Identification of a critical binding site for local anaesthetics in the side pockets of K_v1 channels

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Abstract
Background and Purpose: Local anaesthetics block sodium and a variety of potassium channels. Although previous studies identified a residue in the pore signature sequence together with three residues in the S6 segment as a putative binding site, the precise molecular basis of inhibition of K_v channels by local anaesthetics remained unknown. Crystal structures of K_v channels predict that some of these residues point away from the central cavity and face into a drug binding site called side pockets. Thus, the question arises whether the binding site of local anaesthetics is exclusively located in the central cavity or also involves the side pockets. Experimental Approach: A systematic functional alanine mutagenesis approach, scanning 58 mutants, together with in silico docking experiments and molecular dynamics simulations was utilized to elucidate the binding site of bupivacaine and ropivacaine. Key Results: Inhibition of $K_v1.5$ channels by local anaesthetics requires binding to the central cavity and the side pockets, and the latter requires interactions with residues of the S5 and the back of the S6 segments. Mutations in the side pockets remove stereoselectivity of inhibition of $K_v1.5$ channels by bupivacaine. Although binding to the side pockets is conserved for different local anaesthetics, the binding mode in the central cavity and the side pockets shows considerable variations. Conclusion and Implications: Local anaesthetics bind to the central cavity and the side pockets, which provide a crucial key to the molecular understanding of their K_v channel affinity and stereoselectivity, as well as their spectrum of side effects.

Author keywords
Bupivacaine
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