SOPHION BIOSCIENCE

Application Report

CHO-$Na_V1.5$ use-dependent blockers

The two use-dependent $Na_V1.5$ blockers were tested in single- and multi-hole mode. One blocks open Na_v channels (flecainide) and one blocks inactivated Na_v channels (lidocaine) and the differences were evident in all protocols used.

Summary

The voltage-dependent sodium channel, $Na_V1.5$, was tested on the QPatch in single-hole and multi-hole mode. In this study, we wanted to determine the best test protocol to distinguish between two antiarrhythmic drugs with different modes of action.

Introduction

The voltage-dependent sodium channel, $Na_V1.5$, was tested on the QPatch in single-hole and multi-hole mode. Single-hole mode is a classic patch clamp experiment where one cell is in whole-cell configuration, whereas multi-hole mode comprises up to 10 cells in whole-cell configuration. The multi-hole mode, therefore, tests the summed current of up to ten cells.

Here we wanted to determine the best test protocol to distinguish between two antiarrhythmic drugs with different modes of action: flecainide, which blocks open Na_V channels, and lidocaine, which blocks inactivated Na_v channels. Therefore, four different voltage protocols were set up to test for: 1) steady-state inactivation, 2) open-channel block, 3) recovery from inactivation, 4) state- versus use-dependence. Each protocol was tested in both single-hole and multi-hole mode to compare the capabilities of the QPatch in the two modes.

Results

Each of the voltage protocols described in Materials & Methods were employed on the QPatch in both single- and multi-hole mode.

Figure 1 and Figure 2 show IV plots for steady-state inactivation and activation in dose-response experiments with flecainide and lidocaine respectively (protocol 1). The figures show that both flecainide and lidocaine shift the voltage of half-maximal inactivation ($V_{½}$) towards more hyperpolarized potentials, but that only flecainide has a significant effect on the maximal current amplitude.

