krebs solution

Lenses were incubated at 37 °C in Krebs solution, control osmolarity 300 mOsm, that was equilibrated with 5% CO_2 and adjusted to pH 7.4 prior to use. Krebs solution composition was (in mM): 119 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$, 1 $MgCl_2$, and 5.5 glucose. In specified experiments lenses were incubated in hyperosmotic Krebs solution in which osmolarity was increased to 350 mOsm by addition of mannitol. Osmolarity was checked, and adjusted is necessary, by using a 5004 Micro-Osmette osmometer (Precision Systems Inc., MA).

2.3. Lenses

Pig eyes were purchased from the Meat Science Laboratory at the University of Arizona, or from Hatfield Quality Meats (Philadelphia, PA), or from West Valley Processing (Buckeye AZ) and the use of animal tissue for research was approved by the Institutional Animal Care and Use Committee at the University of Arizona. Human donor eyes were purchased from the National Disease Research Interchange (Philadelphia, PA) and their use was approved by the Institutional Review Board at the University of Arizona. To obtain the lens, the rear of the globe was dissected open and the suspensory ligaments were cut so the lens could be removed intact and undamaged, then placed in Krebs solution. Lenses were incubated at 37 °C for at least 3 h in control Krebs solution before experiments commenced. In specified experiments the capsule-epithelium was removed from the intact lens by using fine forceps to create a small tear, then peeling it from the fiber mass.

2.3.1. RNA isolation

Total RNA was isolated with an RNeasy Mini kit (Qiagen, CA, USA). Freshly isolated lens capsule-epithelium was lysed in RLT buffer containing 1% β-mercaptoethanol and homogenized using a battery operated hand-held Kimble Kontes tissue homogenizer (DWK Life Sciences, NJ). The tissue lysate was placed onto a QIAshredder column and centrifuged for 2 min at 14 000 rpm. The eluent was placed onto an RNeasy mini column in order to bind RNA. After washing, the RNA was eluted by 50 μl of RNase-free water and then quantified at 260/280 nm using a spectrophotometer (NanoDrop technologies, Inc., Wilmington, DE).

2.3.2. First-strand cDNA synthesis and polymerase chain reaction

RT-PCR was carried out as described earlier (Pelis et al., 2009). Briefly, 0.5 μg total RNA was reverse transcribed to cDNA by using SuperScript III Reverse Transcriptase (Thermo Scientific, USA) following manufacturer's protocol on an Applied Biosystem Gene Amp PCR System (Model 9700; Thermo Scientific, USA). Complementary DNA (5 μl) was used for PCR reaction for gene amplification using

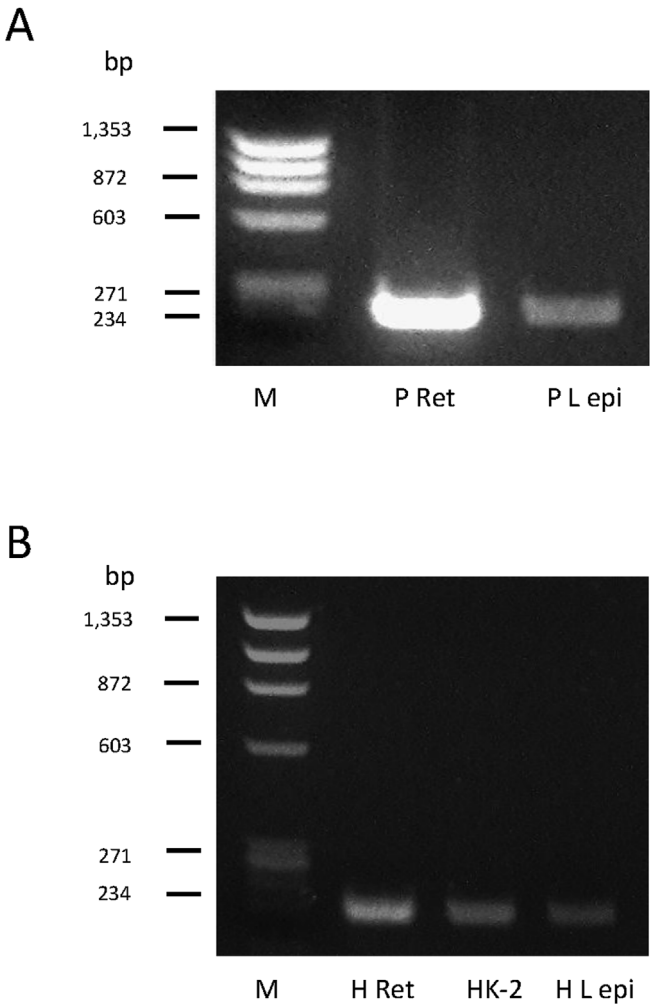

Fig. 1. RT-PCR detection of the TRPV1 message in porcine and human lens epithelium. Human or porcine retina and human kidney were included as positive controls. M = molecular marker; P Ret = Pig retina; P L epi = Pig lens epithelium; H Ret = Human retina; HK-2 = Human Kidney-2 cells; H L epi = Human lens epithelium.

Platinum Pfx DNA Polymerase kit (Thermo Scientific, USA) following manufacturer recommended protocol. The studies used custom-designed TRPV1 primers (Integrated DNA Technologies, Inc., IA, USA) for pig (forward: 5′- GGACAAGCTGTGGGAATCAT-3′, reverse: 5′- TGGGA TTCGCTACCTTTCAG-3′) and for human (forward: 5′- AAGCCCAGGA AAACACCTTT-3′, reverse: 5′- CTGCTGCAACAGCTTGATTC-3′). We used a 2-min hold at 94 °C and then 35 30sec cycles of denaturing at 94 °C, 30 s annealing at 55 °C, and an extension at 72 °C for 1 min. At the end of the reaction PCR product was analyzed by electrophoresis on an agarose gel (2%) containing ethidium bromide (0.2 μg/ml). φX174 DNA Marker Hae III Digest was used as base pair standards. Signals were visualized by UV exposure employing a benchtop UV transilluminator (UVP Inc., USA). Images were captured using a high resolution camera.

2.4. Western blot

The capsule-epithelium was homogenized in ice-cold lysis buffer (pH 7.5) with the composition (in mM): 50 HEPES, 150 NaCl, 1 EDTA, 10 sodium fluoride, 10 sodium pyrophosphate, 2 sodium orthovanadate, 10% glycerol, 1% sodium deoxycholate and 1% Triton X-100 as well as a protease inhibitor cocktail (Thermos Fisher Scientific, IL) and phosphatase inhibitor 1 and 2 cocktails (EMD Millipore, MA). Using a