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Characterization of human iPSC-derived cardiomyocytes (Cor.4U) on an automated planar patch clamp set-up (QPatch)

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Introduction

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC – CM) are providing new, highly predictive strategies to assess cardiotoxicity *in vitro* and can thus reduce costs for cardiac safety assessment in drug development (Ma et al., 2011).

In the present study, we characterized Cor.4U® cells using Sophion's 48-channel QPatch both in voltage and in current clamp mode.

Electrophysiological investigation of hiPSC-CM requires a high quality set up that offers the possibility to both record action potentials in current clamp mode and to isolate individual ion channel currents using voltage clamp.

The QPatch fulfills these requirements and does not rely on the use of a seal enhancer. Many automated patch clamp systems rely on the use of fluoride in the internal solution to gain sufficiently high seal resistances. However, fluoride is also known to chelate Ca^{2+}, inhibit proteases and hyperpolarize the gating of sodium channels and may thus affect the recordings (Cummins et al., 2009).

As the number of cardiomyocytes are usually limited, it is critical to minimize the cell consumption per experiment. QPatch is endowed with a feature that helps to reduce the number of cells needed per experiment. Using this feature, 200 µl of cell suspension is sufficient to run a 48-well QPlate.

Conclusion

In this study, we used the automated patch clamp set-up QPatch 48 (Sophion) to characterize hiPSC – CM (Cor.4U; Ncardia).

We succeed in isolating voltage-gated Na^+ (I_{Na}) and Ca^{2+} (I_{Ca}) currents, where application of the nifedipine, resulted in a concentration–dependent inhibition of I_{Ca}.

Using current clamp, both paced and spontaneous action potentials could be elicited. Here, tetracaine and nifedipine caused shortening of the AP while the hERG channel inhibitor, E-4031, prolonged the duration of APs.

Comparison of a physiological and a fluoride–based internal solution revealed significantly lower I_{Ca}, which was further manifested in a significantly shorter AP duration. The exact reason for these discrepancies has yet to be determined but it is possible that the low solubility of CaF or the effect of F^- on cAMP contributes to this observation.

Methods

Cell culture
Cor.4U® human iPS cell-derived cardiomyocytes were cultured and harvested according to Ncardia's guidelines. Cell density was between 1 and 2 mio/ml and 200 µl of cell suspension were sufficient for one 48-well QPlate.

Cell preparation
Different harvesting protocols were tested. Briefly, the cell detachment solution detachin did not properly separate the cells. A two-step protocol with 5 min accutase and 12 - 18 h collagenase type II incubation resulted in a well separated preparation with good seal resistances, but results were variable.

Electrophysiology
Series resistance (R_S) was 80% compensated (400 µs) using Sophion's patented compensation algorithm.

Data analysis
Data were analyzed using Sophion's Assay Software and Origin (OriginLab).

High fidelity voltage clamp recordings of Cor4U® demonstration of the presence of I_{Na}, I_{Ca} and I_{Kr}

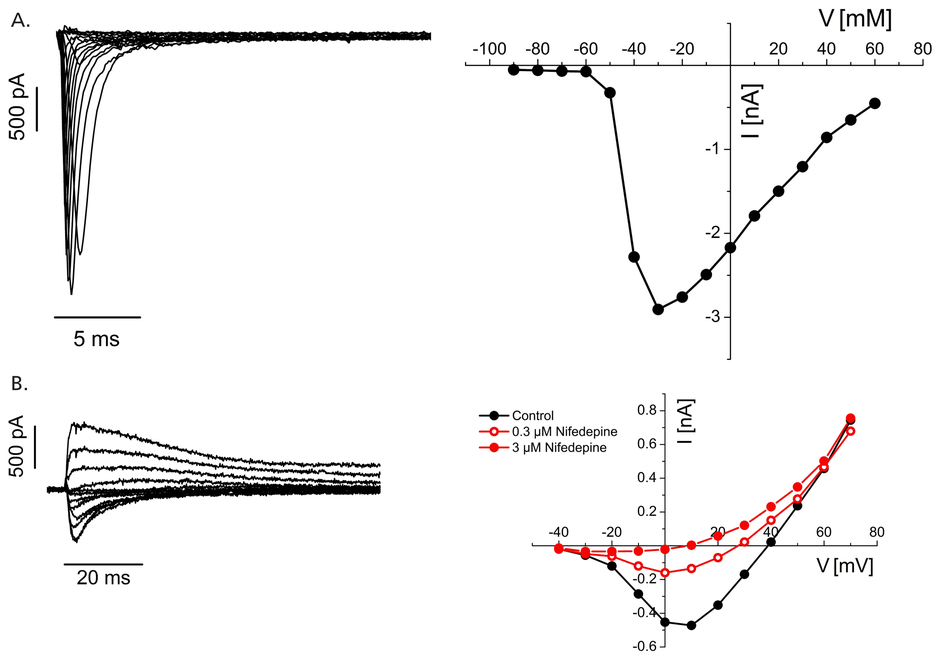


Fig. 1: A) Typical sodium currents (I_{Na}) (left) and the corresponding current-voltage relationship of the peak current (right). The average peak current at -20 mV of n = 45 cells was – 6 ± 6 nA (SD). Voltage protocol: Step from -90 mV to + 60 mV in 10 mV increments. Holding potential: -120 mV.
B) Left: Typical Ca_V and K_V – mediated current. Left: Current response traces. Black: Control condition, red: Nifedipine. Average current at +10 mV was – 140 (± 280 pA (SD), n = 30 cells). Voltage protocol: Pre-pulse to -40 mV (not shown) followed by voltage steps from –40 mV to +70 mV in 10 mV increments. Holding potential: -120 mV.

