Mechanosensitivity of TRPC6 ion channels

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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, transient receptor potential canonical-6 (TRPC6), is a calcium permeable cation channel (PCa/PNa ~ 6) located in the T-tubules of ventricular cardiomyocytes (Allen & Ward, 2011). Abnormal TRPC6 activity has been implicated in cardiac hypertrophy, a maladaptive process likely triggered by increased mechanical stress (Seo *et al.*, 2014). As a result, it is important to elucidate the gating mechanism of TRPC6. In addition to activation by diacylglycerol (DAG) TRPC6 has been reported to be also activated by mechanical force (Spassova *et al.*, 2006; Wilson & Dryer, 2014), though this has not been supported by other studies (Anishkin *et al.*, 2014). In this study, we investigated the mechanosensitivity of TRPC6 overexpressed in HEK293 cells using the patch clamp technique. Three TRPC6 constructs were utilised: N- terminal fusion GFP, C-terminal fusion GFP and non-GFP fusion TRPC6. We have successfully transfected these constructs into HEK293 cells with a 60% transfection efficiency rate using Lipofectamine® 3000 reagent. Confocal images of C-GFP TRPC6 demonstrated that the channel is localised in the plasma membrane. However, N-GFP and non-GFP TRPC6 both distributed uniformly throughout the cells suggestive of impaired trafficking. In our patch clamp experiments we recorded TRPC6 single channel activity in cell-attached and whole-cell configurations. By applying negative pressure (suction) of -50 mmHg to the patch pipette we were able to record single channel activity of TRPC6 channels. Moreover, the channels were spontaneously active without application of negative pressure to the patch pipette suggesting that the membrane tension resulting from the gigaohm seal formation was sufficient to activate TRPC6. Furthermore, by applying specific activators/inhibitors we were able to increase/decrease the channel activity. These results demonstrate that TRPC6 expressed in the plasma membrane of HEK293 cells is mechanosensitive. Future studies will focus on purifying and incorporating the TRPC6 channel protein into liposomes to establish if TRPC6 is intrinsically mechanosensitive (Patel *et al.* 2010).

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