Application Report:

QPatch 16

Ligand-gated ion channels

AR_PUBLIC14467-4

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Ligand-gated ion channels on QPatch 16

Introduction

This report presents QPatch 16 in two studies based on whole-cell current recordings from ligand-gated ion channels (LGIC's) expressed in HEK-293 cells:

- $GABA_A$ receptors ($\alpha_1\beta_2\gamma_2$) that were targeted with GABA (an agonist), bicuculline (an antagonist), and chlordizepoxide (a modulator)
- ASIC1a (acid sensing ion channel 1a) that was targeted with protons (an agonist) i.e. bv extracellular pH perturbations

The QPatch 16 uses four pipette heads that afford more efficient assays and faster throughput for ion channel drug discovery, especially for LGIC assays.

Materials & Methods

Cells and patch-clamping: The QPlate contains 16 individual patch-clamp sites that are operated asynchronously and in parallel. Ringer's solutions and compounds are applied by four pipettes. HEK-293 cells expressing the target ion channel were kept in culture medium in the stirred reservoir for up to four hours. Prior to testing, the cells were transferred to an on-board mini centrifuge, spun down and washed in Ringer's solution twice before being applied to the pipetting wells in the QPlate. Gigaseals were formed upon execution of a combined suction/ voltage protocol. Further suction lead to whole-cell configuration. Solutions and compounds were applied through the glass flow channels in the QPlate. All currents were recorded at a patch potential of -70 mV. Liquid flow was laminar with exchange time constants in of 50-100 ms (Figure 1).

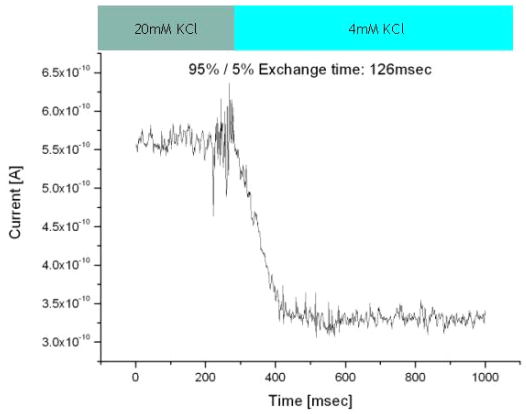

Figure 1. Estimation of liquid exchange time of the fluidics system. The figure shows the shift in whole-cell hERG channel current in response to reduction of $[K^+]$ in the extracellular from 20 to 4 mM. Current decay indicates exchange time of extracellular fluid. Average: ~100 msec (n=10

After application all fluids were collected in the built-in waste reservoir (70 µL) in the QPlate.

Ringers: Extracellular Ringer's solution consisted of (in mM): 145 Na^+, 4 K^+, 2 Ca^{2+}, 2 Mg^{2+}, 155 Cl^-, 10 HEPES (pH 7.4). Intracellular Ringer's solution consisted of (in mM): 120 K^+, 1.8 Mg^{2+}, 123.6 Cl^-, 10 EGTA, 10 HEPES (pH 7.2).

Protocols

Agonist protocol: Figure 2A shows the sequence of exposures to extracellular Ringer's solutions indicated. Green boxes labelled 0 indicate saline. Blue boxes labelled A – D indicate increasing concentrations (400 nM –

50 µM) of the agonist (GABA) added for 5 seconds. Antagonist protocol: Figure 2B shows the sequence of extracellular Ringer's solutions indicated. Olive boxes labelled 0-3 indicate pure saline (0) or saline added the antagonist (bicuculline) at increasing concentrations (1-3) for 60 seconds each. Blue boxes indicate 2-second exposures to saline containing the agonist (GABA) at a standard concentration (A = 10 µM), and the antagonist (bicuculline) at increasing concentrations (1-3). Modulator protocol: similar to B, except that only one concentration of the modulator (chlordizepoxide, librium) was employed (20 µM).

