Protocol-Dependent Differences in IC$_{50}$ Values Measured in Human Ether-Á-Go-Go–Related Gene Assays Occur in a Predictable Way and Can Be Used to Quantify State Preference of Drug Binding

William Lee, Monique J. Windley, Matthew D. Perry, Jamie I. Vandenbeng, and Adam P. Hill

Victor Chang Cardiac Research Institute (W.L., M.J.W., M.D.P., J.I.V., A.P.H.) and St Vincent's Clinical School (W.L., M.J.W., M.D.P., J.I.V., A.P.H.), University of New South Wales, Darlinghurst, New South Wales, Australia

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ABSTRACT

Current guidelines around preclinical screening for drug-induced arrhythmias require the measurement of the potency of block of voltage-gated potassium channel subtype 11.1 (Kv11.1) as a surrogate for risk. A shortcoming of this approach is that the measured IC$_{50}$ of Kv11.1 block varies widely depending on the voltage protocol used in electrophysiological assays. In this study, we aimed to investigate the factors that contribute to these differences and to identify whether it is possible to make predictions about protocol-dependent block that might facilitate the comparison of potencies measured using different assays. Our data demonstrate that state preferential binding, together with drug-binding kinetics and trapping, is an important determinant of the protocol dependence of Kv11.1 block. We show for the first time that differences in IC$_{50}$ measured between protocols occurs in a predictable way, such that machine-learning algorithms trained using a selection of simple voltage protocols can indeed predict protocol-dependent potency. Furthermore, we also show that the preference of a drug for binding to the open versus the inactivated state of Kv11.1 can also be inferred from differences in IC$_{50}$ values measured between protocols. Our work therefore identifies how state preferential drug binding is a major determinant of the protocol dependence of IC$_{50}$ values measured in preclinical Kv11.1 assays. It also provides a novel method for quantifying the state dependence of Kv11.1 drug binding that will facilitate the development of more complete models of drug binding to Kv11.1 and improve our understanding of proarrhythmic risk associated with compounds that block Kv11.1.

Introduction

The voltage-gated potassium channel subtype 11.1 [Kv11.1 (or hERG (human ether-á-go-go-related gene))] carries one of the main repolarizing currents that contribute to the cardiac action potential—the rapid component of the delayed rectifier current (I$_{Kr}$) (Perrin et al., 2008b). Drugs that block Kv11.1 are the most common cause of acquired long QT syndrome, where drug-induced prolongation of repolarization can result in the fatal polymorphic cardiac arrhythmia torsade de pointes. As a result of these proarrhythmic side effects, a range of structurally unrelated drugs, including antihistamines, antibiotics, and antipsychotics were withdrawn from the market. In 2005, safety guidelines were put in place that mandated screening for Kv11.1 block for all new chemical entities to identify potentially proarrhythmogenic drugs during early preclinical development (Food and Drug Administration, HHS, 2005). Although these guidelines have been successful in preventing new proarrhythmic drugs unknowingly coming to market, this has come at the cost of an unnecessarily high attrition rate of drugs in development (Sager et al., 2014) (i.e., current safety guidelines are very sensitive but not very specific).

At the preclinical level, a safety margin is determined by comparing the electrophysiologically determined the IC$_{50}$ of Kv11.1 to the C$_{max}$ of the drug, where the closer these two values are, the higher the risk (Redfern et al., 2003). One criticism of current guidelines is that they do not specify what methods, including cell types, voltage protocols, and temperatures, should be used to measure IC$_{50}$. Several studies have demonstrated significant differences in measured IC$_{50}$ values due to variations in all these factors (Kirsch et al., 2004; Yao et al., 2005; Milnes et al., 2010; Windley et al., 2018); in some cases, as much as a 60-fold difference in IC$_{50}$ values was observed.

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ABBREVIATIONS: ANOVA, analysis of variance; CiPA, comprehensive in vitro proarrhythmic assay; CL, interpulse time; Fso, fractional state occupancy; Fso$_{max}$, state occupancy fraction for the closed state; Fso$_{max}$, fractional state occupancy fraction of the inactivated state; ΔIC$_{50}$, difference in log IC$_{50}$ values; IC$_{50}$, rapid component of delayed rectifier current; I$_{Kr}$, drug affinity in inactivated state; I$_{Ko}$, drug affinity in open state; IC$_{50}$, relative drug affinity; K$_{s}$, voltage-gated potassium channel subtype 11.1; RO/II, ratio of the fractional state occupancy of the open state versus the inactivated state, V$_{h}$, holding potential; V$_{r}$, repolarizing ramp.
observed with different voltage protocols (Potet et al., 2001; Rezzazadeh et al., 2004). A recent publication from the comprehensive in vitro proarrhythmia assay (CiPA) initiative (Ferrini et al., 2016) acknowledged this issue and noted that it is not possible to predict protocol dependence in advance. Therefore, if a “true” IC50 value cannot be reliably determined, then a safety margin is not an effective way of identifying and eliminating risk (Lee et al., 2017).

To address this issue, CiPA has been proposed as a new safety paradigm in understanding torsade de points and assessing proarrhythmia risk (Sager et al., 2014). One of the key objectives of CiPA is to use detailed in vitro electrophysiological characterization of drug interaction with Kv11.1 to build in silico models to predict arrhythmia risk. One factor that is likely important for this approach, is understanding the state dependence of binding (Lee et al., 2017). It has been shown that for some drugs, the affinity can be as much as 30-fold greater for the inactivated state (Ko) relative to the open state (Ko) (Suessbrich et al., 1997; Ficker et al., 1998; Perrin et al., 2008a; Wu et al., 2015), whereas other drugs have no apparent state preference (Hill et al., 2016). Furthermore, previous studies have shown that two drugs with opposite state preference (104-fold difference in previous studies have shown that two drugs with opposite state preference (Hill et al., 2014). Furthermore, Perrin et al., 2008a; Wu et al., 2015), whereas other drugs have no apparent state preference (Hill et al., 2014). Furthermore, previous studies have shown that two drugs with opposite state preference (104-fold difference in Ko vs. Ki) but the same IC50 values can cause up to a 56-millisecond (~15%) difference in the extent of prolongation of the action potential duration at an IC50 dose (Lee et al., 2017). Thus, state preference is an important consideration when determining whether a Kv11.1-drug interaction is likely to be proarrhythmic.

