Tmem16a-generated Ca^{2+}-activated Cl^- currents exhibit similar regulatory properties to those recorded in vascular myocytes

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Ca^{2+}-activated Cl^- channels (Cl_{Ca}) are small conductance anion channels activated by a rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). The channels display outward rectification and time-dependent properties, especially at low $[Ca^{2+}]_i$, due to voltage-dependent alteration of the Ca^{2+} sensitivity of the channel. Opening of Cl_{Ca} channels is believed to serve an important excitatory function in vascular smooth muscle cells (VSMCs) by mediating membrane depolarization, Ca^{2+} influx and increased tone. In arterial myocytes, Ca^{2+}-activated Cl^- currents ($I_{Cl(Ca)}$) exhibit pronounced rundown after seal rupture, a process that is strongly attenuated by omitting ATP, or by replacing ATP by its non-hydrolyzable form AMP-PNP, from the pipette solution (Angermann *et al.*, 2006). Additional evidence suggested that the regulation may involve at least one phosphorylation step implicating CaMKII (Greenwood *et al.*, 2001), calcineurin and PP1/PP2A (Greenwood *et al.*, 2004; Ayon *et al.*, 2009). Recently three groups of investigators independently identified *Tmem16a* as a novel candidate gene encoding for Cl_{Ca} channels (Caputo *et al.*, 2008; Schroeder *et al.*, 2008; Yang *et al.*, 2008). This gene is highly expressed in vascular myocytes (Davis *et al.*, 2010). Whether *Tmem16a*-encoded Cl_{Ca} channels are similarly regulated to those recorded in VSMCs is unknown and is the focus of this study.

Whole-cell patch clamp experiments were carried out 48 h after transient transfection of mouse *Tmem16a* in HEK 293 cells. Typical voltage- and time-dependent $I_{Cl(Ca)}$ were elicited by cell dialysis with a solution set to 500 nM free $[Ca^{2+}]$. $I_{Cl(Ca)}$ displayed pronounced rundown following seal rupture in cells dialyzed with 5 mM ATP; after 10 min, $I_{Cl(Ca)}$ amplitude was down to 38.9±3.1% (n=10) of the initial level at t=0. Omission of ATP from the pipette solution abolished the rundown and led to a small but significant up regulation of $I_{Cl(Ca)}$ after 10 min of cell dialysis (131.9±18% from initial level, n=9). Finally intracellular application of the non-specific PP1/PP2A inhibitor okadaic acid (30 nM) abolished the delayed recovery of $I_{Cl(Ca)}$ seen in the absence of intracellular ATP (42.9±11.85 from initial level; n=6). Five potential consensus sites for phosphorylation by CaMKII have been identified on mouse TMEM16A. Single-point mutation of one these sites found to lie near the speculated pore region of TMEM16A, Threonine 610, to an Alanine, failed to alter the time course of rundown of $I_{Cl(Ca)}$ in the presence of 5 mM ATP (n=12) and suggested that it is not the site potentially phosphorylated by CaMKII. Mutational analysis of the other four consensus sites is in progress. Taken together, these results suggest that similar to Cl_{Ca} channels in VSMCs, *Tmem16a*-evoked $I_{Cl(Ca)}$ also appear to be subjected to down-regulated by phosphorylation.

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