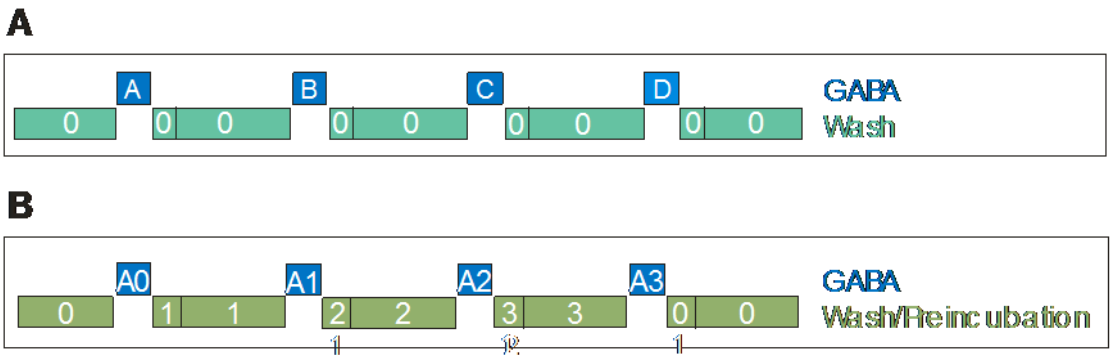

Figure 2. Agonist (A) and antagonist (B) application protocols.

Data analysis

Recorded ion channel whole-cell currents were stored in an integrated Oracle database along with data on suction pressure, series resistance, seal resistance and capacitances (C_{fast} and C_{slow}). Drug effects were analysed as function of time (I-t plot) and concentration (dose-response relationship). Data analysis was accomplished with the QPatch Assay Software. For the currents used for I-t and concentration-response analyses the leak currents had been subtracted.

Results

$GABA_A$ study

The left panel in Figure 3 shows $GABA_A$ currents in response to five increasing concentrations of γ-amino-butyric acid (GABA). The right panel shows the complete concentration-response relationship as determined with the QPatch Analysis Software.

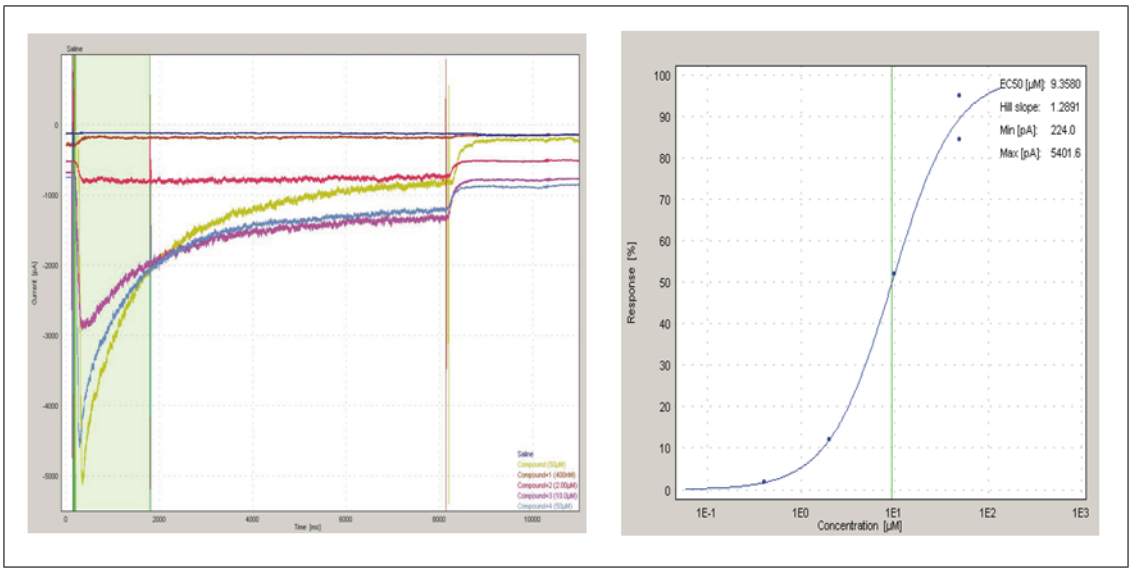

Figure 3. GABA application experiments (left) and concentration-response relationship (right).

The mean EC_{50} was 11.2±2.9 µM (N=6). The mean Hill slope = 1.7±0.6. The mean rise-time for $GABA_A$ receptor currents measured at 50 or 100 µM GABA was 66 msec, ranging from 40 to 100 msec.