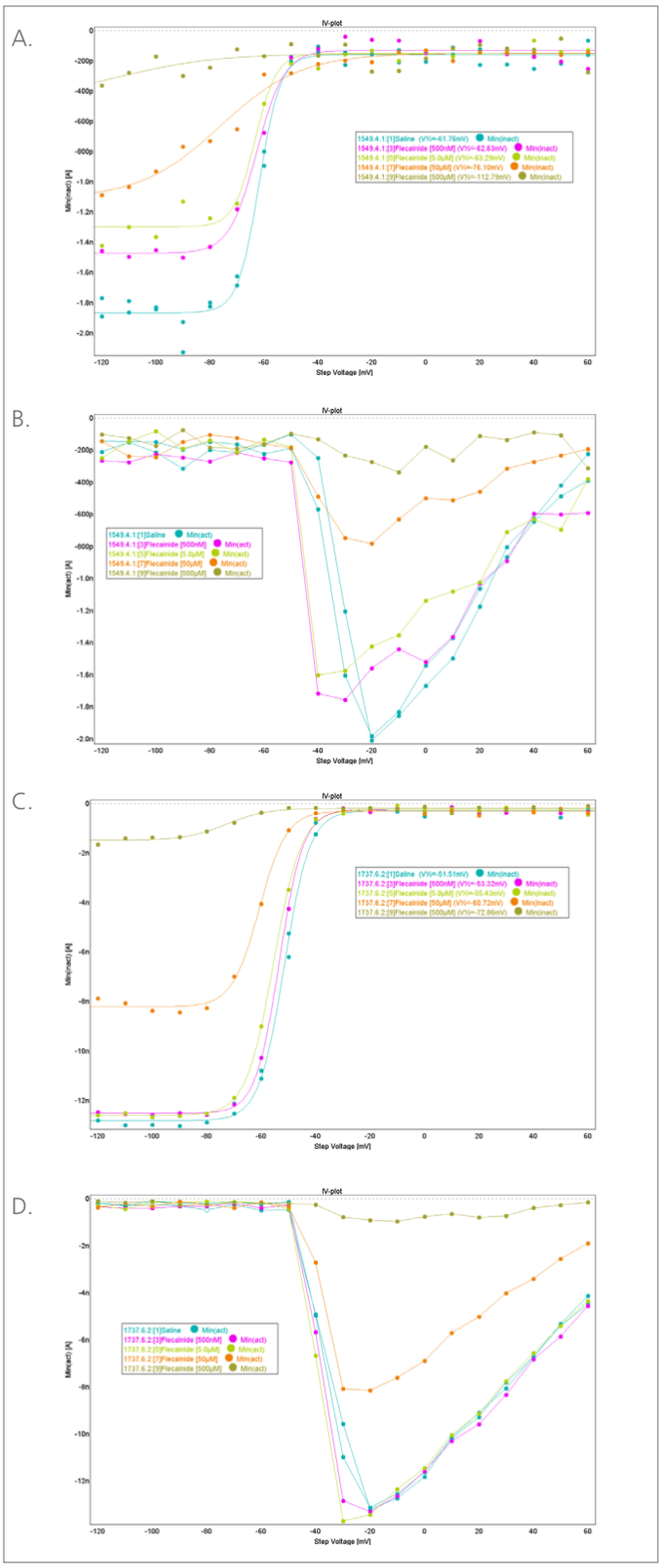


Fig. 1. Steady-state inactivation (Fig. A and C) and IV for activation (Fig. B and D) for flecainide. Fig. A and B are single-hole data, Fig. C and D are multi-hole data.

This is further emphasized in the next experiments shown in Figure 3 and Figure 4 where lidocaine and flecainide were tested with a standard protocol for open-channel block (protocol 2). Here it is evident that flecainide produces a strong effect on the $Na_V1.5$ ion channel already at 50µM, whereas lidocaine does not have an effect until 500µM. The data is summarized in Table 1.

It is also worth noting that the single-hole and multi-hole data is very similar. This is somewhat surprising for the IV-data, given that multi-hole experiments do not allow Rs compensation and we, therefore, could expect a systematic error in the voltage applied to the cells.

In a hypothetical example, say the R_{series} on an individual cell is 5MΩ and the current is 1nA. This would result in a voltage error of 5mV on the measurement site, in both single-hole and multi-hole mode. However, since only single-hole mode allows compensation of this, we expect shifts in the IV curves of multi-hole experiments; but we can see from this data that the shifts are very small.

