Whole-cell patch clamp recording for cardiac potassium ion channels $K_V1.5$, $K_VLQT1/minK$, $K_V4.3$ and Kir2.1 on QPatch automated patch clamp system

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Abstract

Cardiac ion channel activity is important to generate cardiac action potentials in appropriate timing and duration. Drug-induced impairment of those ion channels cause abnormal cardiac activity such as QT interval prolongation, ventricular arrhythmia and in most serious cases, sudden death. These effects are one of the leading causes for drug withdrawal from the market or denied regulatory approval of new therapeutic candidates. Although ICH guideline E14/S7B Q&A published in February focused on I_{Kr} (hERG) as well as I_{Na} ($Na_V1.5$) and $I_{Ca,L}$ ($Ca_V1.2$), the other cardiac ion channel targets including many potassium channels such as I_{Kur} ($K_V1.5$), I_{to1} ($K_V4.3$), I_{Ks} ($K_VLQT1/minK$) and I_{K1} (Kir2.1) are also important to understand the drug effect on the generation of action potentials in cardiomyocytes. To establish the screening assay for those cardiac potassium channels, we have established the whole cell experiment method using QPatch automated patch clamp system.

Voltage protocols customized for each ion channel were applied to measure biophysical properties and current stability. The currents elicited by the designated voltage protocols were measured in each ion channels and the half-inactivated voltages ($V_{1/2}$) were calculated using Boltzmann fitting. The average inactivation $V_{1/2}$ for $K_V4.3$ and $K_V1.5$ was -39.5 mV and -9.5 mV, respectively. The reference compounds for $K_V4.3$ (Flecainide IC_{50}=11.6μM), $K_V1.5$ (4-Aminopyridine IC_{50}=125.1μM), and $K_VLQT1/minK$ (Chromanol 293B IC_{50}=17.4μM, XE-991 IC_{50}=1.7μM) were also assessed. $K_VLQT1/minK$ is known to have severe rundown of the currents. The smallest rundown condition was observed in the modified intracellular solution and extracellular solutions as well as shorten voltage protocols. The remaining currents after vehicle solution application for 6 times (approximately 500 seconds) in the best successful combination were 67% in average of groups of experiments.

The optimized assays on QPatch enable to measure the biophysical properties of those four ion channels and assessed the pharmacological effect of reference compound for each of them. QPatch allows the assessment of a novel compounds proarrhythmic risk on those cardiac ion channels other than hERG, $Na_V1.5$ and $Ca_V1.2$.

Materials and Methods

Cells: HEK-$K_V1.5$, HEK-$K_V4.3$, HEK-$K_VLQT1/minK$, HEK-Kir2.1 cell lines were kindly provided by ChenneloSearch Technology. The cells were cultured according to the vendor's SOP and were harvested by Sophion standard procedure and then automatically washed and resuspended in extracellular buffer using the cell preparation unit of QPatch.

Solutions: The following solutions were used for the denoted ion channel.
[$K_V1.5$, $K_V4.3$, Kir2.1] *Extracellular solution (in mM)*: 145 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 Glucose, 305 mOsm, pH7.4 with NaOH.
[$K_VLQT1/minK$] Based on the above solution, the following modification was made for rundown validation test: EC1: 1mM $CaCl_2$; EC2 0mM $CaCl_2$; EC3 0mM KCl; EC4 0mM $MgCl_2$. EC3 was used for further test.
[$K_V1.5$, Kir2.1] *Intracellular solution (in mM)*: 120 KCl, 5.374 $CaCl_2$, 1.75 $MgCl_2$, 31.25/10 KOH/EGTA, 10 HEPES, 4 Na_2-ATP, 280mOsm, pH 7.2 with KOH.
[$K_V4.3$] *Intracellular solution (in mM)*: 120 KF, 20 KCl, 10 HEPES, 10 EGTA, 295 mOsm, pH 7.2 with KOH.
[$K_VLQT1/minK$] *Intracellular solution (in mM)*: 120 KF, 20 KCl, 10 HEPES, 10 EGTA, 10 EDTA, 295 mOsm, pH 7.2 wit KOH.

Voltage protocols: all experiments used two types of voltage protocols for electrophysiological characterization (biophysics) and pharmacological treatment (pharmacology). The details of voltage protocols for each ion channels are described in the table 1 and illustrated in figure 1.

Table 1: Voltage protocol details

Target	Voltage protocol for biophysics	Voltage protocol for pharmacology
$K_V1.5$	5000ms square pulses from a holding potential at -80mV to a various potentials in range from -70mV to 70mV with 10mV increment followed by a second pulse at 60mV for 1000ms were delivered every 35s.	step pulse from a holding of -80mV to 20mV for 1000ms was delivered every 10s.
$K_V4.3$	300ms square pulses from a holding potential at -80mV to a various potentials in range from -120mV to 30mV with 10mV increment followed by a second pulse at 0mV for 200ms were delivered every 10s.	step pulse from a holding of -80mV to 60mV for 300ms was delivered every 10s.
$K_VLQT1/minK$	2000ms square pulses from a holding potential at -100mV to a various potentials in range from -60mV to 70mV with 10mV increment followed by a second pulse at -40mV for 1000ms were delivered every 15s.	step pulse from a holding of -100mV to 60mV for 2000ms followed by a second pulse at -40mV for 1000ms was delivered every 15s.
Kir2.1	300ms square pulses from a holding potential at -20mV to a various potentials in range from -150mV to 0mV with 5mV increment were delivered every 3s.	step pulse from a holding of -20mV to -120mV for 500ms followed by a ramp from -120mV to 40mV over 2000ms was delivered every 15s.