Bicuculline study

The left panel in Figure 4 shows $GABA_A$ currents in response to four increasing concentrations of the inhibitor bicuculline (50 nM to 50 µM). The right panel shows the concentration-relationship for bicuculline as determined with the QPatch Assay Software. The mean IC_{50} was 0.92±0.43 µM (N=7) at 10 µM GABA. The mean Hill slope = 1.33±0.48.

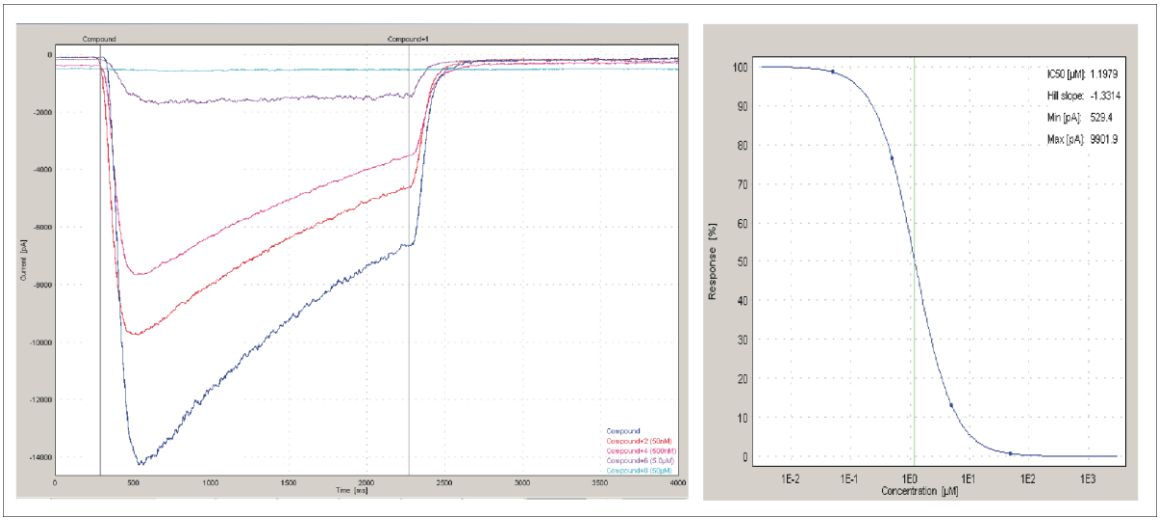

Figure 4. Bicuculline application experiments (left) and concentration-response relationship (right).

Chlordizepoxide study

Figure 5, left panel, shows original whole-cell $GABA_A$ current recordings from saline (dark blue trace), reference (GABA, red and yellow traces), and compound (20 µM chlordizepoxide, a known $GABA_A$ activator, purple trace),

and finally an additional reference (GABA, blue trace). The green shaded area (limited by the green cursors) between 250 and 2000 ms defines the time range within which peak currents are recorded.

The right panel shows the maximal peak current amplitudes from the original traces (blue points), the leak currents (green points), and the leak-corrected currents (red points).