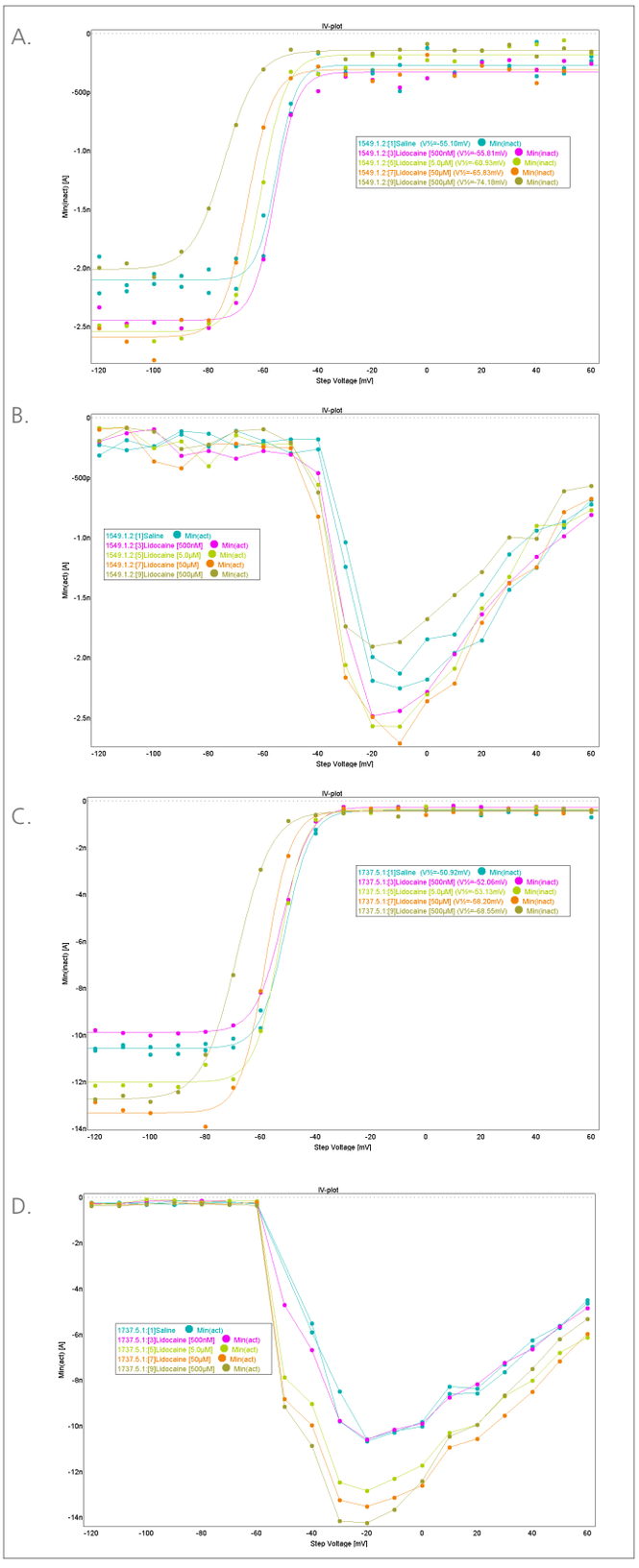


Fig. 2. Steady-state inactivation (Fig. A and C) and IV for activation (Fig. B and D) for lidocaine. Fig. A and B are single-hole data, Fig. C and D are multi-hole data.

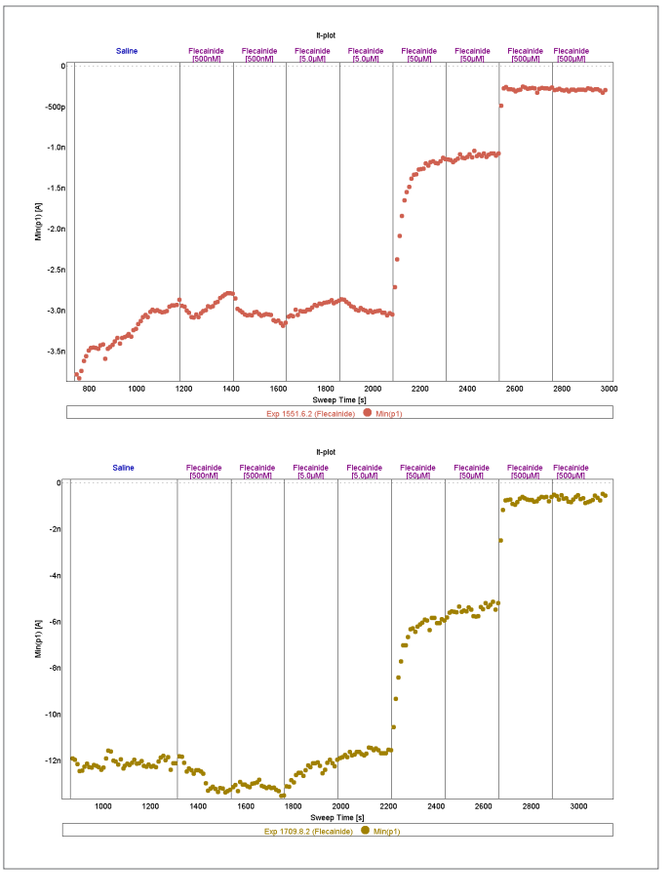


Fig. 3. IT plot for open-channel block for flecainide (simple depolarization to -10mV), top is single-hole and bottom is multi-hole data.

Fig. 4. IT plot for open-channel block for lidocaine (simple depolarization to -10mV), top is single-hole and bottom is multi-hole data.

