

N-Glycosylation-dependent block is a novel mechanism for drug-induced cardiac arrhythmia¹

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SPECIFIC AIMS

I_{Kr} is an important repolarizing potassium current in the human ventricle. There is considerable evidence to support the hypothesis that the native channel is composed of the pore-forming subunit HERG and the β subunit, MiRP1. Genetic mutations in both genes are found in patients with congenital and acquired prolongation of the QT interval, which predisposes to a specific form of polymorphic ventricular tachycardia known as Long QT syndrome. Of particular interest is the case of a MiRP1 single-nucleotide polymorphism resulting in a T to A amino acid mutation at position 8, T8A, that is normal at baseline but able to impair MiRP1/HERG function if associated with the antibiotic sulfamethoxazole (SMX). This study was directed at investigating the molecular mechanism by which T8A susceptibility arrhythmia mutant causes SMX high-affinity block.

PRINCIPAL FINDINGS

1. MiRP1 protects HERG from the inhibitory effect of SMX

Our first step toward the investigation of the molecular mechanisms determining SMX block was directed at ascertaining whether susceptibility to the drug is an intrinsic property of HERG or, rather, is conferred by association with MiRP1. HERG channels alone were blocked by SMX; more important, they were fourfold more susceptible than those formed with MiRP1 ($K_i=0.34\pm 0.02$ mg/mL vs. 1.24 ± 0.09 mg/mL). Thus, MiRP1 exerts a protective action on the complex by significantly decreasing SMX susceptibility in the physiological range (0.1–0.3 mg/mL). HERG alone or with T8A exhibited similar sensitivity ($K_i=0.30\pm 0.02$ mg/mL with T8A).

2. Defective glycosylation is the primary cause for SMX high-affinity block

The T to A mutation occurs in an N-glycosylation site of MiRP1 (NFT) that therefore is predicted not to be functional, a notion confirmed by Western blot analysis of HA epitope-tagged T8A subunits. Single-point muta-

tions in the site or its neighbors were associated with increased block only when they impaired glycosylation of the site (Fig. 1A). Treatment with the specific glycosylation inhibitor neuraminidase increased SMX affinity only with WT channels (Fig. 1B–D). Taken together, these data suggest that imperfect glycosylation of T8A is the primary cause for SMX high-affinity block. Glycosylation is a well-recognized factor affecting intracellular retention; therefore, the lack of T8A at the plasma membrane (and consequent formation of homomeric HERG channels) might provide a natural explanation for why HERG channels alone or with T8A are inhibited to a similar extent. The mutant, however, exhibited normal biochemical characteristics and was found to reach the plasma membrane and coassemble with HERG. Thus, we conclude that the mechanism by which defective glycosylation increases drug susceptibility must have structural origins.

3. Glycosylation-dependent block is a general mechanism of block

Block of HERG channels by SMX and other agents, including divalent cations, exhibit marked similarities. This suggests that all these molecules might interact with some common domain. To test this hypothesis, we elected cadmium as test ion and characterized its effects. The presence of SMX in the test solution (0.5 mg/mL) caused an apparent decrease of HERG sensitivity to cadmium of ~fourfold, a change not expected with independent binding processes (as a result of current normalization, $K_i=0.14\pm 0.01$ mM in the absence and $K_i=0.61\pm 0.08$ mM in the presence of SMX, respectively). Moreover, we found that like with SMX, coassembly with WT but not with T8A diminished channel sensitivity to cadmium ($K_i=0.28\pm 0.07$ mM and

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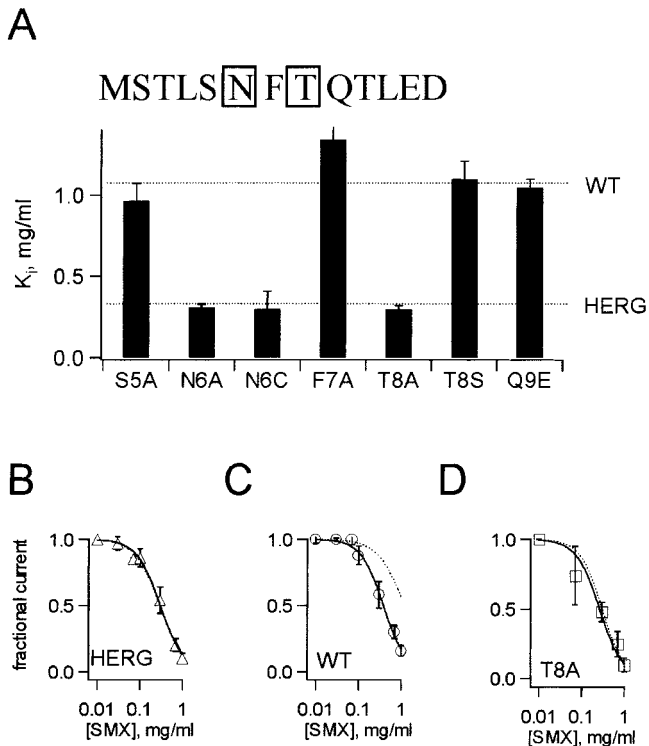


