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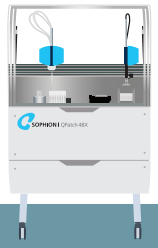

Application Report

Measurements of I_{K1} currents and paced action potentials in hiPSC derived cardiomyocytes

Recorded in physiological Ringer's using QPatch

Summary

Measurements of cardiac action potentials (APs) are of special interest for disease modeling, drug discovery and cardiac safety[1–4]. However, the immature phenotype and heterogeneous ion channel expression of hiPSC-derived cardiomyocytes (CMs), as compared to acutely isolated, primary CMs has limited the physiological relevance of their electrophysiological characterization, in general, and high throughput measurements, in particular[5–7]. This includes the presence of the pacemaker current I_f (routinely expressed in immature CMs and lost in adult CMs) and reduced densities of hyperpolarizing current I_{K1}[8,9].

However, the maturation of hiPSC-CMs has improved recently, and the paradigm of impaired I_{K1} expression in hiPSC-CMs has been challenged[10]. Here, we recorded I_{K1} currents and paced action potentials in about 50% of the investigated hiPSC-CMs, using two QPatch assays for automated patch clamp (APC) measurements of "mature" hiPSC CMs in physiological Ringer's:

1. I_{K1} currents
2. Paced action potentials

Both assays run with ~20% success rate (of 48 measurement sites). The increased throughput of action potential measurements compared to manual patch clamp (10 cells per measurement plate) allowed us to measure concentration-response effects of standard compounds such as nifedipine, E4031 and Bay K8644. These measurements were performed in physiological solutions without the use of fluoride or other types of seal-enhancer. Measurements of I_{Na}, I_{Ca} and I_{Kr} from the same cell line have been reported previously[11,12].

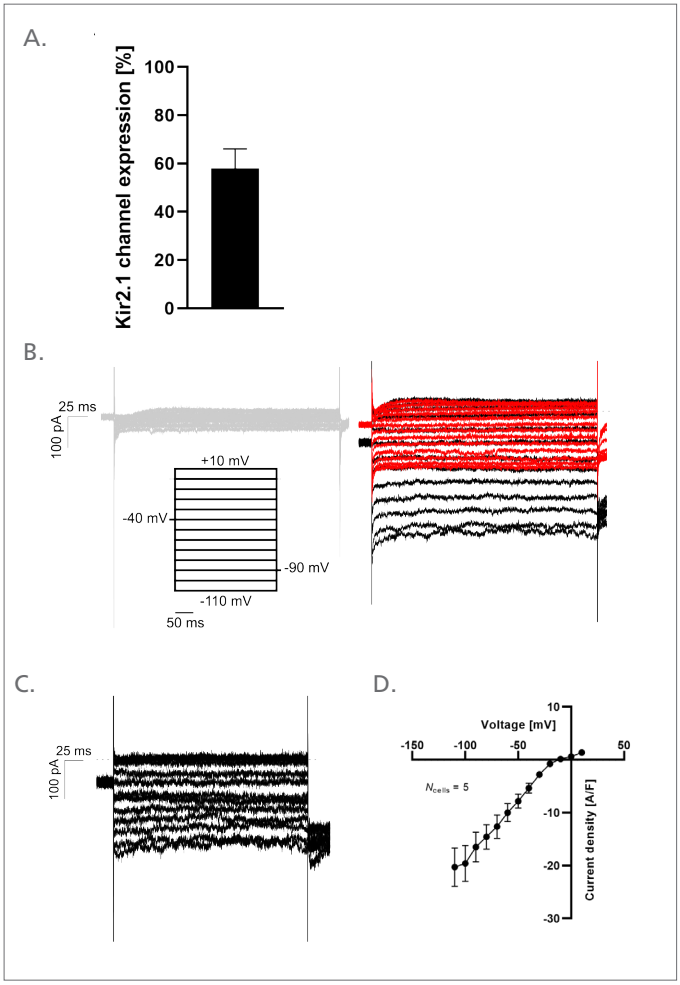

Fig. 1: Measurements of I_{K1} in hiPSC-CMs in physiological solutions. A) The percentage of successful experiments with I_{K1} current. Data is avg ± sd from two measurement plates. B) Representative I_{K1} current in response to a voltage step protocol (see methods). The current was measured in 4 mM extracellular K^+ (grey, left) and 75 mM extracellular K^+ before (black) and after (red) addition of 1 mM $BaCl_2$ (right). C) The Ba^{2+} sensitive I_{K1} current was calculated by subtracting the insensitive current (red trace in B) from the total I_K current (black trace in B). D) The I_{K1} current-voltage relationship was plotted by extracting the steady state current density from the Ba^{2+} sensitive I_{K1} current (shown in C) and plotted as a function of step voltage.