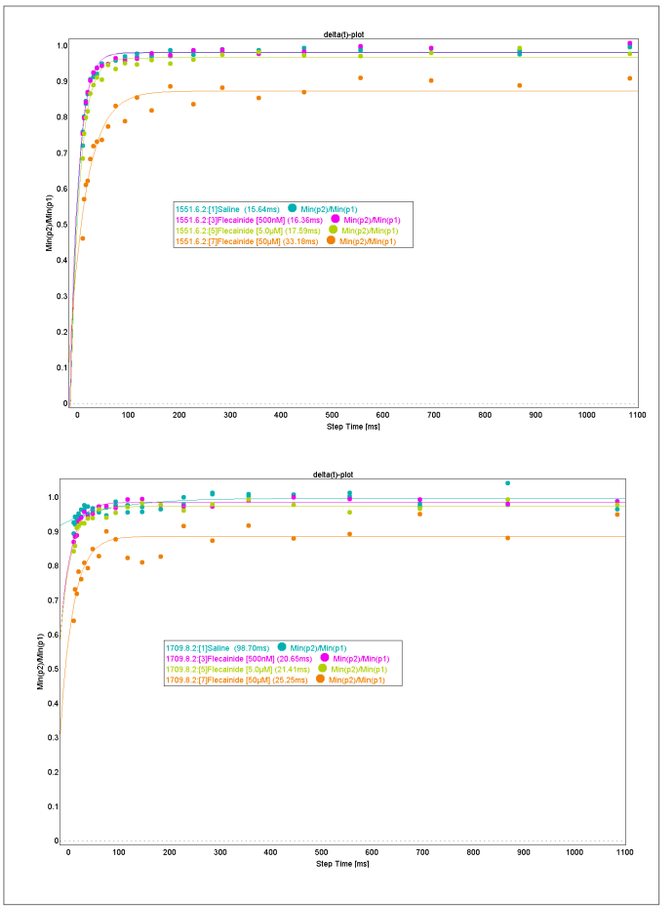


Fig. 5. Recovery from inactivation for flecainide. Peak 2/peak 1 is plotted as a function of the time increment between peaks. Top is single-hole data, bottom is multi-hole data.

In Figure 5 and Figure 6, we tried a voltage protocol to test for recovery from inactivation (protocol 3). The protocol is made up of two depolarizations where the time between them is increased by 25% with each sweep. The ratio between the last and first peak (peak2/peak1) is plotted as a function of the time between them, and the data is fitted to the exponential equation to produce a time constant for recovery from inactivation. The time constants are summarized in Table 1.

It can be seen in Figure 6 that lidocaine increases the time constant for recovery from inactivation, suggesting that lidocaine keeps the ion channels in the inactivated state. Flecainide (Figure 5) does not have as strong an effect, consistent with its reported affinity for open channels.

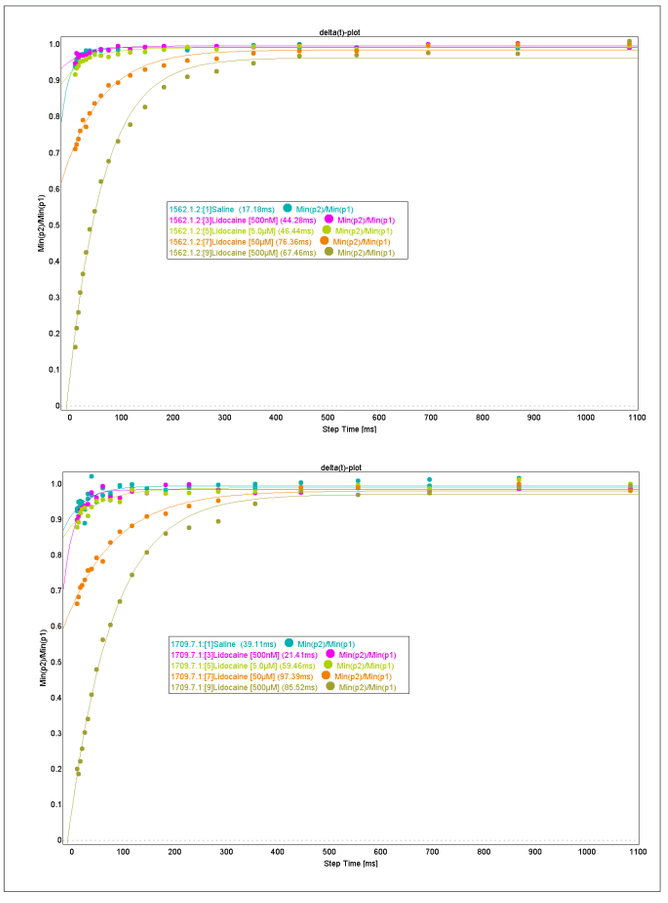


Fig. 6. Recovery from inactivation for lidocaine. Peak 2/peak 1 is plotted as a function of the time increment between peaks. Top is single-hole data, bottom is multi-hole data.

Finally, we tested the pulse-train protocol for determining state- and use-dependence (protocol 4), in both single-hole and multi-hole mode.

