Effect of temperature on stretch-induced cardiac action potential shortening in the rat heart: involvement of TREK-1.

Kelly DR MacKenzie L and Saint DA

Department of Physiology, School Of Molecular & Biomedical Sciences, The University of Adelaide

INTRODUCTION

Mechano-electric feed back (MEF) is the mechanism by which stretch may directly alter heart electrophysiology. As a result, MEF is thought to underlie many cardiac arrhythmias associated with pathological conditions. The phenomenon of MEF is made possible by the presence of stretch-activated ion channels (SACs) on the surface membrane of heart cells. Of the stretch activated ion channels known, both TREK-1 and non-selective stretch-activated cation channels (SACs) are thought to contribute to MEF in the rat heart. These channels, when activated by stretch, alter the cardiac action potential through alterations in $[Ca^{2+}]i$, I_{K+} and I_{Na+}. Since K^+ currents contribute to the resting membrane potential and are important to modulation of cardiomyocyte excitability and termination of the cardiac action potential, TREK-1 may play an important role in MEF modulation of cardiomyocyte behaviour. To date, no compounds have been described that selectively block or activate TREK-1, making difficult the definitive identification of their role in MEF. However, it is known that TREK-1 channels are temperature and stretch sensitive, with lower temperatures reducing single TREK-1 channel open probability and thus the size of the TREK-1 K^+ current.

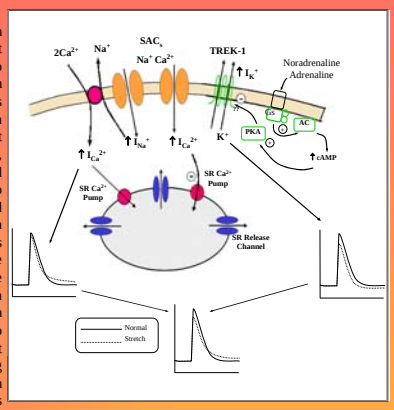


Figure 1. Overview of MEF pathways thought to take place in in cardio-myocytes.

AIMS

The present study aimed to evaluate the contribution of non-selective cation and TREK-1 stretch-activated channels to changes in the rat cardiac action potential duration during stretch by taking advantage of the temperature dependent nature of the TREK-1 K^+ channel.

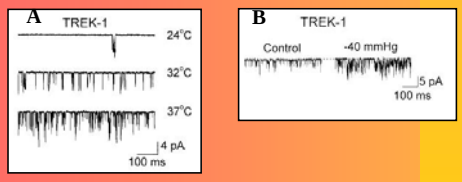


Figure showing the effects of temperature (A) and negative pipette pressure (B) on TREK-1 single channel recordings from COS-7 cell line. Data taken from Kang D, Choe C, Kim D. Thermosensitivity of the two-pore domain K+ channels TREK-2 and TRAAK. J Physiol. 2005 Apr 1;564(Pt 1):103-16.

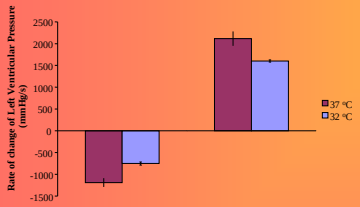
Hypothesis

The reduced basal channel activity of TREK-1 channels at lower temperatures may enable the exaggeration of stretch-induced increases in TREK-1 channel activity and thus the effects of MEF on the cardiac action potential. **(The reduction in channel activity at lower temperatures will enable greater increases in activity upon stretch and thus facilitate the observation of changes in the action potential.)**

METHODS

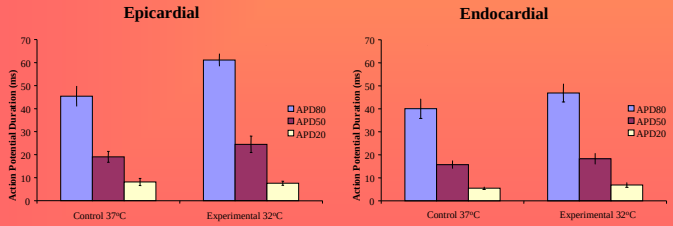
6 male Sprague Dawley rats (450-490g) were heparinized with 3500UI heparin and killed after 5 min by cervical dislocation. Hearts were then retrogradely perfused on a Langendorf system (ADInstruments, Australia), a small water filled latex balloon attached to a transducer and calibrated syringe was inserted into the left ventricle through the left atrium. Hearts were paced at 4 Hz, twice threshold using bi-polar pulses and allowed to stabilise at a constant coronary perfusion pressure of 60mmHg with modified Hepes buffer solution at 37ºC. Following stabilisation, hearts were subjected to three consecutive diastolic pressures (control: 0-5mmHg, 20-25mmHg and 50-55mmHg) at two randomised perfusion temperatures (32°C or 37°C). During manipulation of left ventricular diastolic pressure, endocardial and epicardial monophasic action potentials (MAP) were recorded and their durations at 20, 50 and 80% repolarisation (APD_{20-80}) calculated. The perfusate temperature was then changed to the corresponding control or experimental group and the MAP recordings repeated. This protocol enabled MAP recordings to be made from each heart at 32 and 37oC in a random order.