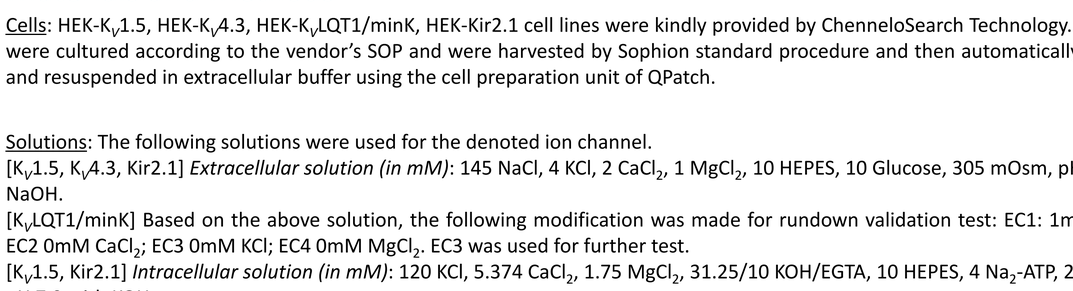

Figure 1: Schematics of voltage protocol settings for biophysics (top) and pharmacology (bottom)

Results

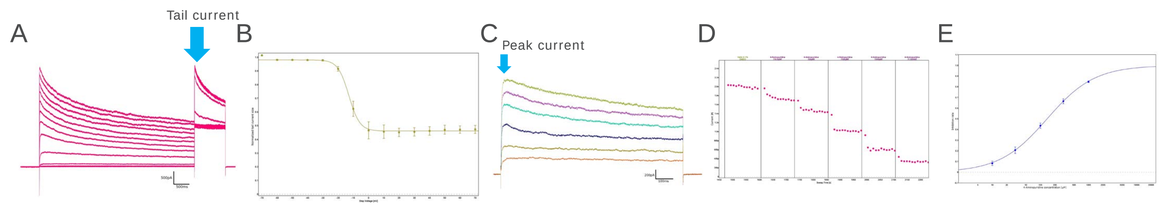

Figure 2: Measurement of $K_V1.5$ current. A) Representative current trace for multiple voltage steps. B) Normalized tail current size (mean±S.E.) vs. step voltage plot. C) Representative current traces for each drug concentration. D) Peak current size vs. time plot. E) Dose response plot of normalized peak $K_V1.5$ current suppressed by 4-Amynopyridine.

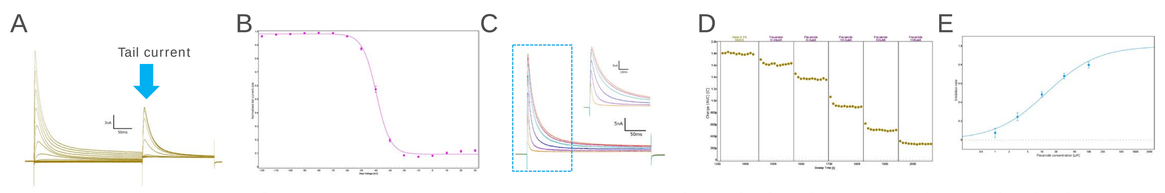

Figure 3: Measurement of $K_V4.3$ current. A) Representative current trace for multiple voltage steps. B) Normalized tail current size (mean±S.E.) vs. step voltage plot. C) Representative current traces for each drug concentration. D) Charge transfer amount (measured by AUC) through $K_V4.3$ channel vs. time plot. E) Dose response plot of normalized charge suppressed by flecainide.

