Membrane trafficking and localization, residence time and degradation of KCa3.1 in a polarized epithelium

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Hypertension, intestinal and kidney diseases are major clinical problems in New Zealand, especially in Maori and Pacific Island populations. The intermediate-conductance, Ca^{2+}-activated K^+ channel, KCa3.1, plays a critical role in controlling vascular tone, blood pressure and proper function of intestinal and kidney epithelial cells. It is has been recently demonstrated that the number of KCa3.1 channels at the membrane of colonic cells is reduced by 75% in patients with ulcerative colitis (Al-Hazza *et al.*, 2012). Additionally, the lack of KCa3.1 in knock-out mice resulted in mice with increased blood pressure, highlighting the crucial importance of KCa3.1 in regulation of blood pressure (Si *et al.*, 2006). Understanding how we can modify the time these channels remain at the membrane will alter physiological function. However, very little is known about the trafficking of KCa3.1 in polarized epithelial cells. We have developed a novel-tagged KCa3.1 channel that allows us to rapidly and exclusively label these channels to determine the membrane residence time and intracellular fate(s) of these channels (Balut *et al.*, 2010a,2010b; Gao *et al.*, 2010; Hamilton, 2010). Therefore, in an attempt to address this lack of basic knowledge of physiology of KCa3.1, we examined the membrane localization, residence time and degradative pathway of KCa3.1, for the first time in a polarized epithelium.

First, we established a stably transfected Fischer rat thyroid cell line (FRT) expressing the biotin-ligase acceptor peptide (BLAP) sequence into KCa3.1 (KCa3.1-BLAP) and the biotin ligase (BirA) with an endoplasmic reticulum (ER) retention motif (BirA-KDEL) which allows the labeling of surface KCa3.1 (Winter *et al.*, 2011). We have previously demonstrated that the BLAP sequence does not alter the trafficking, gating or calcium-dependent activation of KCa3.1 (Gao *et al.*, 2010). Here, we examined cell surface expression and cellular location of KCa3.1-BLAP by immunoblot (IB) and immunofluorescence (IF). BirA-KDEL biotinylates KCa3.1-BLAP within the ER of the FRT cells, therefore, membrane surface KCa3.1-BLAP channels will be biotinylated at the lysine (K) within the BLAP sequence. At the membrane, we label the channels with streptavidin that binds to the biotin for IB experiments or with streptavidin conjugated to a fluorophore for IF experiments. FRT were grown in F12 medium supplemented with 10% fetal calf serum, and penicillin/streptomycin at 37°C in 5% carbon dioxide. FRT cells were seeded onto filters and grown to confluence forming a polarized epithelium (3-4 days post seeding). Non-transfected FRT cells served as controls in all experiments.

In order to determine the membrane localization of KCa3.1 in the polarized FRT cells, cells were grown on filters and incubated with either apically-applied streptavidin or basolaterally-applied streptavidin. Untransfected cells were used as a negative control. We clearly demonstrated, for the first time, that KCa3.1-BLAP was exclusively trafficked to the basolateral membrane of FRT cells in a polarized epithelium (n=5, both IF and IB). Next, we examined the residence time of KCa3.1 in which FRT cells were incubated with basolaterally-applied streptavidin and returned back to 37°C for 0, 1, 3, 5, 8 hours. After 1-2 h, membrane surface KCa3.1-BLAP channels were localized into intracellular vesicles (by IF, n=5) and by 3-4 h the presence of intracellular KCa3.1-BLAP was reduced by half (by IB, n=5) as evident by relative protein determined by densitometry. Finally, in order to determine the degradative pathway of KCa3.1-BLAP, we examined the effects of the lysosomal inhibitors, leupeptin (100 μM) and pepstatin (1μg/ml), on the intracellular fate of KCa3.1-BLAP. Again, stably transfected FRT cells were grown on filters, labeled with basolaterally-applied streptavidin incubated in the presence or absence of leupeptin and pepstatin (n=5). The presence of leupeptin and pepstatin reduced the degradation of KCa3.1-BLAP suggesting the channel is degraded *via* a lysosomal-dependent pathway.

Our results clearly demonstrate that KCa3.1 is exclusively trafficked to the basolateral membrane of polarized FRT cells, the channels have a half-life of 3+ hours and that the channels are degraded in the lysosome. This work was supported by an Aim Fund grant from the Department of Physiology (KLH) and NIH (DCD).

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