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BK channels in mammalian inner hair cells (Jutta Engel)

The mammalian inner hair cell (IHC) transduces sound into depolarization, followed by transmitter release resulting in neuronal activation. BK channels of the mature IHC are responsible for fast repolarization of the receptor potential and for the small time constant of the IHC, enabling it to respond very quickly to changes in membrane potential. Although BK currents of the IHC are largely insensitive to Ca^{2+} influx they show a dependence on intracellular Ca^{2+} under certain experimental conditions. We hypothesize that there are two different subpopulations of BK channels in the IHC, (i) a smaller one with the channels distributed along the basolateral synaptic pole and affected by Ca^{2+} influx through $Ca_v1.3$ Ca^{2+} channels and (ii) a larger one with channels clustered in the apical neck region, which is affected by Ca^{2+} release of adjacent Ca^{2+} stores. Using the patch-clamp technique, whole-cell BK currents will be studied under conditions of different filling states of ryanodine-sensitive and IP_3-sensitive Ca^{2+} stores and defined intracellular Ca^{2+} buffering. Ca^{2+} stores will be emptied by the respective agonists and store refilling will be inhibited by SERCA inhibitors. BK channel currents will also be studied in inside-out patches excised from either the neck region or the basolateral region and will be compared in terms of biophysical properties and sensitivity towards applied Ca^{2+} concentrations.

To clarify the localization of the two pools of BK channels in IHCs, immunohistochemistry with fluorescent antibodies will be performed. The results will be compared with live cell labeling using fluorescently labeled toxins, e.g. iberiotoxin, which bind from the outside. To analyse the spatial relationship of BK channel clusters in the IHC neck region with underlying endoplasmatic reticulum (ER), double immunofluorescence labeling with BK antibodies and ER markers will be performed. To increase spatial resolution and to identify the structures underlying the apical IHC BK channel clusters, this analysis will be extended by electron microscopy, which will be done in collaboration with M. Schweizer, ZMNH (Center for Molecular Neurobiology in Hamburg/Germany) and/or by using cryoelectron microscopy in collaboration with Howard S. Young. We will also test for the presence of the BK channel modulator LRRC26 in IHCs using transcript analysis and immunohistochemistry. Finally, because BK channels are not expressed in IHCs of a variety of deaf mutants, e.g. in myosin VI-deficient mice, their neck subsurface ER structures shall be analyzed and compared with normal BK-expressing IHCs.