Modulation of Hslo1 BK Channels by Aminoglycoside Antibiotics

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Large-conductance calcium- and voltage-activated potassium (BK) channels control smooth muscle tone and neurotransmitter release. BK channels consist of a voltage-sensing domain (VSD), a calcium-sensing domain (CSD), and a pore/gate domain (PGD). To elucidate structure and function of the so-called “C-linker” (res. #327 to 340 in human slo1 U101058) connecting PGD and CSD, we analyzed the impact of amino-acid exchanges within this area on inhibition of BK channels by the aminoglycoside antibiotic neomycin. Slo1 variants were expressed in HEK293 cells and analyzed in excised membrane patches. At a concentration of 100 μM, neomycin reversibly reduced hSlo1 BK currents at 200 mV to 30%. At low voltages, however, neomycin activated BK channels - a phenomenon largely masked by its profound pore blocking effect at high voltages. Assuming that the polycationic neomycin blocks the channel pore, we introduced mutations in the PGD increasing the net charge by one per subunit. For S317R, neomycin did not block at 200 mV and the voltage dependence of activation was left-shifted by almost 40 mV. For E324A, neomycin reduced the current at 200 mV to 81%, while a left-shift of 17 mV was measured. These data suggest that neomycin must have more than one interaction site with BK channels and that it not only blocks the pore but also interferes with gating. Moreover, alteration of the C-linker structure, which was known to be essential for channel gating, almost completely abolished BK inhibition by neomycin. The C-linker has thus to be considered a central part of the PGD, determining pore properties as well as channel gating.

Single Aspartate Substitutions at Multiple Deep-Pore Positions Lead to Constitutively Open BK Channels

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The pore-lining amino acids of ion channel proteins reside on the interface between a polar (the pore) and a non-polar environment (the rest of the protein). Manipulating the charge states of their side-chains, by tilting the energy balance about gating-related structural dynamics. For example, charged side-chains at the M314 position in the BK channel stabilize an open conformation. The M314D mutant, in particular, led to constitutive channel activities. We have now scanned the S6 region (I308 to N328) with aspartate replacement. We found 3 other positions where aspartate substitutions led to constitutively open BK channels in nominal zero calcium and neutral pH. These mutants are L312D, A313D and A316D. Together with M314, these positions are all located within the deep-pore region of the channel, which is immediately intracellular to the selectivity filter but more extracellular to the lower-pore region that aligns with the Kv channel “bundling crossing”. These findings provide further evidence that the BK channel pore is different from that of Kv channels, where aspartate substitution at a single position (as opposed to multiple positions in BK) in the lower-pore region (as opposed to the deep-pore region in BK) led to constitutive channel activities. The aspartate mediated constitutive activities in Kv channels is believed to result from the disruption of specific hydrophobic interactions within the pore-lining segment.