The protocol employs 8 depolarizations to -10mV at a frequency of 4Hz (i.e. peaks 1-8). This is followed by a 650ms step to -60mV (approx. $V_{½}$), and a final depolarization to -10mV (peak 9). Thus, "peak 1" -current is comparable to the simple open channel block (protocol 2), whereas the current at "peak 8" is a measure of use-dependent block, and peak 9 determines state dependency.

The peak currents from thew first, eighth (use dependence) and ninth peak (state dependence) are plotted in Figure 7 for flecainide and Figure 9 for lidocaine.

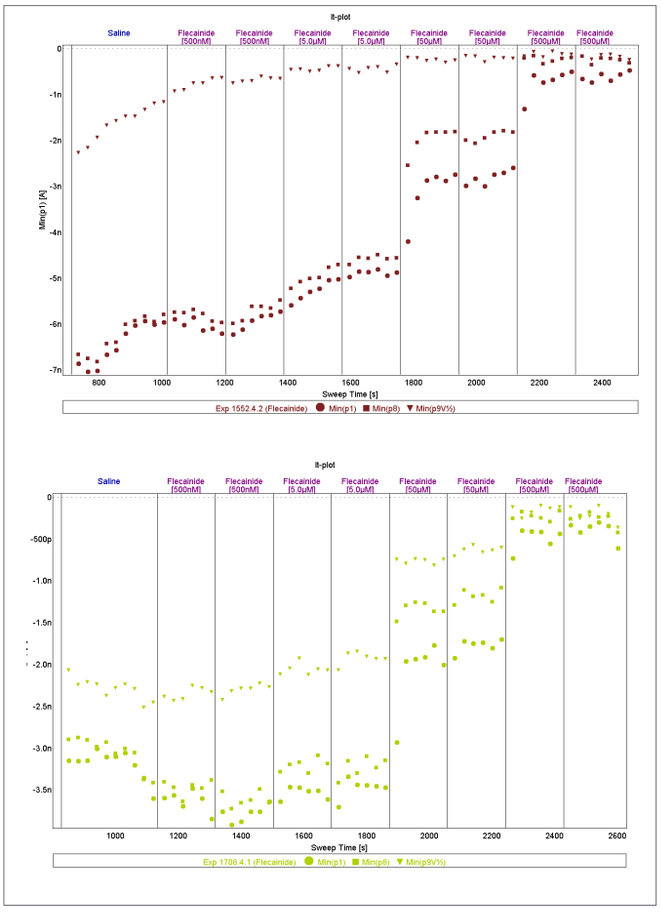


Fig. 7. IT-plot of peak 1 (circles), peak 8 (squares) and peak 9 (triangles) in a dose-response experiment with flecainide. Top: single-hole data, bottom: multi-hole data.

