Application Report:

Cor.At® cells

Embryonic stem cell derived cardiomyocytes

For drug discovery stem cell derived cardiomyocytes provide an exciting new tool for e.g. cardiac target discovery and cardiac safety studies. Axiogenesis has developed the Cor.At® cells, cardiomyocytes derived from transgenic mouse embryonic stem cells, and we have tested them on the QPatch.

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Embryonic stem cell derived cardiomyocytes, Cor.At® cells, tested on QPatch

Introduction

Embryonic stem cell-derived cardiomyocytes have many of the phenotypic properties of authentic cardiomyocytes, and great interest has been shown in their possibilities for modeling human diseases. For drug discovery stem cell derived cardiomyocytes provide an exciting new tool for e.g. cardiac target discovery and cardiac safety studies. Axiogenesis has developed the Cor.At® cells. These cells are cardiomyocytes derived from transgenic mouse embryonic stem cells and are 99.9% pure. This report presents recordings of three essential currents; I_K, I_{Ca}, and I_{Na} from the Cor.At® cells obtained with the QPatch system.

Materials & Methods

Cells

The Cor.At® cells from Axiogenesis are derived from the embryonic stem cell line called D3, which is generated from the blastocyst stage of a mouse embryo (Doetschman etl al. 1985). The Cor.At® cells were received in frozen stocks containing either 1 or 5 mio. alive cells. The cells were kept in a -150 degree nitrogen freezer until cells were thawed and cultured for experimental use. Spontaneous contractile properties of the Cor.At® cells were observed within 24 hours after thawing and culturing. The cells were thawed and cultured according to the specifications described from the standard operating procedure generated in collaboration between Sophion and Axiogenesis. A final preparation of 100-200 µl of cell suspension containing 2-5×10^6 cells/ml was applied to the QPatch for each experiment run.

Ringer's solutions

Intracellular ringer (I_{Ca} and I_{Na}) in mM: 120 CsCl, 3 $MgCl_2$, 10 EGTA, 5 HEPES, 5 MgATP. pH adjusted to 7.3 with CsOH.

Extracellular ringer (I_{Ca} and I_{Na}) in mM: 120 NaCl, 5 KCl, 3.6 $CaCl_2$, 1 $MgCl_2$, 20 TEA-Cl, 10 HEPES. pH adjusted to 7.4 with TEA-OH.

Intracellular ringer (I_K) in mM: 50 KCl, 80 KAspartate, 1 $MgCl_2$, 10 EGTA, 10 HEPES, 3 MgATP. pH adjusted to 7.3 with KOH.

Extracellular ringer (I_K) in mM: 135 NMG, 5 KCl, 3.6 $CaCl_2$, 1 $MgCl_2$, 3 $NiCl_2$, 10 HEPES. Adjusted to 7.4 with KOH.

Compounds

TTX (Alomone Laboratories), nifedipine (Sigma) and isoproterenol (Sigma) were dissolved in DMSO, and diluted to final concentration in the extracellular ringer. The highest DMSO concentration was 0.1%.

Results

Typical I_K, I_{Ca}, and I_{Na} were recorded from the Cor.At® cells cultured for 24 hours (Figure 1-3). From RNA isolation experiments (done by Axiogenesis) it is known that the sodium ion channel expressed in Cor.At® cells is $Na_V1.5$. This subtype is mainly expressed in cardiomyocytes and has very low sensitivity to tetrodotoxin (TTX) (Figure 1). The L-type Ca channel $Ca_V1.2$ has been detected at RNA level in the Cor.At® cells

(Axiogenesis). The I_{Ca} current was modulated by isoproterenol suggesting functional β-adrenergic stimulation pathway in the Cor.At® cells (Figure 2). The potassium current recorded from the Cor.At® cells was characterized by its voltage-dependent fast activation and inactivation typically for transient outward potassium current, I_{to}.