In this study, we use both in silico and in vitro approaches to explore the extent to which protocol-dependent differences in Kv11.1 drug-binding affinity can be explained by state-dependent drug binding. Additionally, we demonstrate that: 1) measuring potency with a range of voltage protocols that result in different ratios of state occupancy can be used to predict and quantify the state-dependent drug-binding characteristics of a drug; and 2) that the potency of a drug measured using specific individual protocols can be predicted from IC50 values measured using other protocols. Importantly, either of these predictions can be made based on simple voltage protocols that are readily amenable to automated, high-throughput patch-clamp platforms. Our work provides a novel method for quantifying the state dependence of Kv11.1 drug interaction from simple measures of potency and identifies how state-preferential drug binding is a major determinant of the protocol dependence of IC50 measured in preclinical Kv11.1 assays.

Materials and Methods

In Silico Modeling. A Markov state model of drug binding to Kv11.1 (Fig. 1A; Supplemental Fig. 1) was used to simulate drug block of IK (Lee et al., 2016). This model has three closed states (C0, C1, C2), an open-conducting state (O), and an inactivated state (I); and drugs can bind to either the open state (OD) and/or the inactivated state (ID). Transitions between the non-drug-bound states are expressed as rate constants (kf for the forward rate and kb for the backward rate) and are of the format shown in eqs. 1 and 2:

\[
k_f = \alpha Q e^{-\beta V_m}/(E_{V_m})
\]

(1)

\[
k_b = \beta e^{-\beta V_m}/(E_{V_m})
\]

(2)

where \( R \) is the gas constant 8.314, \( T \) is the temperature in Kelvin, \( F \) is the Faraday constant, \( V_m \) is he membrane voltage in millivolts.
least 80% in all experiments. External bath solution contained (in mM): 130 NaCl, 5 KCl, 1 MgCl₂, 1 CaCl₂, 1.25 glucose, and 10 HEPES, adjusted to pH 7.4 with NaOH. Single-use patch pipettes were made from borosilicate glass (Harvard Apparatus, Holliston, MA) and pulled using a vertical two-stage puller (PP-830; Narishige, Tokyo, Japan) with resistance of 2–4 MΩ. Pippettes were filled with an internal solution containing (in mM): 120 potassium gluconate, 20 KCl, 1.5 Mg₂ATP, 5 EGTA, and 10 HEPES, adjusted to pH 7.4 with KOH. Data were corrected for a calculated liquid junction potential of −15 mV. Data

Fig. 1. (A) Markov state model of Kᵥ11.1-drug interaction. Parameters describing voltage-dependent Kᵥ11.1 gating transitions are presented in Supplemental Fig. 1. (B) Voltage waveforms and their gating state occupancies [open (red), inactivated (blue), closed (black)] for each of the 13 voltage protocols (numbers indicated in parentheses) used. ID, drug bound to the inactivated state; OD, drug bound to the open state.
were acquired with pCLAMP 10 acquisition software (Molecular Devices). Leak currents were subtracted off-line and data analysis was performed using Clampfit (Molecular Devices), Prism (version 7; GraphPad Software, San Diego, CA), and Matlab (MathWorks).

**Pharmacology.** Verapamil, terfenadine, cisapride, and clozapine were purchased from Sigma-Aldrich and dissolved in DMSO. The maximum final concentration of DMSO in the recording solution was less than 0.01% (v/v), which is well below the 0.1% (v/v) shown to have no effect on Kv11.1 activity (Walker et al., 1999).

Drugs were delivered via a Dynaflow Resolve microfluidic device (Cellecetricon, Möln达尔, Sweden), allowing the delivery of discrete solutions of various drug concentrations under laminar flow with a solution exchange time of less than 30 milliseconds. Current amplitudes were measured when steady state had been achieved. Five drug concentrations were used to construct a Hill curve and calculate an IC50 value for verapamil, cisapride, and clozapine. Three concentrations were used for terfenadine as drug binding has very slow kinetics, and we wished to avoid significant current rundown while allowing sufficient time for drug binding to reach steady state.

**Voltage Protocols.** We studied 13 voltage protocols that reflected the range of commonly used published voltage protocols as well as incorporating variations in cycle length, which allowed sampling of the Kv11.1 at different amplitudes and durations of state occupancy. Voltage waveforms and their corresponding state occupancies for the open (red), inactivated (blue), and closed (black) states are shown in Fig. 1B. All protocols had a holding potential (Vh) of −80 mV, unless otherwise stated. Protocol 1 was a two-step protocol with an initial step (V1) to +20 mV for 2 seconds followed by a −40 mV step (V2) for 4 seconds and a total interpulse time (CL) of 12 seconds. (Milnes et al., 2010). Protocol 2 was also a two-step protocol with V1 = +20 mV for 500 milliseconds, Vh = −50 mV for 200 milliseconds, and CL = 2 seconds (Yao et al., 2005). Protocol 3 was a ramp protocol with V1 = +20 mV for 500 milliseconds, followed by a repolarizing ramp (Vr) of −0.5 V/s, CL = 2 seconds (Yao et al., 2005). Protocol 4 was the voltage waveform of an O’Hara Rudy action potential (O’Hara et al., 2011). Protocols 5–8 consisted of a step ramp with V1 = +20 mV for 200 milliseconds, Vh = 1 V/s, and CL of 500 milliseconds, and 1, 2, and 10 seconds, respectively. Protocol 9 was a step-ramp protocol with a long V1 step of 5 seconds at +20 mV, followed by Vh = 1 V/s and CL = 10 seconds. Protocol 10 was a two-step protocol with V1 = +40 mV for 200 milliseconds, V2 = −120 mV for 600 milliseconds, and CL = 1 second, resulting in a negative hook-tail current (Perrin et al., 2008a). Finally, protocols 11–13 were performed with direct application of the drug during voltage holding at Vh = 0 (Hill et al., 2014), +20, and −20 mV, respectively, and CL = ∞. In vitro experiments and in silico machine-learning algorithms, a subset of six of the above protocols were used; this included protocols 1, 8, 10, 11, 12, and 13. Specific details of individual protocols as well as evoked Kv11.1 currents from in silico and in vitro experiments are shown in Supplemental Figs. 2–14.

To quantify the state occupancy fraction of open state (Fso.open) and inactivated state (Fso.inact) for each voltage protocol, a time integral of state occupancy was calculated for each state as follows:

\[
Fso = \frac{\int_0^{CL} \text{State Occupancy} \, dt}{CL}
\]

(6)

Where State Occupancy represents the state occupancy for each of the three states (Open = O, Inactivated = I, or Closed = C1 + C2 + C3) defined in the Markov model above in the section “In Silico Modeling,” and CL is the total interpulse time, as stated above. The ratio of the fractional state occupancy of the open state versus the inactivated state (RO/I) for each voltage protocol was therefore defined in eq. 7:

\[
R_{O/I} = \frac{Fso_{open}}{Fso_{inact}}
\]

(7)

**Machine Learning.** A subset of six voltage protocols was chosen that maximized the differences in state occupancy for the three states and demonstrated the largest variation in in silico IC50 measurements. This subset was used to simulate and measure IC50 values using the randomly generated 2000 training and 1000 validation theoretical drug sets. These sets of IC50 values were then used to train and validate machine-learning algorithms to predict drug-binding characteristics including state-preference and protocol-dependent affinity. All machine-learning prediction algorithms were generated and validated using the Matlab Neural Network fitting application (MathWorks). Training data sets were normalized (z-score) to have a mean of 0 and a S.D. of 1. Validation data sets were z-score transformed using the mean and S.D. of their respective training set.