Figure 4: Effect of temperature on left ventricular contractility.



Reducing perfusate temperature to 32ºC significantly decreased cardiac contractility. Rate of cardiac contraction and relaxation fell by 24.2% and 36.8% respectively.

Figure : Effect of temperature on Left ventricular MAPs

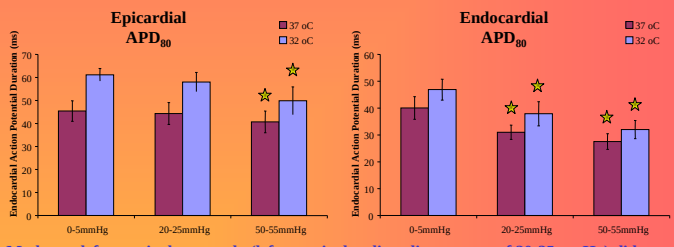


Under control conditions endocardial APD_{80} was 40.3 ±4.2ms, while the epicardial APD_{80} was 45.4 ±4.4ms at 37°C . Decreasing cardiac temperature to 32ºC increased both endocardial and epicardial APD_{80} to 46.9 ±3.9ms and 61.1 ±2.7ms respectively.

RESULTS

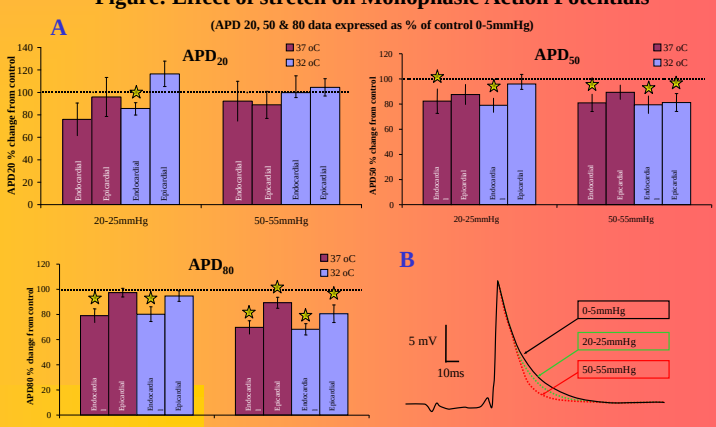
Criteria for an acceptable experiment was a stable MAP recording with a stable resting membrane potential and a MAP amplitude of at least 5 mV. Values for Action Potential Duration (APD) at 20, 50 and 80% repolarisation were recorded for each action potential, data presented as mean ± SEM . During temperature manipulations, coronary perfusion pressure and flow rate remained constant at 60.3 ± 0.5 mmHg and 10.9 ± 0.7 ml/min respectively.

Figure: Effect of stretch on Monophasic Action Potentials



Moderate left ventricular stretch, (left ventricular diastolic pressure of 20-25mmHg) did not significantly alter epicardial APD_{80} at either temperature. By contrast, endocardial APD80 reduced by 21 ±5.5% and 19.8 ±5.9% from control values at 37ºC and 32ºC respectively. **(Stars denote a significant decline, p<0.05, in APD_{80} when compared to results obtained at a diastolic pressure of 0-5mmHg at the corresponding temperature)**