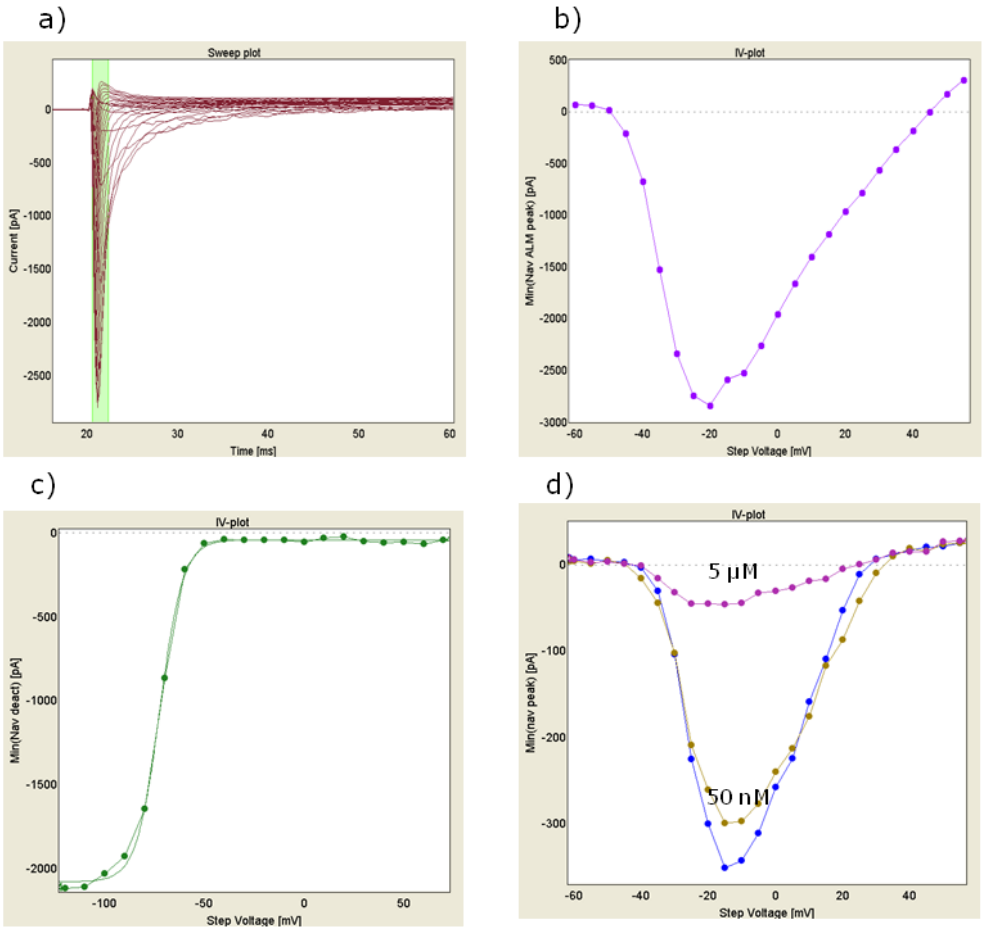

Figure 1. I_{Na} whole cell currents stimulated with a voltage step protocol from –120 mV to +60 mV (1000 ms duration) from a holding potential of -100 mV. a) I_{Na} activation current sweeps. The peak current was plotted as a function of the step voltage in b). c) Peak tail current obtained at +10 mV plotted as a function of the step potential and data fitted to the Boltzmann equation. $V_{0.5}$ was determined to -73 mV (-62 mV ± 13, n=7). d) I_{Na} whole cell currents stimulated with a voltage step protocol from –60 mV to +55 mV (100 ms duration) from a holding potential of -90 mV. Current-voltage relationship obtained in the presence of TTX (50 nM and 5 µM, respectively).

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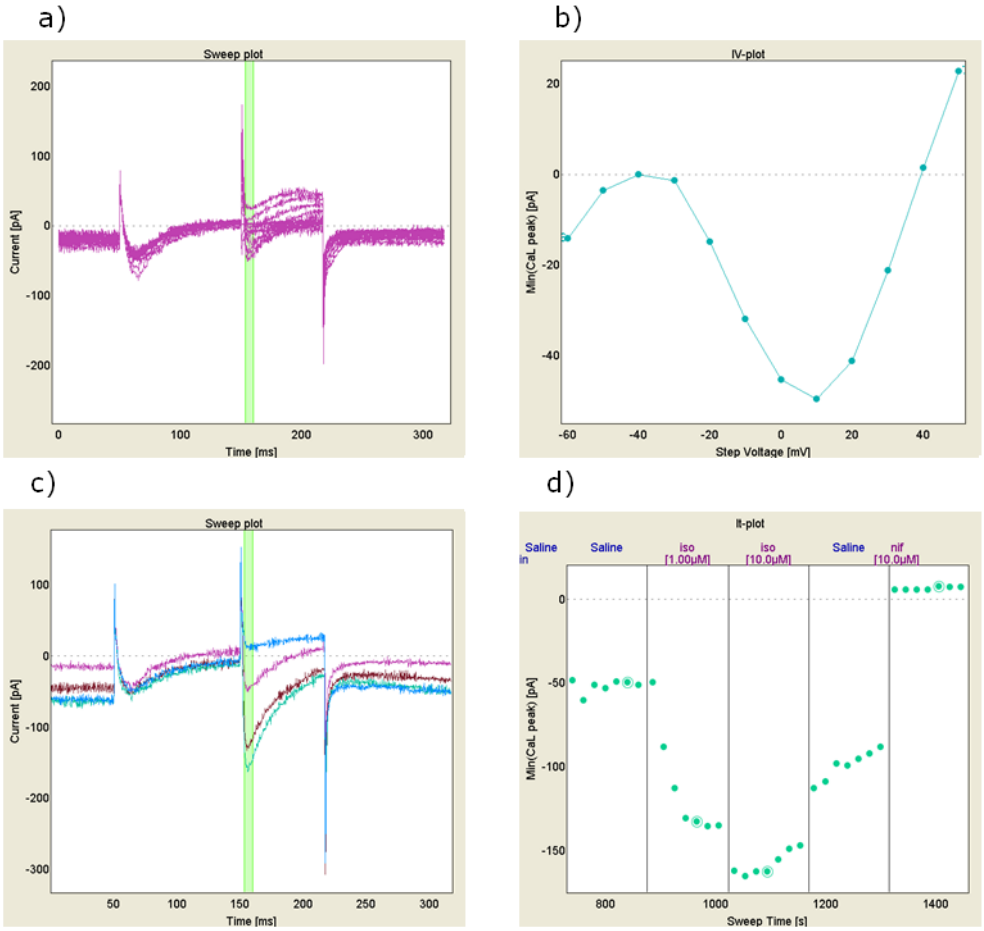