Neural networks were constructed using between 10 and 100 neurons within one hidden layer. The neural network which best fitted the training data set was then tested for overfitting using the corresponding validation data set.

**Statistical Methods.** Analysis of variance (ANOVA) was used for statistical analysis between normally distributed data for multiple
groups. \( P \) values of <0.05 were considered significant. Statistical analyses were performed in Matlab (MathWorks).

**Results**

**In Silico Variations in Measured Drug Affinity to Kv11.1.** To test the influence of state preferential drug binding on variation in measured Kv11.1 affinity, we simulated block of Kv11.1 using two voltage protocols that have been used in previously published studies (Milnes et al., 2010; Hill et al., 2014). We simulated dose-response curves for an inactivated state-preference drug with a 1000-fold higher affinity for the inactivated state versus open state \((K_0/I = 10^{-3})\). Example simulated currents in response to increasing concentrations of the inactivated state-preference drug for the two protocols are shown in Fig. 2B. For each protocol, a Hill curve was fitted to the simulated data to derive an IC\(_{50}\) value for channel block. The inactivated state-preference drug had a 50-fold higher affinity for Kv11.1 when measured using the +20-mV V\(_{h}\) protocol compared with the 0.1-Hz step-ramp protocol \(8\) (Fig. 2C, left panel) \((\text{IC}_{50} = 1 \text{ and } 50 \text{ mM, respectively})\). As a comparison, we also simulated block using a non-state-preference drug, which showed no difference in Kv11.1 affinity between the two protocols \((\text{IC}_{50} = 100 \text{ mM})\) (Fig. 2C, right panel).

To probe the protocol dependence of measured Kv11.1 affinity in more detail, we extended our study to a panel of 6561 theoretical drugs with varying state preferences and a larger array of 13 voltage protocols (Fig. 1B; Supplemental Figs. 2–14). For each combination of drug and protocol, a dose-response curve was simulated (as shown in Fig. 2B) and an IC\(_{50}\) value derived through fit of the Hill equation. For each drug, we then calculated the difference in log IC\(_{50}\) values \((\Delta\text{IC}_{50})\) between pairs of protocols and grouped them with respect to state preference (Fig. 2B: inactivated state-preference \((K_0/I < 0.5)\) are colored blue; open state-preference \((K_0/I > 2)\) are colored red; and minimal-state preference \((0.5 < K_0/I < 2)\) are colored yellow) (Fig. 3). This pairwise analysis of protocols gave a total of 78 comparisons.

We first examined the distribution of \(\Delta\text{IC}_{50}\) values measured using the same two protocols shown in Fig. 2 (protocols 8 and 12; Fig. 3A). We observed a wide range of \(\Delta\text{IC}_{50}\) values, from \(-2.0.9\) to 1.7 which equates to a difference in IC\(_{50}\) for protocol 8 versus protocol 12 of 8-fold less or 50-fold higher. Overall, drugs tended to be grouped by state preference. Although all drugs tended to have a higher affinity when measured with protocol 12, this effect was most pronounced for inactivated state-preference drugs. For reference, the inactivated state-preference drug example from Fig. 2 is marked as with a * symbol, and the non-state-preference drug is marked with a † symbol. Other

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**Fig. 3.** Differences in IC\(_{50}\) values between two protocols for 6561 theoretical drugs. Drugs are grouped by preferential binding to the open state \((K_0/I > 1, \text{red})\), inactivated state \((K_0/I < 1, \text{blue})\), and minimal state \((0.5 < K_0/I < 2, \text{yellow})\) preference. (A) Shows a wider landscape of comparison between protocol 8 and protocol 12. The inactivated state-preference drug and non-state-preference drug examples are marked with * and †, respectively. The difference in IC\(_{50}\) measured as the difference in log_{10}[IC\(_{50}\)] \((\Delta\log[\text{IC}_{50}])\) between these two protocols spans over a wide range with some grouping with respect to drug state preference. (B) Comparison between IC\(_{50}\) values from protocol 5 and protocol 12, showing more distinct grouping with respect to state preference. (C) Comparison between IC\(_{50}\) values from protocol 5 and protocol 8; no obvious grouping is seen with respect to drug state preference due to the very similar waveform of the two voltage protocols. But up to a 20-fold variation in IC\(_{50}\) is still observed, indicating mechanisms other than state preference can influence drug affinity to Kv11.1. (D) Comparison between IC\(_{50}\) values from protocol 12 and protocol 13: very distinct grouping with respect to drug state preference is seen over a smaller range. The right side panels (A–D) show the waveforms of the two voltage protocols being compared for their respective histogram.
protocol comparisons gave much clearer separation of drugs according to their state preference. A pairwise comparison of protocols 5 and 12 (Fig. 3B) resulted in a clear bimodal separation of drugs based on open/inactivated state preference, with inactivated state-preference drugs displaying a higher affinity and open-state-preference drugs having a lower affinity for protocol 12 relative to protocol 5. Consistent with our previous observations, non-state-preference drugs had minimal or no difference in measured IC50 values between protocols. It is important to note that subtle differences in the protocols being compared can significantly affect the observed distributions for ΔIC50. For example, in Fig. 3, A and B, protocol 12 is compared against protocols 8 and 5, respectively. The only difference between protocols 5 and 8 is the CL (500 vs. 10,000 milliseconds), yet the observed distributions (compare Fig. 3, A and B) are clearly different, highlighting that it is not solely the “test pulse” component of the protocol that is important in defining the measured potency. Conversely, when protocols 5 and 8 are compared against each other (Fig. 3C), there is no observable clustering of drugs based on their state preference. However, the fact that there were still observable differences in ΔIC50 values between two protocols for different drugs (maximum ΔIC50, −1.3 or +0.1) suggests that state preference of binding is not the only factor that influences protocol-dependent affinity.