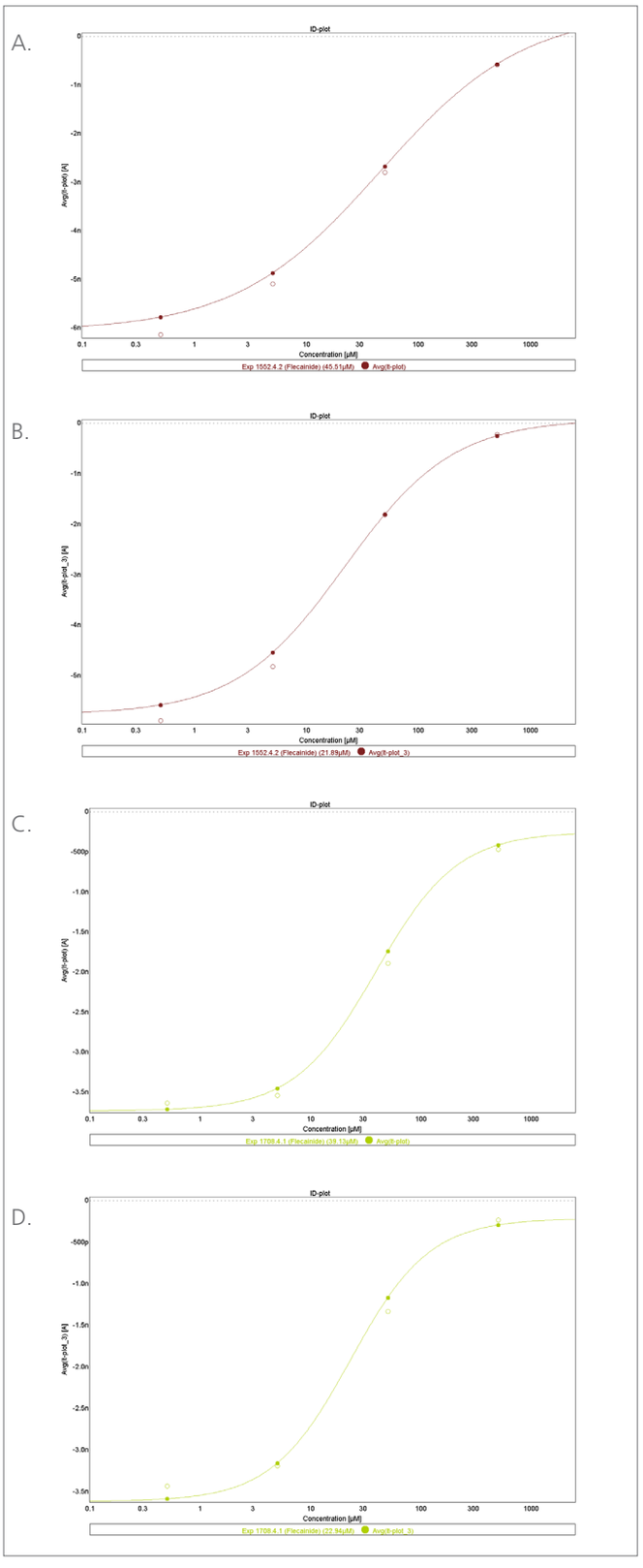


Fig. 8. Hill fits for flecainide. Fig. A and C are fits for current at peak 1, Fig. B and D are fits for current at peak 8. Fig. A and B are single-hole data, Fig. C and D are multi-hole data

Figure 8 shows dose-response plots with Hill fits for peaks 1 and 8 for flecainide (summarized in Table 1). It is evident that flecainide is a lot more potent at peak 8, after the pulse train than at peak 1. The effect is not, however, further enhanced after the $V_{½}$ step.

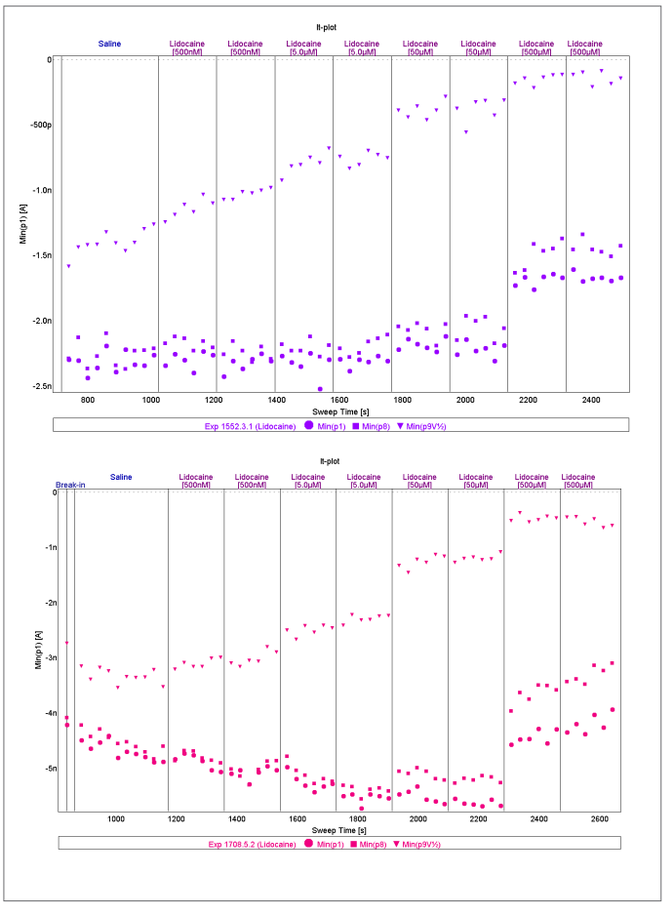


Fig. 9. IT-plot of peak 1 (circles), peak 8 (squares) and peak 9 (triangles) in a dose-response experiment with lidocaine. Top: single-hole data, bottom: multi-hole data.

