A Protocol to Differentiate Drug Unbinding Characteristics from Cardiac Sodium Channel for Proarrhythmia Risk Assessment

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Abstract and Introduction

Background. Drugs that block cardiac sodium channel ($Na_V1.5$) reduce the rate of action potential upstroke and consequently slow the cardiac conduction, manifested by QRS widening and/or PR interval prolongation on the surface electrocardiogram (ECG). Class I antiarrhythmic drugs (AADs) are $Na_V1.5$ blockers that have been used to treat cardiac arrhythmias. According to the Vaughan-Williams classification, the Class I AADs can be further classified into Class IA, IB and IC subgroups. Class IC AADs (flecainide and encainide) are associated with increased mortality in patients with structural heart diseases. Thus, identifying drug-$Na_V1.5$ channel interaction characteristics to distinguish subgroups of Class I AAD is important for proarrhythmic risk assessment. Indeed, FDA recently issued notifications of Post-Marketing Requirements for several antiepileptic drugs that are neuronal sodium channel blockers to request sponsors to characterize drug-$Na_V1.5$ channel interaction characteristics for comparison with Class I AADs.

There is currently no standardized protocol to characterize drug binding to and unbinding from $Na_V1.5$ channels. Reviewing data generated by different protocols can be challenging, because electrophysiology results are dependent on protocols used. To enable a more efficient review process, protocol standardization is needed. *This study tested the utility of one protocol to characterize unbinding kinetics of reference drugs in the Class IA (quinidine), 1B (mexiletine), and IC (flecainide) AAD subgroups from $Na_V1.5$ channels.*

Methodology. Whole cell recordings of $Na_V1.5$ currents were performed on $Na_V1.5$ overexpression cells. Channel properties, block potencies, and blocking kinetics of the aforementioned drugs were assessed at room temperature and near physiological temperature.

Results. Peak $Na_V1.5$ current exhibited large magnitude, extremely fast activation and inactivation, and required a high degree of series resistance compensation to maintain adequate voltage control. Mexiletine, quinidine and flecainide manifested fast, intermediate, and slow dissociation rates, respectively, at room temperature and near physiological temperature. Both association and dissociation rates of these three drugs increased by 3~5 times at physiological temperature compared with room temperature. However, the potencies of the three drugs on inhibiting $Na_V1.5$ current were not impacted by recording temperature.

Discussion. The dissociation time constants of quinidine, mexiletine and flecainide as determined using this protocol are consistent with their classification in the Class IA, IB, and IC subgroups. Adoption of this protocol for proarrhythmia risk assessment based on drug-$Na_V1.5$ channel interactions should facilitate the review process.

Materials and Methods

Cell Culture. A HEK293 cell line stably expressing human $Na_V1.5\alpha$ and β1 subunits was used (SB Drug Discovery).
Electrophysiology. Cells were recorded at room temperature (23 ± 2°C) and near physiological temperature (37 ± 2°C), using manual whole-cell patch clamp method. Protocol for drug potency study can be found at www.fda.gov/media/151418/download.

Results and Discussion

- The amplitude of the $Na_V1.5$ current is ~60% larger at near PT than at RT. The apparent rate constant of activation or inactivation at 0 mV was ~3X faster at near PT compared with that at RT (Fig. 1).
- The potencies of the representative Class I drugs (mexiletine, quinidine and flecainide) on $Na_V1.5$ current were not temperature sensitive (Fig. 2).
- Time constant of channel recovery from drug block could be determined using the protocol shown in Figure 3.
- Mexiletine, quinidine and flecainide exhibited fast, intermediate, and slow dissociation rates, respectively, at RT and near PT. Both association and dissociation rates of the these drugs increased by 3~6X at near PT compared at RT (Fig. 4 and Table 1).

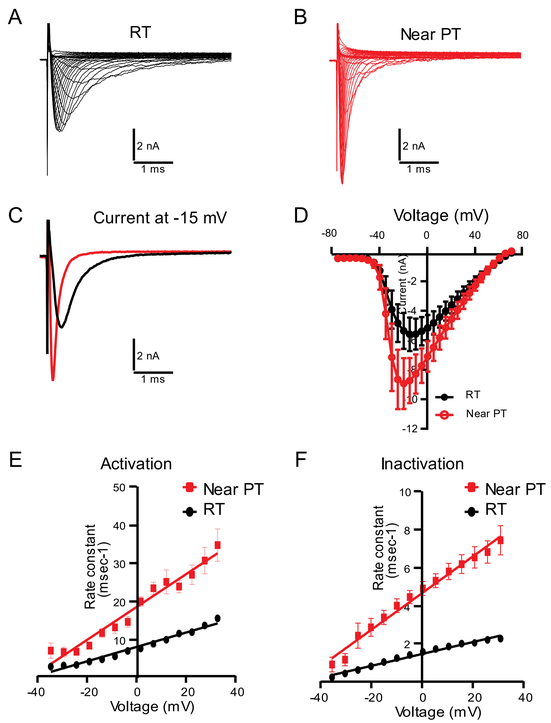


Figure 1. Effects of temperature on $Na_V1.5$ current. **A, B.** Example current traces from the same cell at RT and near PT evoked by increasing depolarization voltage steps from -80 mV in 5 mV increments. **C:** Example current traces evoked by the -15 mV step from the cell shown in A&B. **D.** Current-voltage relationship of $Na_V1.5$ current at RT and near PT. **E, F.** Rate constants of current activation and inactivation at RT and near PT.