Figure: Effect of stretch on Monophasic Action Potentials



(A)Extreme left ventricular stretch (50-55mmHg) significantly reduced the APD_{80} in both epicardial and endocardial recordings at 37°C and 32°C. Epicardial APD_{80} reduced by 10.7 ±4.3% and 19.4 ±7.0% relative to control conditions at 37°C and 32°C respectively. Similarly, endocardial APD_{80} decreased by 30.4 ±5.3% and 31.8 ±4.5% at 37°C and 32°C respectively. **(Stars denote a significant decline, p<0.05, in APD_{20} or APD_{80} when compared to results obtained at a diastolic pressure of 0-5mmHg at the corresponding temperature).** **(B) Endocardial action potential demonstrating changes in duration observed during stretch.**

Endocardial								Epicardial							
Diastolic Pressure	APD_{20}		APD_{20}		APD_{80}			Diastolic Pressure	APD_{20}		APD_{50}		APD_{80}		
20-25 mmHg	⟷	↓	↓	↓	↓	↓		20-25 mmHg	⟷	⟷	⟷	⟷	⟷	⟷	
50-55 mmHg	⟷	⟷	↓	↓	↓	↓		50-55 mmHg	⟷	⟷	⟷	↓	↓	↓	
	37 °C	32 °C													

Table 1: Summary of statistically significant changes in Endocardial and Epicardial MAP recordings made at 37 and 32 ºC during left ventricular diastolic pressure manipulations. **Downward arrows denote a decrease (P<0.05) in action potential duration (APD) and horizontal arrows denote no significant change. Arrows connecting 37 and 32 °C columns denote statistically similar reductions in APD.**

DISCUSSION

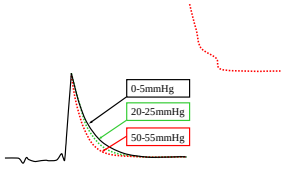
These experiments were prompted by previous demonstrations of MEF in isolated Langendorff rat heart preparations, observed as a reduction in action potential duration. Both TREK-1 (K^+ selective) and non-selective stretch-activated cation channels (SACs) are thought to contribute to MEF in the heart. Since no compounds have been described that selectively block or activate TREK-1, in an attempt to better understand the ionic basis of MEF and the contribution by TREK-1 we recorded MAPs in isolated rat hearts during diastolic pressure manipulations at two different temperatures. This was based on the understanding that TREK-1 channels are inactivated at lower temperatures, but remain responsive to stretch. Our efforts failed to convincingly demonstrate that stretch-induced changes in the action potential, even at extreme tensions, were influenced by temperature.

CONCLUSION:

It was concluded that a change in cardiac temperature did not affect the magnitude of reduction in action potential duration (as measured by APD_{80}) following moderate (20-25mmHg) or extreme stretch (50-55mmHg) for either endocardial or epicardial recordings when compared to their control. Since TREK-1 channels are temperature sensitive, (inactivating at lower temperatures), these results suggest that non-selective stretch-sensitive cation channels may be more important in modifying action potential duration during stretch than TREK-1 in the rat heart.

References:

A Lab MJ. Transient depolarisation and action potential alterations following mechanical changes in isolated myocardium. *Circ Res.* 1980; 14:624-637.
B Kim D. novel Cation-selective mechanosensitive ion channel in the atrial cell membrane. *Circ Res* 1993; 72:225-231.
C Tavi P, Han C, Weckstrom M. Mechanisms of stretch-induced changes in $[Ca^{2+}]_i$ in rat atrial myocytes. *Circ Res.* 1998: 83:1165-1167.
D Le Guennec J-Y, White E, Gainner F, Argibay JA, Garnier D. Stretch induced increase of resting intracellular calcium concentration in single guinea-pig ventricular myocytes. *Exp Physiol.* 1991; 76:975-978.
E DuBell WH, Boyett MR, Spurgeon HA, Talo A, Stern MD, Lakatta EG. The cytosolic calcium transient modulates the action potential of rat ventricular myocytes. *J Physiol (Lond).* 1991;436:347-369.
F Zhang Y, Youm I, Sung H, Lee S, Ryu S, Lee S, Ho W, Earn Y. Stretch-activated and background non-selective cation channels in rat atrial myocytes. *J Physiology.* 2000. 523;3:607—619
G Hai Hu and Frederick Sachs Stretch-Activated Ion Channels in the Heart. *J Mol Cell Cardiol* 29, 1511–1523 (1997)
H Ravelli,F.,Allessie,M.,1997.Effects of atrial dilatation on refractory period and vulnerability to atrial fibrillation in the isolated Langendorff-perfused rabbit heart. *Circulation* 96,1686 -1695.


0-5mmHg
20-25mmHg
50-55mmHg