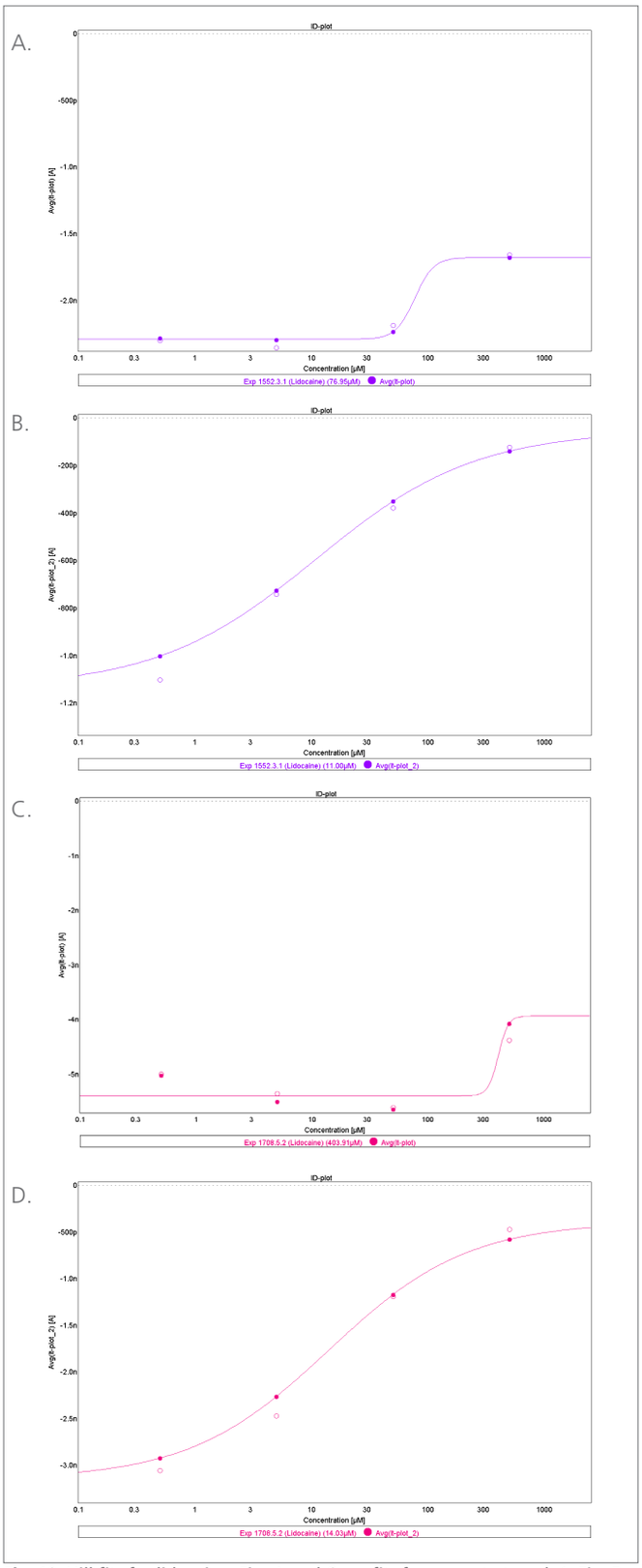


Fig. 10. Hill fits for lidocaine. Fig. A and C are fits for current at peak 1, Fig. B and D are fits for current at peak 9. Fig. A and B are single-hole data, Fig. C and D are multi-hole data

As is evident in Figure 10 and Table 1, the effects of lidocaine are not strong until pulse 9, after the $V_{½}$-step. The IC_{50} values shown for lidocaine at peak 1 and peak 8 are not good estimates (as indicated by the asterisks in the table), because of the small drug effect.

Table 1. Summary of data from all figures.