Finally, we examined an example of a pairwise comparison between nonpulse protocols. A comparison of ΔIC50 for protocols 12 and 13—static Vh values of +20 and −20 mV respectively—is shown in Fig. 3D. We observed a distinct grouping of drugs based on state preference, albeit over a smaller range of ΔIC50 values, from −0.7 to 0.2 difference between the two protocols. In this case, inactivated state-preference drugs had a higher affinity for protocol 12 and open state-preference drugs for protocol 13. It is notable that these two protocols show the greatest separation of drugs based on state preference as they are pure equilibrium measures, meaning that there is no cycling of state occupancy among the open, inactivated, and closed states during the protocol, and consequently there is no influence from drug-binding kinetics.

Given this varied separation of drugs with different pairs of protocols, we next investigated which pairwise comparison gave the greatest ΔIC50 across all our drugs. To do this, we took the maximum ΔIC50 from each pairwise comparison for each drug (Fig. 4). This demonstrated that the maximum ΔIC50 was most often observed for comparisons between protocols 8 and 12, as well as between protocols 8 and 13 (Fig. 4A) (for voltage waveforms, refer to Fig. 1B, protocols 8, 12, and 13). More specifically, we observed that open state-preference drugs tended to have a maximum ΔIC50 between protocols 8 and 13 (for voltage waveforms, refer to Fig. 1B, protocols 8 and 12). This supports the idea that individual voltage protocols sample distinct gating state “space” and hence differentially interact with state preferential drug binding to determine these shifts in IC50.

**Voltage Protocol Dependence of State Occupancy.** To better understand the mechanism behind these observations, we next examined how the state occupancy of the Kv11.1 channel varies with different voltage protocols. We examined the underlying Kv11.1 state occupancy during the three most prominent voltage protocols from Fig. 4. Shown in Fig. 5A are the voltage waveforms for the 0.1-Hz step-ramp protocol (protocol 8) and the two nonpulsed Vh protocols at +20 and −20 mV (protocols 12 and 13, respectively). The respective time-varying state occupancy for the open (red), closed (black), and inactivated (blue) states of the channel during each of these protocols is shown in Fig. 5B. To compare the relative occupancy over time, for each protocol we calculated the state occupancy time integral for each state expressed as a fraction of the total time integral [i.e., Fso, Fso, and state occupancy fraction for the closed state (Fso)] (Fig. 5C) (Materials and Methods).

The relative occupancy of the open versus inactivated states (i.e., RO/I) for the different protocols is shown as a number above each protocol in Fig. 5C. All the protocols we tested had
a \( RO/I < 1 \), indicating that there was a greater probability of channels spending time in the inactivated state than the open state. For the two nonpulsed protocols (protocols 12 and 13), \( RO/I \) decreased with increasing voltage (0.6 and 0.07 for \(-20\) and \(+20\) mV, respectively), consistent with the known voltage-dependent inactivation behavior of \( \text{K}_\text{v}11.1 \). The pulsed protocol (protocol 8) had a close to equal time spent in the open and inactivated states with an \( RO/I = 0.82 \). However, the pulsed protocol (protocol 8) dwelt predominantly in the closed state \( (F_{\text{so\_closed}}, 96.3\%) \) in contrast to the nonpulsed protocols \( (F_{\text{so\_closed}}, 0.03\% \) and 14.2\% for protocols 12 and 13, respectively). In keeping with this, a lower occupancy of the open and inactivated versus closed states was evident for the pulsed protocol (protocol 8) compared with the nonpulsed protocols 12 and 13 (Fig. 5C).

These data suggest that protocol 12, having the lowest \( RO/I \), will be the most sensitive to inactivated state–preference drugs, whereas protocol 8 will be almost equal in sensitivity to open state–preference and inactivated state–preference drugs. Moreover, the observation in Fig. 4 that inactivated state–preference drugs were most likely to have a maximum difference in affinity between protocols 8 and 12 can be attributed to the large difference in \( F_{\text{so\_inact}} \). Similarly, the maximum difference in affinity for open state–preference drugs is seen between protocols 8 and 13 due to the large difference in \( F_{\text{so\_open}} \) values between these protocols.
In Silico Prediction of State Preferential Drug-Binding Characteristics. Our observations so far suggest that the state-dependent binding properties of the drug and the gating state occupancy of the channel (as a function of the voltage protocol) interact in a predictable way to determine the differences in measured potency between protocols. To further test this hypothesis, we sought to demonstrate the corollary of this (i.e., use measured differences in IC$_{50}$ to predict the state preference of a drug. To do this, we measured IC$_{50}$ values for 2000 in silico drugs using protocols 8, 12, and 13 (for voltage waveforms, refer to Fig. 1B, protocols 8, 12, and 13) and from these created a set of three pairwise comparisons of ΔIC$_{50}$. This set of pairwise comparisons was then used to train a neural network. A second set of 1000 different drugs was then used to test the accuracy of our predictions (Fig. 6). Overall, the model performed extremely well, with a Pearson’s correlation coefficient between predicted and true state preference (KO/I) of $R = 0.930$.

We then sought to test whether the same three pairwise comparisons of ΔIC$_{50}$ could be used to predict IC$_{50}$ values for other voltage protocols. We were able to predict IC$_{50}$ values in all cases (protocols 11, 1, and 10) (for voltage waveforms, refer to Fig. 1B, protocols 1, 8, 10, 11, and 12) with a high degree of accuracy (Fig. 7). In silico predictions (shown in black) for protocols 1, 10, and 11 had a correlation coefficients of $R = 0.984$, $R = 0.988$, and $R = 0.999$, respectively.

In Vitro Measurement of Protocol Dependence of IC$_{50}$. We next tested whether drugs with varying state preference also showed different measured potencies in manual patch-clamp electrophysiology assays, depending on the voltage protocol used. Typical K$_{11.1}$ current traces recorded using six of the protocols examined in silico (1, 8, 10, 11, 12, and 13) for voltage waveforms, refer to Fig. 1B, protocols 1, 8, 10, 11, 12, and 13) in response to increasing (cisapride) are shown in Fig. 8. To highlight the differences in measured potency among the protocols, the 60 nM dose of cisapride is highlighted in red in Fig. 8 for each case. For the short pulse protocols (8 and 10), 60 nM cisapride blocked measured current by $\sim 25\%$. In comparison, the same concentration caused a 75%–80% reduction in current amplitude when measured using the long-pulse protocol (protocol 1) or the non–pulse protocols 11, 12, and 13.

