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EDITORIAL COMMENT

How to Boost Efficacy of a Sodium Channel Blocker

The Devil Is in the Details*

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The development of antiarrhythmic therapies is a costly endeavor. Computational models have increasingly been included in the drug development pipeline as a method to screen potentially proarrhythmic compounds and provide a critical link between protein scale perturbations (e.g., ion channel mutations) and tissue scale behavior (e.g., arrhythmia episodes). As experiments have more clearly elucidated ion channel dynamics, computational models too need to incorporate more detailed representations of channel responses.

The earliest models of the cardiac sodium channel ($Na_v1.5$) used Hodgkin-Huxley–type gating variables to reproduce the kinetics associated with channel activation and inactivation. Although these types of models can reproduce important channel properties, a key limitation is that channel activation and inactivation are assumed to be independent. Markov chain models were subsequently developed to address this limitation and to represent multiple distinct closed, open, and inactivated channel states. This framework was also critical to more accurately model drugs that target ion channels. Simply put, a drug may only access its binding site when the channel is in specific states; therefore, representing state-specific binding rates is critical. Moreno et al. (1) previously applied such an in silico approach to explain the paradoxically proarrhythmic response of the sodium channel blocker flecainide.

Sodium channel Markov models have been expanded to account for several variants of gain-of-function mutations in the *SCN5A* gene, encoding the alpha subunit of the $Na_v1.5$ channel, that are associated with long QT type 3 syndrome (LQT3). Although the phenotypes associated with these variants are similar, a late sodium current which prolongs the action potential duration (APD), the mechanism by which the late current manifest differs among variants. This suggests that a "one size fits all" therapy to treat LQT3 may be problematic.

Recent work by Zhu et al. (2) illustrated that the picture is indeed even more complicated. That study used voltage-clamp fluorometry, an elegant technique in which a fluorophore is tethered to each of the 4 voltage-sensitive domains (VSDs) of the $Na_v1.5$ alpha subunit to monitor VSD movement simultaneously with channel current (2). The study found that activation of the VSD of the third $Na_v1.5$ domain (DIII-VSD) varied significantly across 15 LQT3 variants and, critically, that DIII-VSD activation and channel inhibition by the sodium channel blocker mexiletine were correlated. Thus, the complex picture begins to emerge: 1) VSDs regulate channel gating processes and transitions between channel states; 2) VSD kinetics are altered differently for different LQT3 variants; and 3) drug binding depends on VSD activation.

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In this issue of *JACC: Basic to Translational Science*, Moreno et al. (3) for the first time developed a computational model of the sodium channel that incorporated all of these critical details: channel mutations, DIII-VSD movement, and drug binding. The authors focused on 2 LQT3 mutant variants,

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R1626P (RP), which is highly sensitive to mexiletine, and M1652R (MR), which is minimally sensitive to mexiletine (2). They then used voltage-clamp fluorometry to show that the mexiletine-sensitive RP mutant channels had enhanced DIII-VSD activation, whereas the mexiletine-resistant MR mutant channels had reduced DIII-VSD activation. Next, the authors developed a Markov model of the wild-type (WT) and mutant sodium channels, representing distinct drug-free and drug-bound VSD states and gating conformations that accurately reproduced experimental measurements of voltage-, time-, and drug concentration-dependent channel activities, including mexiletine blockage of late sodium current. One key aspect of the modeling approach was that the authors assumed that the intrinsic affinity of mexiletine for the channel was constant between the variants. Thus, the different drug responses of the WT and the 2 mutants emerged from the dynamics of DIII-VSD activation and channel gating.

Bridging the scale from ion channel to cellular response, the authors next incorporated the sodium channel model into a human ventricular myocyte model. Simulations of myocytes with the mutant channels displayed a sustained late sodium current, the hallmark of the LQT3 phenotype. Myocytes with the RP mutant illustrated significant APD prolongation and early after-depolarizations (EADs). Treatment with a maximal clinical dose of 10 μmol of mexiletine abolished EADs and shortened APD, although not to WT levels. In contrast, myocytes with the MR mutant displayed highly irregular repolarization, with significant APD prolongation, EADs, and periods of repolarization failure; 10 μmol of mexiletine delayed the onset of EADs but failed to suppress them or APD prolongation.

Based on the relationship between DIII-VSD activation and mexiletine channel blockage, Moreno et al. (3) then insightfully hypothesized that mexiletine efficacy in the MR mutant could be enhanced or "boosted" by combining mexiletine with an agent that promoted DIII-VSD activation. The authors performed a proof-of-concept demonstration experimentally by introducing a second mutation, R1306C, which, in conjunction with extracellular application of biotin, stabilized DIII-VSD in an activated position. Consistent with the hypothesis, these modifications enhanced late sodium current blockage. The authors then pushed the computational predictions further by altering only DIII-VSD movement rates. The authors designed an in silico mexiletine booster with enhanced DIII-VSD activation. Simulations in MR mutant myocytes with the "boosted" mexiletine showed late sodium current was reduced to nearly WT levels and suppressed EADs and APD prolongation. The authors concluded by scaling predictions to the tissue level, illustrating that EADs are similarly suppressed by boosted mexiletine in a 1-dimensional cardiac fiber.

The authors are to be commended for proposing a novel strategy for developing new antiarrhythmic drugs, as the booster strategy deviates from the typical approach of solely targeting the ion channel pore and draws from considerable insights into the molecular mechanisms underlying drug action on the sodium channel. The study also predicts that the booster combination therapy would enable a greatly reduced drug dose, which in turn mitigates potentially off-target side effects. Although Moreno et al. (3) focused specifically on the cardiac sodium channel, the concept of altering VSD activation to enhance drug binding responses can be applied to other channels and should be explored further as a new approach to increasing drug efficacy.

The proposed strategy illustrated by Moreno et al. (3) introduces a new dimension in ion channel drug-targeting development. However, the translational application of such a strategy faces significant hurdles. The experimental demonstration of enhanced DIII-VSD activation using extracellular application of biotin also required the introduction of an additional point mutation in the *SCN5A* gene and, thus, is far from ideal for patient therapy. A more likely approach would involve the use of a hypothetical molecule that targets the DIII-VSD, but as the authors note, no such molecule has been identified. However, this study can motivate the exploration for and development of agents targeting and modifying channel VSDs.

With increased biophysical detail comes increased model complexity. The full sodium channel Markov model incorporating the combinations of DIII-VSD states, channel gating, and drug-free and drug-bound states results in 40 distinct channel states and introduces more than 80 parameters, despite constraints from microscopic reversibility and additional assumptions. To further motivate the development of such VSD-targeting therapeutics, new in silico predictions demonstrating the robustness of the proposed strategy would be particularly valuable (e.g., by performing parameter sensitivity analysis and population-based simulations).

Further challenges arise after considering the strong evidence for multiple pools of $Na_v1.5$ channels in different regions of the cell, with distinct interacting regulatory proteins and channel kinetics (4). Differences in regulatory proteins in turn may result in different responses to therapeutics modulating DIII-VSD activation. Accounting for distinct $Na_v1.5$

pools is particularly critical in LQT3, as the present authors recently showed that $Na_v1.5$ preferential localization at the intercalated disk can reduce the late sodium current and suppress EAD formation (5).

Despite these additional qualifications, the study by Moreno et al. (3) proposes several significant advances in computational modeling and drug design approaches. The strategy of predicting the drug response of a specific genetic variant and then enhancing drug efficacy by targeting nonporous channel regions could become a critical tool in the design of new antiarrhythmic therapies.

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