Results and discussion

I_{K1} measurements

I_{K1} currents were evoked by increasing extracellular [K^+] from 4 mM to 75 mM and subsequently blocked by addition of 1 mM $BaCl_2$ (see Fig. 1). Subtraction of the Ba^{2+} insensitive current, allowed us to plot the Ba^{2+} sensitive I_{K1} current (Fig. 1C). Finally, we plot the steady state sensitive current density vs. voltage (Fig. 1D).

Action potential measurements

The assay yielded up to 10 cells with paced action potentials per measurement plate (Fig. 2). The threshold potential (V_t), peak potential (V_p), hyperpolarization potential (V_h) and action potential duration at 90% (APD90) were quantified using Sophion Analyzer. The potentials were relatively reproducible between the cells, V_t = -48 mV ± 8 mV, V_p = 35 mV ± 9 mV, V_h = -63 mV ± 6 mV, with relative standard deviation (RSD) between 10% - 26% (Fig. 2C). The APD90 was more variable with RSD of ~ 50%. The action potential duration at 90% (APD90) was quantified before and after addition of 0.3 µM Bay K8644, 10 µM nifedipine or 1 µM E4031 (Fig. 3A-C). Finally, with the achieved success rates it was possible to measure APD90 5-point concentration-response plots for Bay K8644 and nifedipine (Fig. 4).

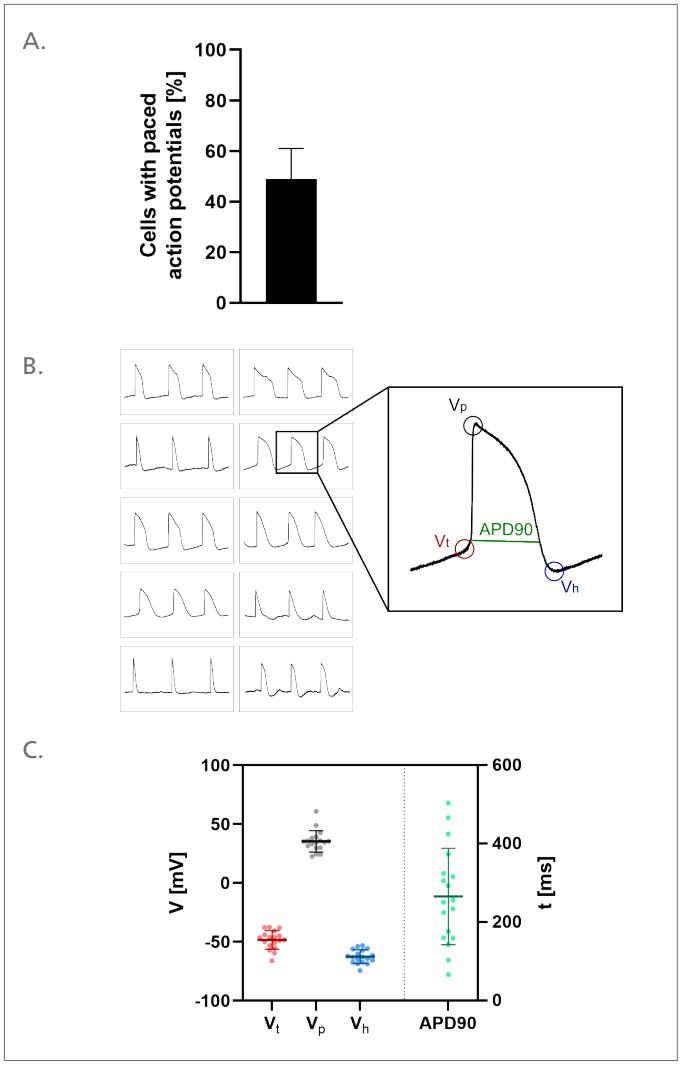

Fig. 2: Measurements of paced action potentials in hiPSC-CMs in physiological solutions. A) The percentage of hiPSC-CMs in which we recorded paced action potentials. Data is avg ± sd of three measurement plates. B) Paced action potentials from 10 individual hiPSC-CMs within a single measurement plate (left) and an expanded action potential displaying the extracted parameters: V_t, V_p, V_h and APD90. C) Plot of extracted parameters, V_t (red), V_p (grey), V_h (blue) and APD90 (green) for 18 individual iPSC CMs with the avg ± sd (solid lines).

