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MECHANOSENSITIVITY OF THE TRPC6 ION CHANNEL

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Human Physiology

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

Victor Chang Cardiac Research Institute

Statement of originality

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision.

The thesis contains no material which has been accepted, or is being examined, for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

Statement of collaboration

I hereby certify that the work embodied in this dissertation has been done in collaboration with other researchers, or carried out in other institutions. I have included as part of the dissertation a statement clearly outlining the extent of collaboration, with whom and under what auspices. HEK293 stretching experiments in Chapter 3 was done by me at the Friedrich-Alexander University of Erlangen-Nuremberg, Erlangen, Germany. TIRF microscope images in Chapter 4 were acquired by Dr. Massimo Vassalli at the University of New South Wales, Sydney, Australia. Dr Vassalli was a visiting Endeavour Fellow from the Institute of Biophysics National Research Council, Genova, Italy. Purified mouse TRPC6 protein used in Chapter 5 was acquired from Dr. Valeria Vasquez and Dr. Julio F. Cordero-Morales laboratory (The University of Tennessee, Knoxville, TN, USA). The electrophysiology with this protein was done by me at the Victor Chang Cardiac Research Institute (VCCRI), Sydney, Australia. The isolation of the cardiomyocytes from normal and TAC induced mice were acquired by Yang Guo at the VCCRI, Sydney, Australia. Stretching of the hypertrophy induced cardiomyocytes in Chapter 6 was done together with Dominik Schneidereit at the VCCRI, Sydney, Australia. Dominik was a visiting PhD student from the Friedrich-Alexander University Erlangen-Nuremberg Erlangen-Nuremberg. The rest of the work was done by me at the VCCRI, Sydney, Australia.

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Publications

Conferences

- Australian Physiological Society (AuPS) and Australian Society for Biophysics (ASB) Meeting in Adelaide, Australia, Dec 4-7, 2016, (*Oral*)
- The Victor Chang Cardiac Research Institute International Symposium, Sydney, Australia, Nov 6-8, 2016, (*Poster*)
- Cardiac Mechano-Electric Coupling and Arrhythmias, Freiburg, Germany, Sep 21-24, 2016, (*Poster*)
- Biophysical Society (BPS) 60th Annual Meeting, Los Angeles, USA, Feb 27-Mar 2, 2016, (*Poster*)
- The 39th Annual Meeting of the Australian Society for Biophysics. Armidale, Australia, Nov 22-25, 2015, (*Oral*)
- The 23d St Vincent's Health Australia Sydney Precinct Research Symposium. Sydney, Australia, Sep 10-11, 2015, (*Poster*)
- The 10th European Biophysics Congress, Dresden, Germany, 18-22 Jul 2015, (*Poster*)
- The Gage Ion Channels and Transporters Conference, Canberra, Australia, Apr 15-17, 2015, (*Poster*)

- The European Molecular Biology Laboratory (EMBL) Australia PhD Symposium, Sydney, Australia, Dec 3-5, 2014, (*Poster*)
- The 22nd St Vincent's Health Australia Sydney Precinct Research Symposium. Sydney, Australia, Sep 11-12, 2014, (*Oral & Poster*)
- Mechanosensory Transduction Satellite in conjunction with International Union for Pure and Applied Biophysics (IUPAB). Goldcoast, Australia, Jul 31 - Aug 2, 2014, (*Poster*)

Abstracts

- Nikolaev Y., Rohde P., Laver D., Martinac B. Mechanosensitivity of TRPC6 Ion Channel Reconstituted in the Liposomes. Biophysical Journal, 110.3 (2016): 610a-611a
- Nikolaev Y., Rohde P., Cox C., Laver D., Martinac B. Mechanosensitivity of TRPC6 ion channels. *Biophysical Journal*, 108.2 (2015): 564a

Original articles

- Nikolaev Y., Rohde P., Cox C., Laver D., Martinac B. Mechanosensitivity of TRPC6 ion channels., (Manuscript in preparation, to be submitted to the FEBS journal)
- Bavi N., Nikolaev Y., Bavi O., Ridone P., Martinac A., Nakayama Y., Cox C., Martinac B. Principles of mechanosensing at the membrane interface. *Springer Series in Biophysics*, (in press)
- Friedrich O., Schneidereit D.*, Nikolaev Y.* et al. Adding Dimension to cellular Mechanotransduction: Advances in Biomedical Engineering of-multi-axial Cell-Stretch Systems and their Application to cardiovascular Biomechanics and Mechano-Signaling. *Prog Biophys Mol Biol*, (2017); *equally contributed

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- Nikolaev Y., Dosen P., Laver D, Van Helden D, Hamill O. Single mechanically-gated cation channel currents trigger action potentials in neocortical and hippocampal pyramidal neurons. *Brain Research*, (2015), 1608:1-13