The same set of in vitro experiments were also carried out for three other drugs (clozapine, verapamil, and terfenadine), dose-response curves constructed for each of the six protocols (Fig. 9, A–D, left-side panels), and IC$_{50}$ values derived from fits of the Hill equation (Fig. 9, A–D, right-side panels) (for voltage waveforms, refer to Fig. 1B, protocols 1, 8, 10, 11, 12, and 13). Maximums of 2.79-fold, 8.32-fold, 1.93-fold, and 3.09-fold ΔIC$_{50}$ values, respectively, were measured between protocols for verapamil, cisapride, clozapine, and terfenadine. Overall, significant differences between the IC$_{50}$ values measured using the six protocols were observed for verapamil, cisapride, and terfenadine ($P = 0.0297$, 0.0005, and 0.001, respectively; ANOVA), but not for clozapine ($P = 0.3406$). This result suggests that verapamil, cisapride, and terfenadine may have state preferential binding properties. Furthermore, verapamil, and cisapride demonstrated a higher affinity when measured with protocol 12 compared with protocol 8 (1.77-fold and 5.96-fold ΔIC$_{50}$, respectively), consistent with our in silico model observations for inactivated state–preference drugs (Fig. 2; Fig. 3A).

To understand these observed differences in IC$_{50}$ values, we again considered the channel state occupancy during the individual voltage protocols. For consistency between in vitro experiments and in silico state occupancy calculations, the state occupancies in Fig. 9E were calculated for 22°C by scaling the transition rates in the Markov model (Fig. 1) for temperature (see Materials and Methods). For cisapride, there is a correlation between measured potency and F$_{so_{inact}}$ (i.e., lower IC$_{50}$ values were measured for protocols with greater F$_{so_{inact}}$ values (protocols 11, 12, and 13) (for voltage waveforms, refer to Fig. 1B, protocols 11, 12, and 13), and the highest IC$_{50}$ values were measured for protocols with the lowest F$_{so_{inact}}$ values (protocol 8), consistent with preferential binding to the inactivated state. In addition to this, among the pulsed protocols, block by cisapride is less potent when measured with the short-pulsed protocols 8 and 10 (for voltage waveforms, refer to Fig. 1B, protocols 8 and 10), which have lower F$_{so_{open}}$ and F$_{so_{inact}}$ values compared with the long-pulsed protocol 1 (for voltage waveform, refer to Fig. 1B, protocol 1) (Fig. 9E). This suggests that there is a requirement for a longer channel opening time in order for the drug-bound states (either open or inactive) to reach their true equilibrium. Overall, a similar pattern was observed for verapamil, though to a lesser degree (Fig. 9A).

For terfenadine, although there are significant differences in the potency of block measured among protocols, the correlation between IC$_{50}$ and F$_{so_{inact}}$ is not apparent. In particular, the relationship is disrupted by the downward shift in IC$_{50}$ measured using protocols 8 and 10 (e.g., compared with cisapride) for (voltage waveforms, refer to Fig. 1B, protocols 8 and 10). We note that these protocols have a greater proportion of F$_{so_{inact}}$, suggesting that drug remaining bound to the closed state or drug trapping may be a modulating factor in the measured IC$_{50}$ value for terfenadine. Finally, for clozapine, the potency of the block was the same.
regardless of the state occupancy of the voltage protocol used (Fig. 9C).

**In Silico Prediction of In Vitro Drug-Binding Characteristics.** Since there appears to be a qualitative agreement between state occupancy and measured potency for most of the drugs tested, we next sought to use our in silico trained prediction algorithm (Fig. 6) to predict the state preference ($KO/I$) of the four drugs using the in vitro measurements of IC$_{50}$ from protocols 8, 12, and 13 (for voltage waveforms, refer to Fig. 1B, protocols 8, 12, and 13). $KO/I$ predictions for verapamil, cisapride, clozapine, and terfenadine are shown in Table 1.

Similarly, we used the algorithms from Fig. 7 to predict the measured IC$_{50}$ values of other in vitro voltage protocols 1, 10, and 11 (for voltage waveforms, refer to Fig. 1B, protocols 1, 10, and 11). Predictions for our four in vitro drugs (verapamil, cisapride, clozapine, and terfenadine) are shown in Fig. 10 in red. To put these in vitro predictions in context, we added the predictions of our 1000 theoretical in silico drugs from Fig. 7 in gray. A similar prediction accuracy was achieved for the four in vitro drug predictions (shown in red) as for the in silico drug predictions. Notably, in vitro IC$_{50}$ prediction algorithms were better for the nonpulse protocol (protocol 11) (Fig. 10A) and the long-pulse protocol (protocol 1) (Fig. 10B), compared with the short-pulse protocol (protocol 10) (Fig. 10C). Overall, our accuracy of in vitro IC$_{50}$ prediction using our in silico prediction algorithm, was within one logarithmic unit of the measured in vitro IC$_{50}$ in all but one case. Details of in vitro measured and in silico predicted IC$_{50}$ values, respective Hill coefficients, as well as $KO/I$ values are shown in Supplemental Table 1.

**Discussion**

In this study, we have shown that state-dependent drug binding to K$_{11.1}$ channels is a significant factor in determining the range of IC$_{50}$ values measured for individual compounds using different voltage protocols. Furthermore, these differences occur in a predictable manner as a function of the state occupancy of the channel. As a result, both the state preference of a drug as well as its potency measured using other voltage protocols can be inferred from simple equilibrium measures of block.

**Factors Contributing to Protocol-Dependent Potency of Block.** The variability in potency of K$_{11.1}$ block measured using different voltage protocols has been reported in several studies (Kirsch et al., 2004; Yao et al., 2005; Milnes et al., 2010). For example, the IC$_{50}$ for cisapride varies over a 60-fold range, depending on the protocol used (Potet et al., 2001; Rezazadeh et al., 2004). Our data demonstrate that the synergy of the state preference in drug binding as well as voltage protocol–dependent state occupancy results in such observed variations in affinity. Therefore, drugs that preferentially bind to a specific gating state will have a higher measured affinity for K$_{11.1}$ when using a voltage protocol that favors that gating state, and vice versa. In contrast, for drugs that have no state preference there is no dependence of coefficient $R$ to the identity line for each prediction algorithm. The predicted and experimentally measured IC$_{50}$ values are shown as the $z$-score–transformed $\Delta$log[IC$_{50}$].
the measured IC$_{50}$ on the voltage protocol used. However, the data in Fig. 3 demonstrates that variations in IC$_{50}$ values can also occur regardless of state preference, suggesting that the kinetics of drug binding can also have an impact on measured potency. Drugs that block Kv11.1 require channel opening to gain access to their binding site in the inner cavity of the channel pore (Ficker et al., 1998; Walker et al., 1999). Therefore, drugs with slow binding kinetics will have a relatively lower affinity when measured using protocols where occupancy of the open/inactivated states is short (relative to the on rate for drug binding) and have a relatively higher apparent affinity when measured using voltage protocols with long depolarization steps or nonpulsed protocols that promote prolonged occupancy of the open and inactivated states. In our in vitro data, this phenomenon is particularly evident for cisapride (Fig. 9B), which has a lower potency measured using protocols with high proportion of Fso$_{closed}$ (95% and 78%, respectively) (for voltage waveforms, refer to Fig. 1B, protocols 8 and 10) than when measured using all other protocols that have lower Fso$_{closed}$. Conversely, for terfenadine, a trapped drug (Kamiya et al., 2008; Windley et al., 2017), the potency measured with protocols 8 and 10 (for voltage waveforms, refer to Fig. 1B, protocols 8 and 10) is increased relative to the other protocols, consistent with the accumulation of block in the closed state. This ability to identify truly trapped drugs, as opposed to those such as cisapride that display "virtual trapping" as a result of slow unbinding kinetics (Windley et al., 2017), is important since it has been suggested that "true" trapping may confer an additional arrhythmia risk (Di Veroli et al., 2014).