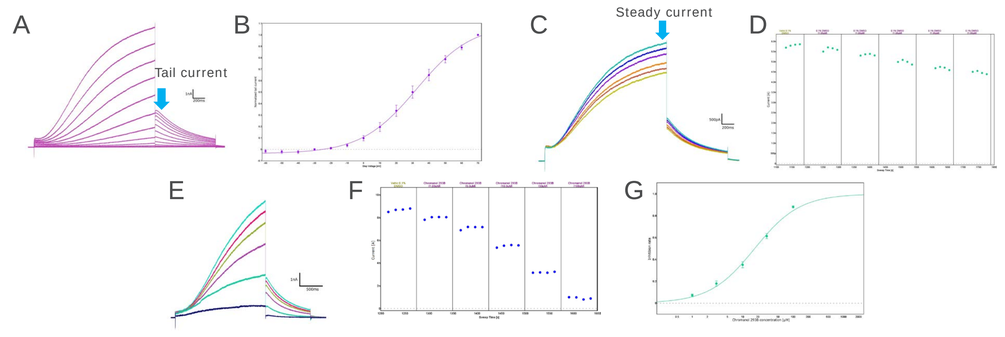

Figure 4: Measurement of $K_VLQT1/minK$ current. A) Representative current trace for multiple voltage steps. B) Normalized tail current size (mean±S.E.) vs. step voltage plot. C) Representative current traces for repetitive vehicle application. D) Average steady current size vs. time plot during vehicle application. E) Representative current traces for each drug concentration. F) Average steady current size vs. time plot. G) Dose-response plot of normalized average steady $K_VLQT1/minK$ current suppressed by chromanol 293B.

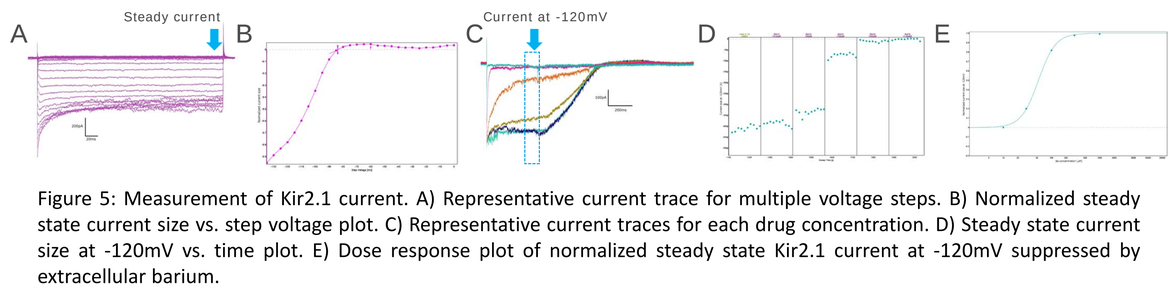

Figure 5: Measurement of Kir2.1 current. A) Representative current trace for multiple voltage steps. B) Normalized steady state current size vs. step voltage plot. C) Representative current traces for each drug concentration. D) Steady state current size at -120mV vs. time plot. E) Dose response plot of normalized steady state Kir2.1 current at -120mV suppressed by extracellular barium.

Table 2: Results of electrophysiology characterization experiments

Ion channel	$V_{½}$ [mV]	V slope [mV]	N	Literature $V_{½}$ [mV]
$K_V1.5$	-9.5±1.4 (inactivation)	-4.2±0.7	25	-10.8[5]
$K_V4.3$	-39.5±0.3 (inactivation)	-5.0±0.2	58	-26.9, -51.0[6,7]
$K_VLQT1/minK$	37.6±1.9 (activation)	17.5±0.5	48	29.4[8]

Table 3: Results of rundown test with multiple modified extracellular solutions

EC	modification of EC	Currrent remain (%)	N
EC1	1mM Ca^{2+}	61.1±4.3	7
EC2	0mM Ca^{2+}	N/A	0
EC3	0mM K+	78.3±2.1	12
EC4	0mM Mg^{2+}	64.4±6.9	9

Table 4: Results of pharmacology experiments

Ion channel	Compound	IC_{50} [μM]	Hill coefficient	N	Z' factor	Literature IC_{50} [μM]
$K_V1.5$	4-Aminopyridine	125.1±13.6	0.86±0.03	11	0.83	270.0[1]
$K_V4.3$	Flecainide	11.6±1.3	0.80±0.02	44	0.90	26.0[2]
$K_VLQT1/minK$	Chromanol 293B	17.4±1.8	1.01±0.04	15	0.79	1.0, 18.0[1,3]
$K_VLQT1/minK$	XE-991	1.7±0.2	0.87±0.02	17	0.76	5.8-11.1[1,3]
Kir2.1	Ba	49.9±7.5	2.35±0.09	3	N/A	16.2[4]

Discussion

Using QPatch II, most of the cells were successfully established the whole cell configuration by suction pulse using automatic whole cell protocol. $K_V1.5$ and $K_V4.3$ showed the very stable current over time when vehicle solution (extracellular solution containing 0.1% DMSO) was repeatedly applied. The biophysical property and pharmacological results were consistent with past results. The $V_{1/2}$ value for $K_V4.3$ was not corresponding to the literature value. This might be due to the difference in the voltage of test pulse. The literature value for this channel also big difference between the reference.

$K_VLQT1/minK$ showed the significant rundown with standard solution. Using the extracellular solution excluding potassium to wash the extracellular potassium around the ion channel contributed to decrease the rundown.

The amount of Kir2.1 data was limited as the cell line did not have enough channel expression for stable assessment. It requires further optimization of the cell culture to stabilize the ion channel expression for this cell line.

Conclusion

Our results demonstrate the feasibility of conducting electrophysiological characterization and pharmacological screening test for those cardiac potassium ion channels using fully automated patch clamp system like QPatch II.

Reference

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Acknowledgements

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