Current clamp recordings of paced and spontaneous action potentials

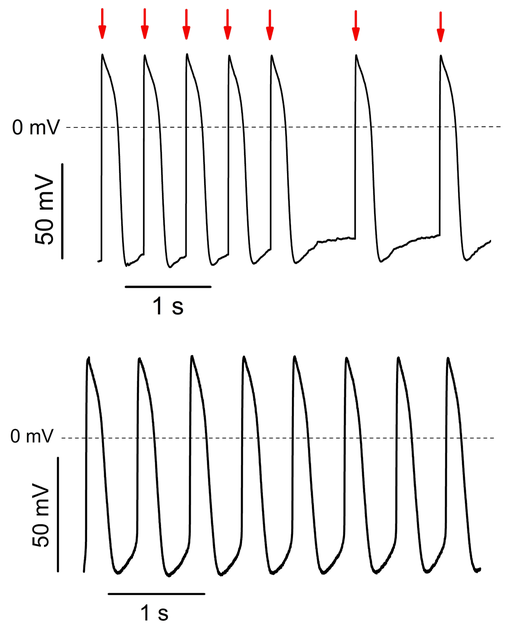
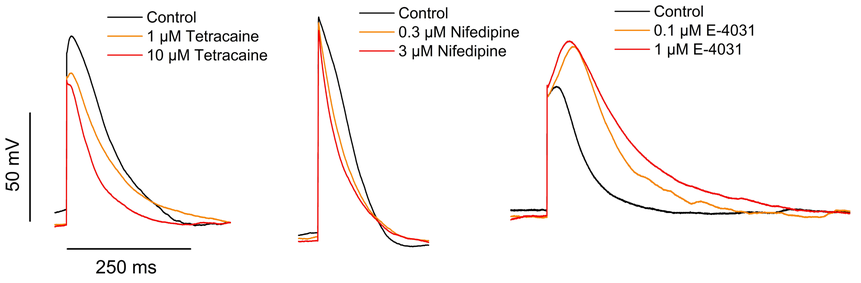


Fig. 2: A) Typical paced action potentials. The average resting potential of n = 27 cells was – 47 ± 14 mV (SD). Red arrows: injection of 1.5 nA for 1 ms from a holding current of 0 pA. First at a frequency of 2 Hz and last two, 1 Hz.
B) Typical spontaneous action potentials in current clamp mode. Current was clamped at 0 pA.

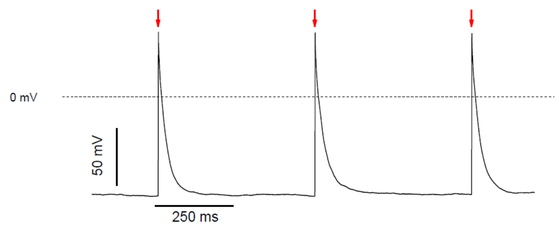
Pharmacological modulation of action potentials



	APD90 [ms] Control	Compound	ΔAPD90[ms]	n
Tetracaine [10 µM]	150 ± 60	120 ± 40	-30 ± 30	5
Nifedipine [3 µM]	180 ± 100	70 ± 50	-110 ± 70	6
E-4031 [1 µM]	170 ± 100	250 ± 110	80 ± 50	4

Fig. 3: A) Effect of different pharmacological agents on paced action potentials recorded in current clamp mode. Pacing: 1 ms pulse of 1.5 nA. B) Summary of compound effects on action potential duration at 90% (APD(90) ±SD).Tetracaine: Na^+ channel inhibitor. Nifedipine: L-type Ca^{2+} channel inhibitor. E-4031: hERG channel inhibitor.

The use of fluoride in the internal solution causes lower I_{Ca} and shortened action potential durations



	Physiological solution Mean ± SD	n	Flouride-based solution Mean ± SD	n
APD90 [ms]	140 ± 120	24	50 ± 40**	15
I_{Na} at -20 mV [nA]	-6 ± 6	30	-4 ± 4	25
I_{Ca} at +10mV [pA]	-140 ± 280	30	30 ± 130**	25

Fig. 4: A) Typical paced action potentials recorded using a fluoride-based internal solution. The use of fluoride in the internal solution resulted typically in much shorter, triangulated action potentials. Red arrows: injection of 1.5 nA for 1 ms from a holding current of 0 pA.
B) Summary of different electrophysiological characteristics recorded in different internal solutions. All cells exhibiting seal resistance greater 100 MΩ were included. ** indicates statistical significance at $p < 0.01$ in a Student`s T test.

Cor.4U® cells were kindly provided by Ncardia

Ncardia
Stem cell experts

References

Cummins et al. (2009). Nature Protocols, 4(8), 1103–1112.
Ma et al. (2011). Am J Physiol Heart Circ Physiol, 301(5), 2006–2017.