Introduction

The human sodium channel hNa\textsubscript{v}1.5, encoded by the SCN5A gene, is crucial for the cardiac action potential upstroke and subsequent signal propagation in the heart. Inhibition of the sodium current by drugs decreases the rate of cardiomyocyte depolarization and the conduction velocity which may lead to serious implications. For these reasons, off-target effects on hNa\textsubscript{v}1.5 are considered a risk marker for drug candidates and the hNa\textsubscript{v}1.5 is one of the most used in vitro assays in cardiac safety testing and is also one of the most important currents in the CiPA paradigm.

Several drugs, known to inhibit the hNa\textsubscript{v}1.5 channel, are either use- or state-dependent and preferentially bind to the open and inactivated state, respectively. When experimenting on voltage-gated ion channels, it is imperative that the voltage applied to the cells is accurate. This is especially important when testing state-dependent compounds. In order to determine compound activity in the most accurate way, all tested cells should have an identical degree of inactivation, a state that will not be achieved by using the same voltage to all experiment sites but rather individual voltages adjusted to the cell’s individual biophysical properties need to be applied.

The Qube, as well as the new QPatch II, are high-throughput automated patch clamp platforms, suitable for studying a wide range of ion channels. Both systems are equipped with the option to run online adaptive protocols, which makes it possible to set each individual cell to a user-defined level of inactivation, e.g. the half-inactivation potential ($V_{\text{1/2}}$) and use that value subsequently in e.g. a preconditioning pulse. Applying this new adaptive protocol feature, we determined Na\textsubscript{v}1.5 $V_{\text{1/2}}$ values for both the open and the inactivated state for a set of known sodium channel inhibitors. We could show that the use of individual $V_{\text{1/2}}$ reduces data variability compared to standard methods and thereby improves the accuracy of drug evaluation.

Conclusions

Here we show how the use of an adaptive protocol with individual $V_{\text{1/2}}$ values for each cell significantly decreases the variability of the relative current compared to the traditional method where all cells are depolarized from an ensemble average $V_{\text{1/2}}$ value.

As a consequence, the new adaptive protocol enables increased control of the state that voltage-gated channels are in during an experiment, leading to improved data quality when testing compounds using the 384-well high-throughput automated patch clamp platform Qube as shown here, or on the new QPatch II instrument which carries the same features.

Methods

Cells expressing hNa\textsubscript{v}1.5 were from CreaCell (La Tronche, France) and cultured according to instructions. All compounds were from Sigma-Aldrich (Søborg, DK) and dissolved in DMSO (final concentration DMSO ≤ 0.3%).

Table 1: pIC\textsubscript{50} for a subset of well-known sodium channel blockers determined using either an adaptive voltage protocol or a predetermined average value for $V_{\text{1/2}}$ (“standard”). As reference the potency was also estimated using a CiPA-like protocol.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Resting</th>
<th>$pIC_{50}$</th>
<th>$V_{\text{1/2}}$</th>
<th>CIPA</th>
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</thead>
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<tr>
<td></td>
<td>Adaptive</td>
<td>Standard</td>
<td>Adaptive</td>
<td>Standard</td>
</tr>
<tr>
<td>Quinidine</td>
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<td>Propafenone</td>
<td>5.01</td>
<td>5.05</td>
<td>5.21</td>
<td>5.3</td>
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</tbody>
</table>

Fig. 1: Use of adaptive $V_{\text{1/2}}$ of inactivation in an experimental protocol. A. $V_{\text{1/2}}$ of inactivation was estimated using a standard two-pulse protocol (insert) and this value was subsequently used in the same experiment (blue dashed line). If the measured value falls outside the accepted range (light blue square) then a fallback value is used (red dashed line). The current traces were elicited by depolarising the cell -90 mV and V\textsubscript{b} to -15 mV respectively. B. Distribution of relative currents from 6 different single-hole QChip384 obtained by applying an adaptive protocol. Insert shows the voltage protocol used in A. C. Boxplot of the normalised current distributions using either an adaptive protocol or a static value determined in a previous experiment (“standard”).

Fig. 2: A. Dose-response relationships for 6 different compounds determined using either an adaptive voltage protocol with or a static ensemble average $V_{\text{1/2}}$ value. Black symbols are for deppolarisations from resting conditions and blue from $V_{\text{1/2}}$ inactivation. Data are mean ± SEM. B. Dose-response relationships for 4 compounds determined using a CiPA-like protocol. $pIC_{50}$ values are listed in table 1. C. Ridge plot of the data in panel A showing the distribution at each individual compound and concentration. D. Scatterplot of data at the concentration closest to the $IC_{50}$ value for each compound. Red symbols are mean ± SD.

Fig. 3: Normalised current distributions for 20, 40, 60 and 80 % inactivation respectively. Using $V_{\text{1/2}}$ for inactivation may not be optimal for different sets of experiments but a different level may be desired and therefore it is possible to arbitrarily set this value. Data in this figure was obtained using a cell line expressing hNa\textsubscript{v}1.7.