Awards

- The Biophysical Society International Travel Award to the 60th Annual Meeting, 2016
- Student Presentation Award at Australian Society for Biophysics, 2015
- Best poster award at Mechanosensory Transduction Satellite in conjunction with IUPAB, 2014
- The Australian Society for Biophysics Student Travel Award, 2014
- Victor Chang Cardiac Research Institute top-up scholarship, 2014
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Abstract

Background: The transient receptor potential (TRP) ion channel family is a diverse group of channels gated by various physical and chemical stimuli. One of the members, TRPC6, is a Ca^{2+} permeable cation channel, which is expressed in ventricular cardiomyocytes (CMs). TRPC6 is gated via the G-protein-coupled receptor pathway leading to generation of diacylglycerol (DAG), which ultimately activates the ion channel. TRPC6 can also be activated by mechanical force, which for example plays a significant role in mechanotransduction of the heart. However, it is still unclear whether TRPC6 is activated directly by membrane tension (the bilayer mechanism) or its activation is mediated via other mechanosensitive membrane structures, such as the cytoskeleton and/or the extracellular matrix (the tether mechanism).

The aim of the study: To determine whether TRPC6 is an inherently mechanosensitive (MS) ion channel.

Methods and Results: First, mechanosensitivity of TRPC6 was evaluated in HEK293 cells by stretching the membrane via application of negative pressure (suction) to a patch pipette. Spontaneously active TRPC6 channel did not respond to the force. The entire-cell TRPC6 currents were revealed in the whole cell configuration and the channels were activated by DAG analogue. Second,

using a stretch device, 15% isotropic stretch was applied to the intact cells attached to the bottom of stretchable PDMS chambers and Ca^{2+} entry via TRPC6 ion channels was demonstrated. The effect, suggested mechanosensitive nature of TRPC6 channel in HEK293 cells, however, it remained unclear whether mechanosensitivity is inherent or promoted via other membrane components.

To answer this question, using the purified TRPC6 protein, liposome reconstitution was carried out. Spontaneous activity of the TRPC6 single channel was demonstrated in the liposome by the patch clamp. The channel was activated according to "force-from-lipids" principle; however, application of stretch did not change the open probability of the channel. Therefore, it has been concluded that TRPC6 is not stretch activated upon application.

Furthermore, the role of TRPC6 in cardiac hypertrophy was investigated. Immunostaining of TRPC6 in hypertrophic CMs revealed that the channel migrates from the intracellular t-tubules to the sarcolemma. Furthermore, the impact of MS channels on the Ca^{2+} homeostasis in the CMs was investigated. A new method was developed, which allowed stretching of hydrogel embedded CM in multiaxial (isotropic) directions and simultaneous measurement of Ca^{2+} fluorescence. Both normal and hypertrophic CMs showed a late mechanical response 300s after the stretch. MS channels were only activated after long-term induced stretch, which suggests their mechanoprotective role in the heart.

The main finding of the thesis is that the TRPC6 ion channel is not inherently mechanosensitive since it is unresponsive to membrane stretch; instead it is activated by "force-from-lipids" principle without involvement of any other membrane components. Since abnormal TRPC6 activity is implicated in cardiac hypertrophy, our findings contribute to a better understanding of pathophysiological mechanisms of hypertrophy and may open up new directions for therapeutic strategy.

Contents

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List of abbreviations

CaM	Calmodulin
CC	coiled-coil
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
CM	cardiomyocyte
CIRB	calmodulin/inositol trisphosphate receptor binding
DDM	n-Dodecyl-beta-Maltoside
DM	decyl maltoside
DMEM	Dulbecco's modified culture medium
DMSO	dimethyl sulfoxide
DR	dehydration/rehydration
FBS	fetal bovine serum
FSEC	fluorescent size exclusion chromatography
FSM	Frank Starling mechanism
GFP	green fluorescent protein
GPCR	G-protein coupled receptor
IMAC	immobilized metal ion affinity chromatography
LB	Luria broth
LPC	lysophosphatidylcholine
LVH	left ventricular hypertrophy
MBP	Maltose-binding protein

MS	mechanosensitive
NFAT	nuclear factor of activated T-cells
NDSB	non-detergent sulfobetaine
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PEI	Polyethylenimine
SA	sino-atrial
SD	standard deviation
SDS	sodium dodecyl sulfate
SFR	slow force response
SOC	super optimal broth with Catabolite
SR	sarcoplasmic reticulum
SUV	small unilamellar vesicles
TAC	transverse aortic constriction
TBS	tris-buffered saline
TCEP	tris(2-carboxyethyl)phosphine
TIRF	total Internal reflection fluorescence
TRP	transient receptor potential
TRPC	transient receptor potential canonical
WT	wild type