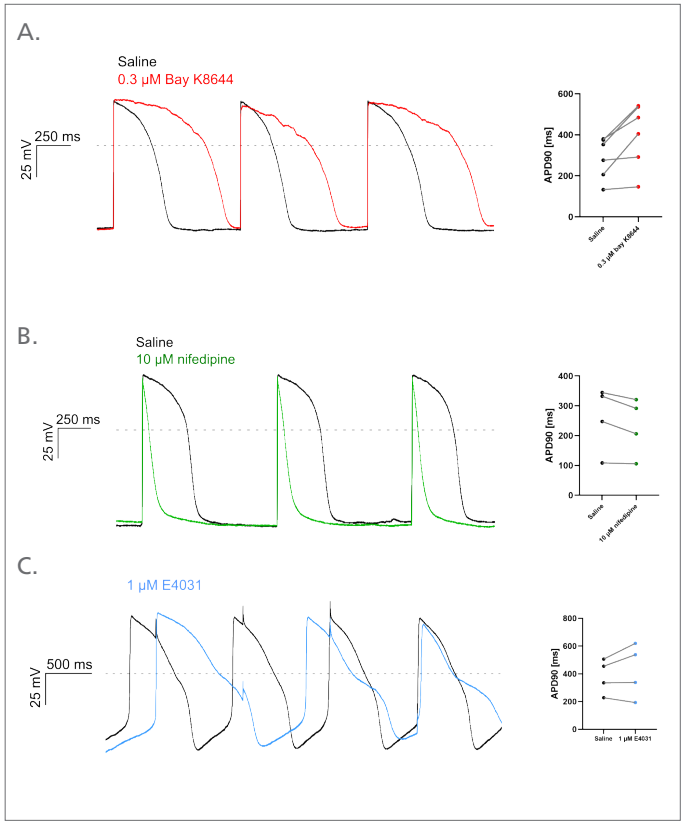

Fig. 3: Measurements of APD90 single-concentration compound effects. Paced action potentials and plot of APD90 values before (control, black traces and data points) and after addition of: A) 0.3 µM Bay K8644 (red trace and data), B) 10 µM nifedipine (green trace and data) and C) 1 µM E4031 (blue trace and data).