Figure 1. Defective N-glycosylation of MiRP1 increases susceptibility to SMX. *A*) Inhibition constants (K_i) calculated by fitting dose-response curves of the indicated MiRP1 mutants to the Hill function: $K_i^n / (K_i^n + [SMX]^n)$. Mutations of N6 or T8 (boxes) disrupt glycosylation of the site and cause high-affinity block. Dotted lines indicate inhibition constants of HERG and WT. *B–D*) Dose-response curves from cells expressing HERG channels alone, with WT, or T8A treated with the N-glycosylation inhibitor neuraminidase. Data fitted to the Hill function with $K_i = 0.3 \pm 0.06$ mg/mL and $n = 1.6 \pm 0.13$ for HERG alone, $K_i = 0.39 \pm 0.08$ mg/mL and $n = 1.6 \pm 0.14$ for WT MiRP1, and $K_i = 0.29 \pm 0.11$ mg/mL and $n = 1.6 \pm 0.14$ for T8A. The dotted lines correspond to the fits of dose-response relationship in the absence of neuraminidase. Treatment affected WT channels, but not HERG channels alone or with T8A.

$K_i = 0.15 \pm 0.01$ mM with WT and T8A, respectively). We conclude that both agents might act to inhibit the protein complex through common mechanisms and shared molecular determinants.

CONCLUSIONS AND SIGNIFICANCE

Our observations are summarized in the schematic shown in **Fig. 2**. We propose that HERG channels have multiple binding sites, each able to interact with a specific molecule. There is no evidence for a common binding site; in contrast, our data support a picture in which the single sites are clustered together to form a structure that we call the variable receptor. The oligosaccharide groups attached to N6 lie in the adjacency of the variable receptor, acting like an umbrella. When the carbohydrates are absent, as with T8A, accessibility to the variable receptor is no longer hampered, resulting in higher affinity. Alternative mechanisms are possible, however; for instance, the carbohydrates might act to induce structural alterations of the variable receptor or its surroundings.

Cardiac arrhythmias are a leading cause of morbidity and mortality; thus, the ability to predict, prevent, and treat these disorders remains a major scientific and medical challenge. The finding that defective glycosylation can affect channel pharmacology is novel and opens new avenues in our thinking about the structure and function of ion channels. Moreover, the information contained in this study might have repercussions for the management of cardiac arrhythmia. In fact, the possibility exists that several well-tolerated drugs might predispose to serious risk of arrhythmia the numerous individuals that carry partially glycosylated T8A MiRP1 subunits. FJ

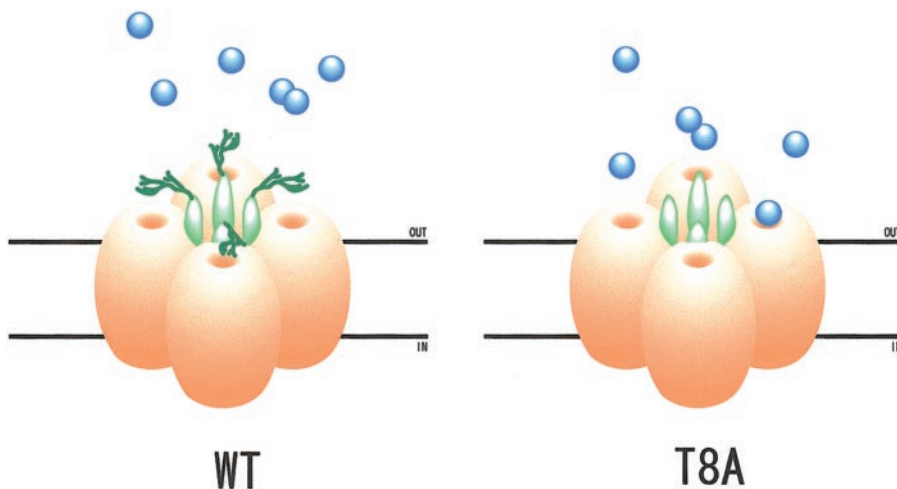


Figure 2. Proposed structural and molecular bases for glycosylation-dependent block. Four HERG subunits (tan) form a single, central ion conduction pathway. There are four MiRP1 subunits (green) facing the conduction pathway. The variable receptor is at the mouth of the pore and contains distinct binding sites (not indicated). Under normal conditions, the carbohydrates attached to MiRP1 (green branches) shield the variable receptors and thus impair SMX (blue balls) binding. Conversely, in channels formed with T8A mutants, SMX accessibility to the receptor is facilitated by the absence of the oligosaccharide groups.