We also noted some variation in the Hill coefficients measured across the various in vitro protocols (see Supplemental Table 1). Although most of the coefficients were close to 1, consistent with previously published reports, there were some exceptions. For example, a lower Hill coefficient was found for verapamil using protocol 8 (Hill coefficient = 0.55) (for voltage waveforms, refer to Fig. 1B, protocol 8), whereas, in general, Hill coefficients for terfenadine were slightly higher than 1 (e.g., the highest Hill coefficient for terfenadine was 1.67 for protocol 13) (for voltage waveforms, refer to Fig. 1B, protocol 13). These observations can perhaps be explained by factors such as the difficulty in accurately measuring the degree of block for some drugs with certain protocols. For example, the kinetics of binding of terfenadine to Kv11.1 have previously been shown to be problematic for measuring steady-state block, since the timecourse of block is so slow (Windley et al., 2016).
In our experiments, an underestimation of the degree of block at low concentrations, when the timecourse of terfenadine block is slowest, would explain the Hill coefficients being greater than 1.

**Predicting Potency across Different Voltage Protocols.** Defining the potency of Kv11.1 block is an important part of existing preclinical safety screening, where a safety index value is determined by comparing the IC50 of Kv11.1 to the Cmax of the drug (Redfern et al., 2003). However, the degree of protocol dependence of measured potency that we and others have demonstrated (Supplemental Tables 2–5), makes this problematic because: 1) a single affinity measure does not represent a true affinity for Kv11.1—it only represents the affinity of a drug for one specific voltage protocol; and 2) even if a single voltage protocol could be agreed upon to standardize drug affinity assays, it would not adequately reflect the wide-ranging effects of external conditions such as heart rate, QT prolongation, and hypokalemia. Our data show that there is a predictable relationship between IC50 values measured using different protocols. Furthermore, using IC50 values measured from just a few simple voltage protocols, it is possible to predict what the potency of a drug will be when measured using other voltage protocols. For in silico data, this can be done with a very high degree of accuracy (Fig. 7), whereas for in vitro data, the degree of accuracy is reduced, perhaps as a result of factors such as drug trapping, which are not implicit in the Markov model used in this study. Nevertheless, this demonstration of a quantifiable relationship between the variable measured potencies of Kv11.1 block is important as it provides a framework for the direct comparison of potencies measured between groups using different assays and may allow for the reinterpretation of legacy data sets obtained using different protocols.

**Prediction of State-Dependent Binding Characteristics.** It has previously been shown that high-affinity binding to Kv11.1 occurs, at least for some drugs, because of preferential binding to the inactivated state (Suessbrich et al., 1997; Ficker et al., 1998; Numaguchi et al., 2000; Perrin et al., 2008a). Furthermore, preferential binding to the inactivated state has been associated with increased prolongation of the action potential duration (Lee et al., 2016), and several drugs that have been withdrawn from market (e.g., astemizole and terfenadine) or had their use severely restricted (e.g., cisapride) preferentially bind to the inactivated state (Perrin et al., 2008a). However, the quantification of state-dependent binding is difficult, since it cannot be readily measured experimentally. Rather, indirect measures relying on changes in affinity that occur as a result of perturbation of the inactivated state using site-directed mutagenesis (Suessbrich et al., 1997; Ficker et al., 1998; Numaguchi et al., 2000; Perrin et al., 2008a), changing potassium concentration (Kamiya et al., 2008), or measuring of drug-binding kinetics (Hill et al., 2014) are routinely used.

**Fig. 9.** In vitro experimental data. (A) Examples of in vitro Kv11.1 currents evoked when voltage clamped using voltage protocols 8 (left) and 12 (right) in the presence of increasing concentrations of drug. (A–D) Dose-response curves (left) and box plot of corresponding IC50 values for each of the six voltage protocols (1, 8, 10, 11, 12, and 13) (n = 4–6) in the presence of verapamil, cisapride, clozapine, and terfenadine, respectively.

The central line shows the median value of each protocol. The mean for each box is depicted with a + symbol. Box plot boxes extend to the 25th and 75th percentile, of the data for each protocol, and the whiskers show the 95% confidence intervals for IC50 values for each protocol. P values for ANOVAs are shown for each drug. (E) Relative proportions of Fso for each of the six voltage protocols, demonstrating underlying differences in state occupancy at 22°C. The relative state occupancy (RO/I) of each protocol representing the ratio of Fso_open to Fso_inact is shown above each column in the figure.
In this study, we show that a machine-learning algorithm, trained using IC\textsubscript{50} values measured from just three simple voltage protocols, can accurately determine the degree of state-preference drugs in silico (Fig. 6). We also used the same algorithm to predict the state preference of four drugs—clozapine, cisapride, verapamil, and terfenadine—based on our in vitro measured IC\textsubscript{50} values. For clozapine, the only drug we tested for which there is a published, fully parameterized in silico model (Hill et al., 2014), meaning that the state-dependent binding properties are unambiguously known, the algorithm correctly identified this as a drug with no state preference (Table 1). For cisapride, the algorithm predicted inactivation state preference (KO/I = 0.26), consistent with previously published studies using mutagenesis, which showed a ratio of 0.37 between the IC\textsubscript{50} value for wild-type K\textsubscript{v}11.1 and the N588K inactivation-deficient mutant K\textsubscript{v}11.1 (Perrin et al., 2008a). The data in the literature for verapamil are mixed. Previously, publications show a low variation in measured IC\textsubscript{50} values from any two protocols (maximum 6-fold difference) (Supplemental Table 2), suggesting that verapamil has weak or nonpreferential state binding to K\textsubscript{v}11.1. This is consistent with our model prediction of KO/I = 1.97. However, other studies have shown that potency of block of the S620T inactivation-deficient K\textsubscript{v}11.1 mutant by verapamil was less potent than for wild type (Zhang et al., 1999), consistent with inactivation state preference. However, this mutation has also been shown to have a gating-independent effect on drug binding (Guo et al., 2006) (i.e., mutation at this residue directly disrupts the drug-binding site), which might confound the interpretation of the effects of this mutant in relation to state preference. Finally, our predicted KO/I for terfenadine was 69.67, suggesting a strong preference for binding to the open state of K\textsubscript{v}11.1. This is inconsistent with published data using either mutagenesis (Perrin et al., 2008a) or potassium inhibition of inactivation (Kamiya et al., 2008) that identified terfenadine as a drug that preferentially binds to the inactivated state. One explanation for this is that terfenadine exhibits closed-state trapping (Kamiya et al., 2008; Windley et al., 2017), which is not accounted for in the Markov model we used, that confounds the in vitro predictions for this drug.