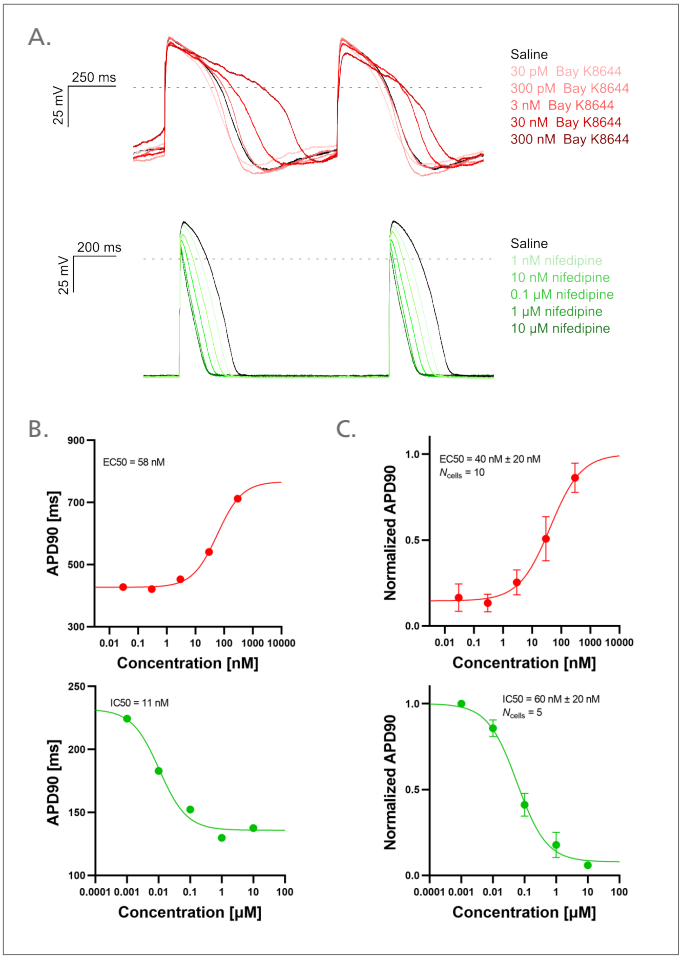

Fig. 4: Measurements of APD90 concentration-response plots. A) Paced action potentials for saline controls (black traces) and in response to increasing concentrations of Bay K8644 (top, traces in shades of red) and nifedipine (bottom, traces in shades of green). B) Plots of APD90 versus compound concentration with Boltzmann fits including EC_{50} or IC_{50} calculated values, recorded in a single cell. C) Concentration-response (average, normalized APD90) relationships with Boltzmann fits including EC_{50} or IC_{50} calculated values. Data points are avg ± sem of N_{cells}.

Conclusion

In summary, it is possible to perform I_{K1} and AP measurements of hiPSC-CMs on QPatch in physiological solutions, yielding up to a 20% success rate. Within the hiPSC-CM population that passed quality criteria, we recorded I_{K1} as well as paced action potentials in ~ 50% of the investigated cells. As 1) the hiPSC-CM quality and maturity increase and 2) cell suspension preparation is further optimised, we expect the success rate and throughput of these measurements to increase further. Eventually, we envision that APC can be used as a characterization tool to assist the continuous development of hiPSC-CMs as well as for cardiac drug screening, disease modelling and possibly even diagnostics.

Methods

Cells were provided by UM Göttingen lab (www.molecular-pharmacology.de). Cell culture and cell suspension preparation according to internal Sophion methods (contact info@sophion.com for further information).

Solutions: **Intracellular solution** (in mM): 5.374 $CaCl_2$, 1.75 $MgCl_2$, 31.25/10 KOH/EGTA, 10 HEPES, 120 KCl, 4 Na2-ATP, pH = 7.2 with KOH, osmolarity adjusted to 285 – 296 mOsm with sucrose. **Extracellular solution** (in mM): 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 KCl, 145 NaCl, 10 Glucose, pH = 7.4 with KOH, Osmolarity 305 mOsm with sucrose. **Extracellular solution with high K^+** (in mM)**:** 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 75 KCl, 75 NaCl, 10 Glucose, pH = 7.4 with KOH, osmolarity adjusted to 305 mOsm with sucrose.

Voltage- and current-clamp protocols:

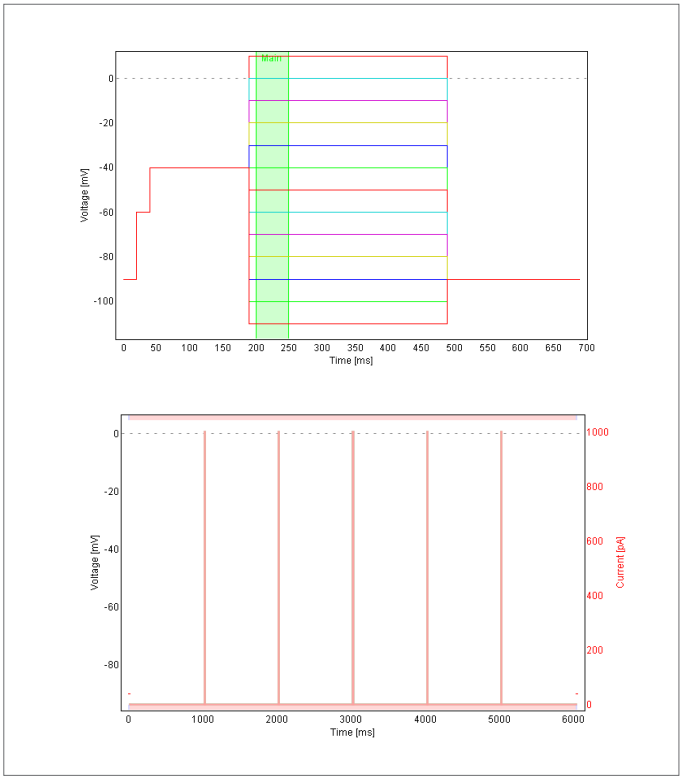

Fig. 5: Voltage step protocol for I_{K1} (top) and current clamp protocol for paced APs (bottom). All analysis was performed with Sophion Analyzer.

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