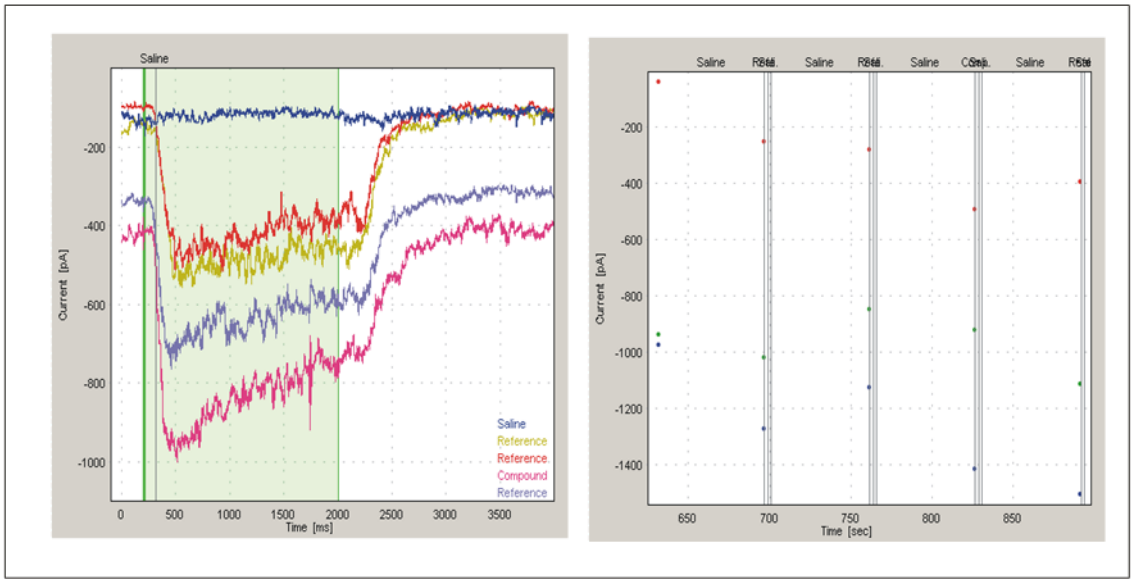

Figure 5. Effect of $GABA_A$ modulator chlordizepoxide (left). Right: I-t plot (for explanation see text).

The mean chlordizepoxide-induced increase in $GABA_A$ current was 59±25 per cent (N=2). The mean rise-time for $GABA_A$ receptor currents when exposed to 20 µM chlordizepoxide was 80 msec.

Acid sensing ion channels (ASIC1a)

The left panel in Figure 6 shows original recordings of ASIC1a currents in response to progressive acidification at four pH values (from pH 7 to pH 4). It is seen that both the current amplitude and the desensitization time constant increase when pH is reduced. The green cursors define the time range within which the maximum current amplitude should be identified.

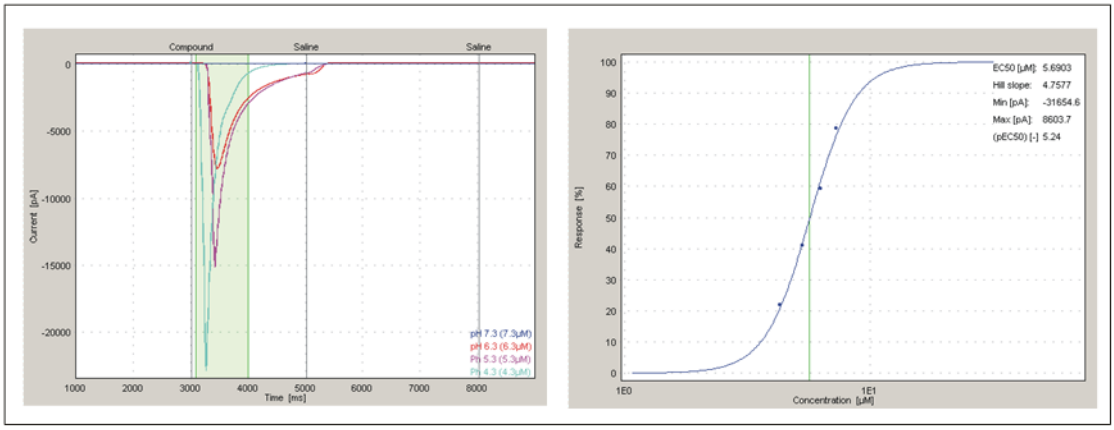

Figure 6. ASIC1a current recordings at decreasing pH values (left). Proton concentration-response relationship (right).

In the experiment shown, the currents were half maximal at pH 6.23, i.e. EC_{50} = 602 nM [H^+], which is similar to literature values (EC_{50}=6.45, Gunthorpe et al, 2001).

References

Gunthorpe, MJ, GD Smith, JB Davis, AD Randall (2001) Pflügers Arch. Eur. J. Physiol. 442:668-674.

Conclusion

The analyses presented here demonstrate that compound screening on ligand-gated ion channels ($GABA_A$ and ASIC1a) can be performed efficiently with the QPatch 16 automated patch-clamp system in order to characterize the effects of agonists, antagonists and modulators. The characterizations were based on I-t and concentration-response relationships, and on rise-time determinations. The EC_{50} and IC_{50} values determined from the concentration-response relations in the present study are comparable to values listed in the literature.