Voltage protocol		Single-hole		Multi-hole	
		Flecainide	Lidocaine	Flecainide	Lidocaine
Steady-state inactivation ($V_{½}$ in mV, Boltzman fit)	Control	-61.8	-55.1	-51.5	-51.0
	500 nM	-62.6	-55.8	-53.3	-52.0
	5 µM	-63.3	-60.9	-55.4	-53.1
	50 µM	-76.1	-65.8	-60.7	-58.2
	500 µM	-	-74.2	-72.7	-68.6
Open channel block (% of block by chain of single depolarization)	500 nM	0	0	0	0
	5 µM	0	0	0	0
	50 µM	60	0	60	0
	500 µM	90	20	100	30
Recovery from inactivation (ms, time constant)	Control	15.6	17.2	-	39.1
	500 nM	16.4	44.3	20.7	21.4
	5 µM	17.6	46.4	21.4	59.5
	50 µM	33.2	76.4	25.3	97.4
	500 µM	-	67.5	-	85.5
State versus use dependence (µM, IC_{50})	Peak 1	45.5	77.0*	39.1	403.9*
	Peak 8	21.9	82.6*	22.9	497.8*
	Peak 9	19.9	11.0	17.5	14.0

Methods

Cells

CHO-$Na_V1.5$ from B'SYS were used for these experiments.

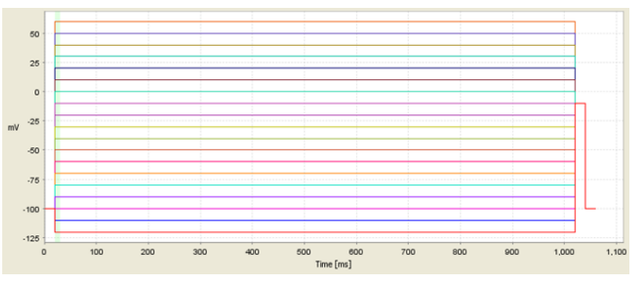
Drugs

Lidocaine and flecainide were dissolved in an ethanol stock solution, such that the final ethanol concentration for the experiments did not exceed 0.1%.

Voltage protocols

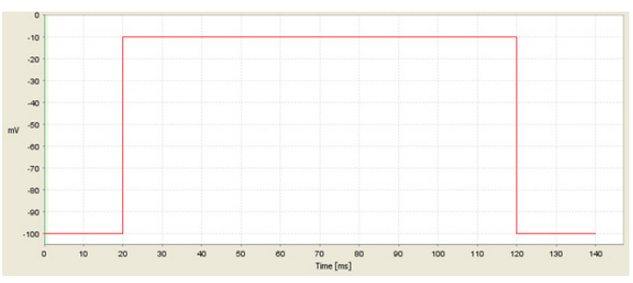
V_{hold}= -100 mV. Data were sampled at 50kHz, 8th order Bessel filter, cut-off frequency 3kHz, and, in single-hole mode, 80% Rs compensation. P/n leak subtraction was employed.

1. Steady-state inactivation



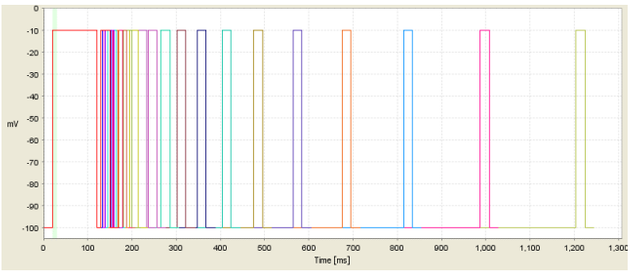
20ms at V_{hold}, 1000ms at potential ranging from -120 to +60 in 10mV increments, 20ms test potential at -10mV.

2. Open-channel block



20ms at V_{hold}, 100ms at test potential of -10mV.

3. Recovery from inactivation



20ms at V_{hold}, two pulses of 100ms at test potential of -10mV, with an incremental increase in time between depolarizations starting at 10ms and increasing by 25% per sweep.

4. State- versus use-dependence

8 depolarizations to -10mV for 50ms, at a frequency of 4Hz, followed by a 650ms step to -60mV (approx. $V_{½}$), and a final depolarization to -10mV for 50ms.

Conclusion

The data presented here show that the QPatch can produce data in multi-hole mode that is fully comparable to those in single-hole mode, both with regard to biophysical and pharmacological characteristics.

The two compounds tested clearly have different modes of action on the $Na_V1.5$ ion channel, and these differences are evident in all the protocols used. The data is fully consistent with flecainide being a use-dependent blocker of open channels, and lidocaine a state-dependent blocker of inactivated channels.

In a screening scenario where one wishes to distinguish between these two modes of action, we would prefer to use protocol 4 (state- versus use-dependence).

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