**Limitations.** The main limitations of this study, in relation to the prediction of in vitro data using an algorithm developed using in silico data, are the characteristics of the available Markov models describing drug binding to K\textsubscript{v}11.1, particularly in relation to drugs that exhibit closed-state trapping. Although there are models in the literature that include either state-dependent binding (Di Veroli et al., 2013; Lee et al., 2016) or trapping (Di Veroli et al., 2014; Li et al., 2017), there are no models that include both. Development of a more complete Markov description of drug binding, which included both state-dependent binding and trapping, would further improve predictions based on in vitro data and, indeed, the quantification of state-dependent binding enabled using the approach reported here will likely facilitate the parameterization of such models.

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**Fig. 10.** In silico neural network algorithms were trained using in silico IC\textsubscript{50} values from protocols 8, 12, and 13 to predict in vitro IC\textsubscript{50} values for other voltage protocols using in vitro measurements of IC\textsubscript{50}. For each panel, predictions vs. true measured IC\textsubscript{50} value for the four in vitro drugs (verapamil, cisapride, clozapine, and terfenadine; shown in red). For context, in silico predictions from Fig. 7 are shown in gray. IC\textsubscript{50} value for protocol 1 (A), IC\textsubscript{50} value for protocol 10 (B), and IC\textsubscript{50} value for protocol 11 (C). An identity line (Y = X) is shown as a dashed green line. The accuracy of the in vitro prediction for each of the three voltage protocols is represented as the root mean squared error (RMSE) for each panel.
TABLE 1

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<td>Clozapine</td>
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<td>Terfenadine</td>
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Note that only clozapine has a fully parameterized model of binding to K11.1 from Hill et al. (2014), and therefore is the only drug that has an in vitro validated value for KO/I.

Second, it is well known that the kinetics of drug binding to K11.1 channels (Windley et al., 2016) as well as the kinetics of K11.1 channel gating (Vandenbergh et al., 2006) are temperature dependent. This is evident in the differences that we observe in the \( I_F \) current at 37°C in silico (Fig. 1B) and 22°C in vitro (Fig. 8). Ideally, therefore, an in vitro data set gathered at 37°C would be desirable for further validation. Despite this, we still achieve good prediction accuracy with our machine-learning models both for state preference and drug affinity. This is consistent with previous published data showing that even though the kinetics of drug binding to K11.1 for the four drugs examined here are temperature dependent, the IC50 values for block are not (Hill et al., 2014; Windley et al., 2016, 2018).

Conclusion. In this study, we have demonstrated that state-dependent drug binding is a major factor in determining the potency of K11.1 block measured using different protocols, with the exact measured potency also affected by factors such as drug-binding/drug-unbinding kinetics and closed-state trapping. As a result, we show that interprotocol differences in measured IC50 occur in a predictable way, meaning the potency of a compound measured using any voltage protocol can be reliably estimated from knowledge of the channel-state occupancy. This is an important step since it allows for the direct comparison of potencies measured using different assays, as well as the reinterpretation of legacy data sets obtained using different protocols as part of previous compound screens. Furthermore, we also show that the state preference of a drug can be inferred from differences in its IC50 values measured from commonly used protocols that are simple enough to be readily deployed on high-throughput, automated patch-clamp platforms. This is the first demonstration of the quantification of state preference as the need for measuring either the kinetics of block/unblock or the disruption of K11.1 inactivation (using mutagenesis or ionic concentration). The ability to simply quantify state preference will facilitate the development of more complete models of drug binding to K11.1 and will improve our understanding of prorarrhythmic risk associated with K11.1 blocking compounds.

Acknowledgments
The authors thank, Mark Hunter, Dr. Stewart Heitmann, and Dr. Melissa Mangala for informative discussion.

Authorship Contributions
Participated in research design: Lee, Vandenbergh, Hill.
Conducted experiments: Lee, Windley, Perry.
Contributed to new reagents or analytical tools: Lee, Vandenbergh, Hill.

References

Performed data analysis: Lee, Hill.
Wrote or contributed to the writing of the manuscript: Lee, Windley, Perry, Vandenbergh, Hill.


**Address correspondence to:** Adam P. Hill, Molecular Cardiology and Biophysics Division, Victor Chang Cardiac Research Institute, 405 Liverpool Street, Darlinghurst 2010, NSW, Australia. E-mail: a.hill@victorchang.edu.au
Protocol-dependent differences in IC$_{50}$ values measured in hERG assays occur in a predictable way and can be used to quantify state preference of drug binding.

William Lee, Monique J. Windley, Matthew D. Perry, Jamie I. Vandenberg, Adam P. Hill

*Molecular Pharmacology*
Supplemental Figure Legend

Supplemental Figure 1. A) Markov state model of Kv11.1-drug interaction. Parameters describing voltage-dependent Kv11.1 gating transitions are presented in the table in (B). Drug binding and unbinding is described as forward and backward rate constants $k_{f,\text{open}}$ and $k_{b,\text{open}}$ respectively for the open state and $k_{f,\text{inact}}$ and $k_{b,\text{inact}}$ for the inactivated state. These drug binding and unbinding rate constants are specified for each individual drug scenario.

Supplemental Figure 2. Protocol 1. Voltage waveform, and example evoked Kv11.1 currents from in silico model and in vitro experiments.

Supplemental Figure 3. Protocol 2. Voltage waveform, and example evoked Kv11.1 currents from in silico model experiments.

Supplemental Figure 4. Protocol 3. Voltage waveform, and example evoked Kv11.1 currents from in silico model experiments.

Supplemental Figure 5. Protocol 4. Voltage waveform, and example evoked Kv11.1 currents from in silico model experiments.
Supplemental Figure 6. Protocol 5. Voltage waveform, and example evoked Kv11.1 currents from in silico model experiments.

