Heteromultimeric channels formed by potassium channel

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Heteromultimeric channels formed by rat brain potassium-channel proteins – SUMMARY

Coexpression of RCK specific mRNAs in several regions of the brain suggested the presence of heteromultimeric potassium channels. These differ in properties compared to several copies of identical subunits in homomultimeric potassium channels, hence provides diversity. The aim of this study was to understand the components and compositions of the heteromultimeric potassium channels. RCK proteins are vital in the formation of the voltage-gated potassium channels. Normally species containing RCK proteins have homomultimeric potassium channels. In this experiment rat's RCK variants: RCK1 and RCK4 were expressed in *Xenopus* oocytes and HeLa cells and tested their sensitivity against a K+ channel blocker (TEA).

Normally K+ channels in HeLa cells do not rectify outward currents. However transfecting with plasmids consisting either cDNA RCK1 or cDNA RCK4, both resulted in outward rectifying K+ current. According to the method by Chen and Okayama, they were cultured by a standard protocol. Same pulse and cells were tested using the whole-cell patch configuration to measure the current. RCK1 and RCK4 channels both mediated a transient K+ outward current. In the presence of TEA, RCK4 is completely insensitive with 50% recovery time of 7. 3(+/-3. 4)s. Whereas RCK1 channels showed high sensitivity with complete blockage of current. However when cotransfection of cDNA RCK1, 4 into HeLA cells resulted in a depolarising voltage step to 0mV, with outward currents consisting of an initial transient current followed by a non-inactivating component. In addition to TEA, similar expression as RCK1 channels with sensitivity and almost half blockage of current at 10mM,

with 50% recovery time of 2. 1(+/-0. 5)s . Further, increase in TEA to 100mM lead to complete blockage of the currents. Therefore due to different expression from homomultimeric RCK4 channels, these suggested that the cotransfection either mediated homomultimeric RCK1 channels or heteromultimeric channels being indistinguishable from RCK1 channels.

Further examining of differing properties in voltage-dependent gating and conductance of the channels mediating the transient currents was done by injecting RCKs into *Xenopus* oocyte with cRNAs. Cell-attached marco patches configuration was used, allowing more voltage control with the presence of 10mM TEA solution. The oocyte was injected with cRNA RCKs and results of peak amplitudes of the transient currents against the voltage obtained were graphed, enabling the analysis of the saturation and inactivation behaviour. Paired conditioning and test pulses both were made to avoid test-pulse involved inactivation. Oocytes with RCK4 specific cRNA resulted in transient current peak showing no saturation until 40mV and began to inactivate during the test pulse, with 16. 5(+-2. 5)s at 50% recovery. Whereas coinjected RCK1, 4 cRNA showed steep and shifted about 15mV towards more positive potential (~20mV) inactivation curve, with 5. 7(+/-1. 8)s at 50% recovery. This implied little inactivation during the test pulse with faster recovery compared to RCK4 specific.

Examining the gating of K+ channels resulted in linear and superlinear shape for coinjected and RCK4 specific oocytes channel opening respectively. Therefore the saturation and response of open gated channel from both coinjected HeLa and transfected oocyte cells support the characteristic of the opening of different channels and not from different activation kinetics.

RCK 1, 4 combinations showed intermediate sensitivity and faster recovery from inactivation to DTX and TEA, compared to homomultimeric RCK1 and RCK4 subunits. Results showed that RCK 1, 4 inactivation were similar to RCK4 and single channel conductance being similar to RCK1. Overall, these results strongly support the suggestion of coexpression of RCK1 and RCK4 subunits which assemble to make a heteromultimeric RCK 1, 4 channels with differing properties from homomultimeric channels.