Figure 3. Measuring drug binding and unbinding from $Na_V1.5$ channel. **A.** Schematic diagram of the stimulation protocol. **B.** Current amplitude during the control period. **C.** Current amplitude during the drug application period. **D.** Normalized current amplitude evoked by the 2 Hz stimulation protocol during the drug application period. **E.** Normalized current amplitude during the variable stimulation frequency period of the protocol to assess off rate of drug unbinding. Data were fit with a mono-exponential function.

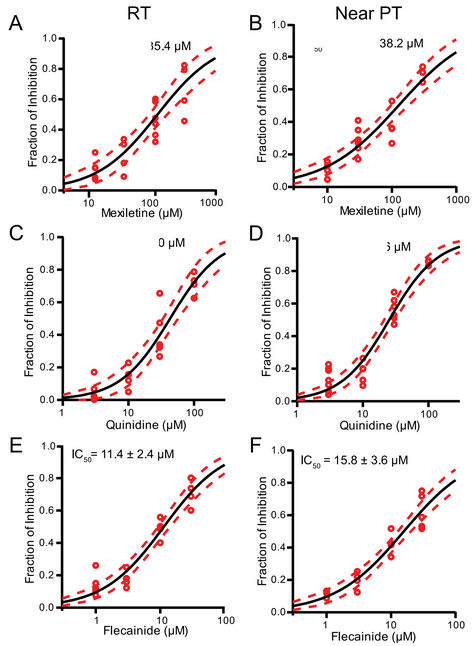
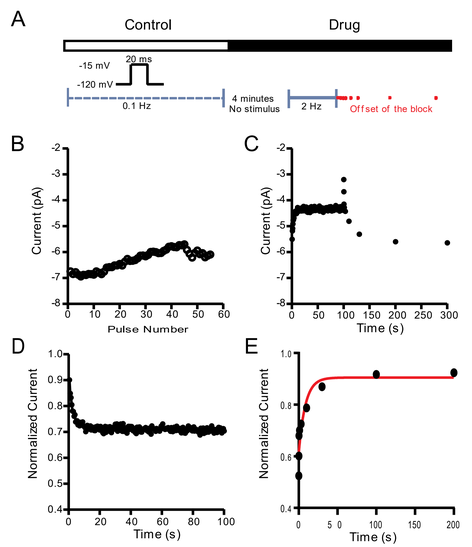


Figure 2. Concentration-inhibition plots of mexiletine (**A, B**), quinidine (**C, D**), and flecainide (**E, F**) on $Na_V1.5$ currents at RT and near PT. Solid curve reflects fit with the Hill equation. Dotted curves represent upper and lower 95% confidence intervals.



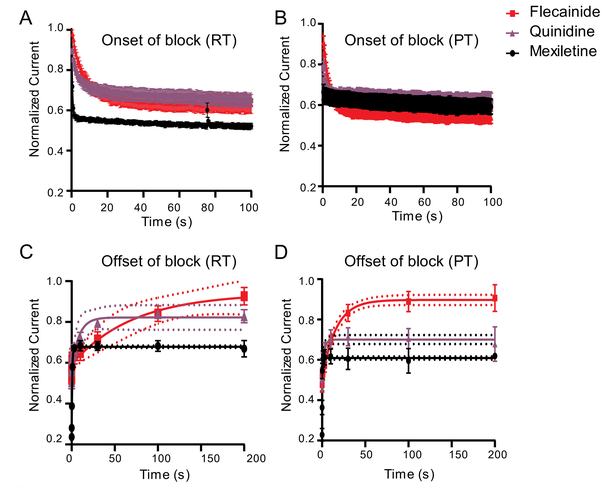


Figure 4. Summary data of binding and unbinding kinetics of quinidine, mexiletine, and flecainide on $Na_V1.5$ channel. **A, B.** Normalized current amplitude obtained in drug during the 2 Hz stimulation period at RT and near PT. **C, D.** Normalized current amplitude in drug during the variable stimulation frequency period to study drug unbinding.

Table 1. Estimated association/dissociation rates of drugs on $Na_V1.5$ channel

Drug	Dose (μM)	τ_{off} (s)		IC_{50} (μM)		K_{off} (S^{-1})		K_{on} ($S^{-1}M^{-1}$)	
		RT	PT	RT	PT	RT	PT	RT	PT
Mexiletine	100	0.671	0.16	107.9	139.7	1.5	6.3	13902	45097
Quinidine	30	10.21	2.84	43.4	25.6	0.09	0.35	2074	13672
Flecainide	10	77.27	15.14	11.4	15.6	0.01	0.06	877	3846

Conclusion

- The dissociation time constants derived from this protocol, whether generated at RT or near PT, separated quinidine, mexiletine and flecainide into Class IA, IB and IC subgroups, respectively.
- By comparing the dissociation time constant of the test article against those of the reference drugs generated concurrently, one can gain a sense which Class 1 subgroup hence proarrhythmia risk level best describes the test article.
- Using this protocol for proarrhythmia risk assessment based on drug-$Na_V1.5$ interactions could facilitate the review process.