Supplemental Figure 7. Protocol 6. Voltage waveform, and example evoked Kv11.1 currents from in silico model and experiments.

Supplemental Figure 8. Protocol 7. Voltage waveform, and example evoked Kv11.1 currents from in silico model experiments.

Supplemental Figure 9. Protocol 8. Voltage waveform, and example evoked Kv11.1 currents from in silico model and in vitro experiments.

Supplemental Figure 10. Protocol 9. Voltage waveform, and example evoked Kv11.1 currents from in silico model experiments.

Supplemental Figure 11. Protocol 10. Voltage waveform, and example evoked Kv11.1 currents from in silico model and in vitro experiments.

Supplemental Figure 12. Protocol 11. Voltage waveform, and example evoked Kv11.1 currents from in silico model and in vitro experiments.
Supplemental Figure 13. Protocol 12. Voltage waveform, and example evoked Kv11.1 currents from in silico model and in vitro experiments.

Supplemental Figure 14. Protocol 13. Voltage waveform, and example evoked Kv11.1 currents from in silico model and in vitro experiments.
**Supplemental Table 1.** In vitro measured IC50s, their 95% confidence intervals and Hill coefficients for the four drugs (verapamil, cisapride, clozapine & terfenadine) are shown for the training protocols (8), (12) & (13) and the test protocols (1), (10) & (11). The test protocols are paired with their in silico IC50 prediction (denoted by *). $K_{O/I}$ and the in silico prediction $K_{O/I}^*$ are also shown for each of the four drugs. IC50 values are show in nM.

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<th>(8)</th>
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**Supplemental Table 2. Published IC<sub>50</sub>s for Verapamil.** The range of IC<sub>50</sub> values (in nM) for each drug are listed above, with the corresponding first author and year of publication. In addition, where available, details of the assay protocol are listed including: cell line, Holding voltage potential (V<sub>H</sub>), 1<sup>st</sup> voltage step voltage and duration (V<sub>1</sub>), 2<sup>nd</sup> voltage step voltage and duration (V<sub>2</sub>), voltage protocol cycle length (CL), extracellular potassium concentration ([K<sub>o</sub>]) and experimental temperature (Temp).

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**Supplemental Table 3. Published IC₅₀s for Cisapride.** The range of IC₅₀ values (in nM) for each drug are listed above, with the corresponding first author and year of publication. In addition, where available, details of the assay protocol are listed including: cell line, Holding voltage potential (Vₜ), 1ˢᵗ voltage step voltage and duration (V₁), 2ⁿᵈ voltage step voltage and duration (V₂), voltage protocol cycle length (CL), extracellular potassium concentration ([K]₀) and experimental temperature (Temp).

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Supplemental Table 4. Published IC\textsubscript{50}s for Clozapine. The range of IC\textsubscript{50} values (in nM) for each drug are listed above, with the corresponding first author and year of publication. In addition, where available, details of the assay protocol are listed including: cell line, Holding voltage potential (V\textsubscript{h}), 1\textsuperscript{st} voltage step voltage and duration (V\textsubscript{1}), 2\textsuperscript{nd} voltage step voltage and duration (V\textsubscript{2}), voltage protocol cycle length (CL), extracellular potassium concentration ([K]\textsubscript{o}) and experimental temperature (Temp).

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**Supplemental Table 5. Published IC₅₀s for Terfenadine.** The range of IC₅₀ values (in nM) for each drug are listed above, with the corresponding first author and year of publication. In addition, where available, details of the assay protocol are listed including: cell line, Holding voltage potential ($V_h$), 1ˢᵗ voltage step voltage and duration ($V_1$, $V_1$), 2ⁿᵈ voltage step voltage and duration ($V_2$, $V_2$), voltage protocol cycle length (CL), extracellular potassium concentration ([K]₀) and experimental temperature (Temp).

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SUPPLEMENTAL FIGURE 1

A.

\[
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C_0 \xrightarrow{k_{f_1}} C_1 \xrightarrow{k_{f_2}} C_2 \xrightarrow{k_{f_3}} O \xrightarrow{k_{f_4}} \Theta \\
& \xleftarrow{k_{b_1}} \quad \xleftarrow{k_{b_2}} \quad \xleftarrow{k_{b_3}} \quad \xleftarrow{k_{b_4}} \quad \xleftarrow{k_{b_5}} \quad \xleftarrow{k_{b_{inact}}} \quad \xleftarrow{k_{b_{open}}} \\
& \text{drug} \quad \text{drug} \\
& \text{ID} \\
& \text{OD}
\end{array}
\]

B.

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SUPPLEMENTAL FIGURE 2
Protocol (1)

Voltage Waveform

In Silico Kv11.1 Current

In Vitro Kv11.1 Current
SUPPLEMENTAL FIGURE 3
Protocol (2)

Voltage Waveform

In Silico Kv11.1 Current
SUPPLEMENTAL FIGURE 4
Protocol (3)

Voltage Waveform

In Silico Kv11.1 Current
SUPPLEMENTAL FIGURE 5
Protocol (4)
SUPPLEMENTAL FIGURE 6
Protocol (5)
Voltage Waveform

Frequency = 2 Hz

In Silico Kv11.1 Current

I / Icontrol (%)
SUPPLEMENTAL FIGURE 7
Protocol (6)

Voltage Waveform

Frequency = 1 Hz

In Silico Kv11.1 Current
SUPPLEMENTAL FIGURE 8
Protocol (7)

Voltage Waveform

Frequency = 0.5Hz

In Silico Kv11.1 Current

$I/I_{control}$ [%]
SUPPLEMENTAL FIGURE 9
Protocol (8)

Voltage Waveform

Frequency = 0.1Hz

In Silico Kv11.1 Current

In Vitro Kv11.1 Current
SUPPLEMENTAL FIGURE 10

Protocol (9)

Voltage Waveform

In Silico Kv11.1 Current
SUPPLEMENTAL FIGURE 11

Protocol (10)

Voltage Waveform

In Silico Kv11.1 Current

In Vitro Kv11.1 Current
SUPPLEMENTAL FIGURE 12

Protocol (11)

Voltage Waveform

In Silico Kv11.1 Current

Drug application

In Vitro Kv11.1 Current

Drug application
SUPPLEMENTAL FIGURE 13
Protocol (12)

Voltage Waveform

+20mV

In Silico Kv11.1 Current

Drug application

In Vitro Kv11.1 Current

Drug application
SUPPLEMENTAL FIGURE 14
Protocol (13)

Voltage Waveform

-20mV

In Silico Kv11.1 Current

Drug application

$I / I_{\text{control}} (%)$

In Vitro Kv11.1 Current

Drug application

$I / I_{\text{control}} (%)$
Supplementary References


