Adaptive voltage protocols increase precision of voltage-gated ion channel measurements on high throughput automated patch clamp platforms

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1 ABSTRACT

Voltage-gated sodium (Na+) channels are studied extensively due to their potential as targets for several indications, such as pain, epilepsy, cardiac and muscle paralysis. Some Na+ channel modulators show state-dependence and bind preferentially to the inactivated state of the channel. The potency of state-dependent compounds are known to vary depending on the percentage inactivation of the channels.

To calculate accurate compound activity the precise value for the V_{1/2} of inactivation should be used for each cell. The adaptive protocol block for the Sophon Qube 384-well automated patch clamp platform has made it possible to separately define the voltage applied to individual wells for both the activation and inactivation of the channels. This enables the generation of more precise data for voltage-gated ion channels.

3 RESULTS

3.1 The adaptive protocol did not change the performance of the assay compared to the standard protocol.

<table>
<thead>
<tr>
<th>Success</th>
<th>State resistance (Ω)</th>
<th>Series resistance (MO)</th>
<th>Cell capacitance (pF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>73%</td>
<td>2.4 ± 1.2</td>
<td>3.1 ± 1.9</td>
</tr>
<tr>
<td>Adaptive</td>
<td>76%</td>
<td>2.1 ± 1.9</td>
<td>2.5 ± 2.0</td>
</tr>
</tbody>
</table>

Table 1. Assay performance parameters. Parameters shown for before and after the online Boltzmann fit in the experimental protocol.

3.2 V_{1/2} of inactivation values derived from online Boltzmann fits were comparable for both experiments, but the resulting current inactivation percentage less variable using the adaptive protocol

![Graph showing V_{1/2} of inactivation and current inactivation values.](image)

3.3 Example Na_{1.1} traces.

![Figure 2: Example Na_{1.1} current traces.](image)

4 SUMMARY

The incorporation of the adaptive protocol did not change the performance of our Na_{1.1} assay compared to the standard protocol. The adaptive protocol significantly decreased the variability of the percent current inactivation. In the standard protocol experiment approximately 80% of the wells had percent current inactivation between 26-66%, whereas in the adaptive protocol experiment this was between 47-61%.

Four known state-dependent compounds were tested as concentration-response changes and all Na_{1.1} in both protocols yielded similar results. However, the compound data at 10 µM was found to be much less variable in the adaptive protocol experiment. In a high throughput screen this reduced variability should lead to increased confidence in the results.

In summary, the new adaptive protocol enables increased control of the state that voltage-gated channels during an experiment on a 384-well high throughput automated patch clamp platform, which leads to reduced data variability and increased confidence in compound testing results.

Acknowledgements
Thanks to Anders Lindqvist at Sophion Bioscience and to the cell culture team at Charles River and their help and support.