Figure 2. I_{Ca} whole cell currents stimulated with a double-pulse voltage protocol. First a 100 ms depolarization step at -40 mV to depict and then inactivate T-type Ca^{2+} channels, then a voltage step protocol from -40 mV to +50 mV (67 ms duration) to record L-type Ca^{2+} current. The holding potential was -100 mV. a) I_{Ca} whole cell current sweeps. The green bar shows the position of the cursor used to measure the peak current. b) The peak current plotted as a function of the step potential. c) Typical I_{Ca} current sweeps at +10 mV recorded in the presence of the β-adrenoceptor agonist, isoproterenol, at 1 µM (brown) and 10 µM (purple), respectively. The current was blocked by nifedipine (10 µM) (blue). e) The peak current in c) plotted as a function of time.

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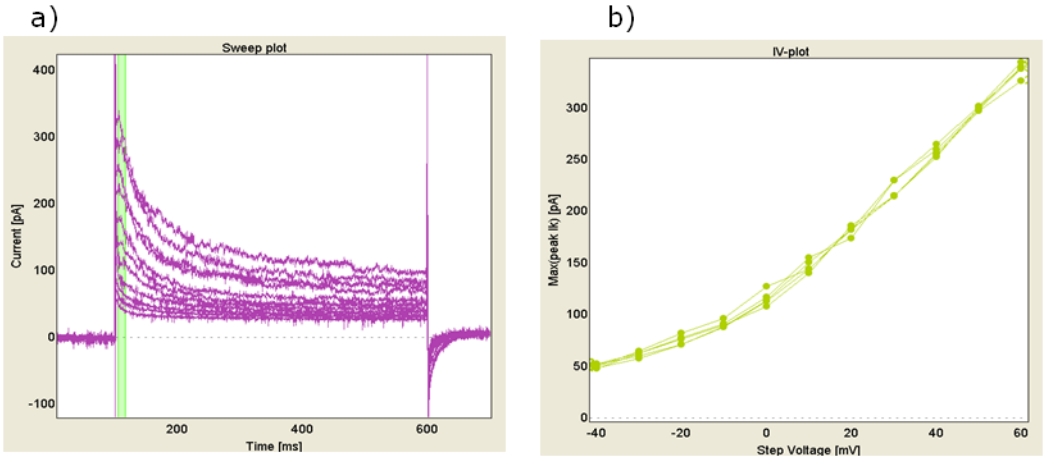

Figure 3. I_K whole cell currents stimulated with a voltage step protocol from –40 mV to +60 mV (500ms duration) from a holding potential of -80 mV. a) I_K whole cell current sweeps. The green bar shows the position of the cursor used to measure the peak current. b) Peak current plotted as a function of step voltage potential.

The current amplitude of I_{Na} and I_{Ca} varied from cell to cell. For I_{Na} the size of the peak amplitude varied from a few hundred pA to several nA. In approximately one third of the cells I_{Na} was undetectable (Table 1). For the I_{Ca} the current amplitude was typically < than 100 pA, and not detectable in approximately half of the cells (Table 1). It is possible the culture duration can affect the current amplitudes but this has not been investigated in details in this study.

Subject	Result
Viability after harvest procedure (%)	87± 3, n= 4*
Size of cell (pF)	17 ± 7, n= 112
Peak I_{Na} (pA) (at -30 mV)	1842 ± 2521, n= 52
Current density I_{Na} (pA/pF)	104 ± 129, n= 52
Cells with recordable I_{Na} amplitude (%)	68 (15/22)
Peak I_{Ca} (pA) (at +10 mV)	35 ± 27, n= 32
Current density I_{Ca} (pA/pF)	2 ± 1.5, n= 32
Cells with recordable I_{Ca} amplitude (%)	55 (12/22)

Table 1. The table summarizes the results from different investigations of the Cor.At® cells and the I_{Na} and I_{Ca} currents recorded with the QPatch.

*Data from 4 different experimental days where the cells were harvested according to the SOP. The viability was checked just before the cells were added to the QPatch.

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Success rates

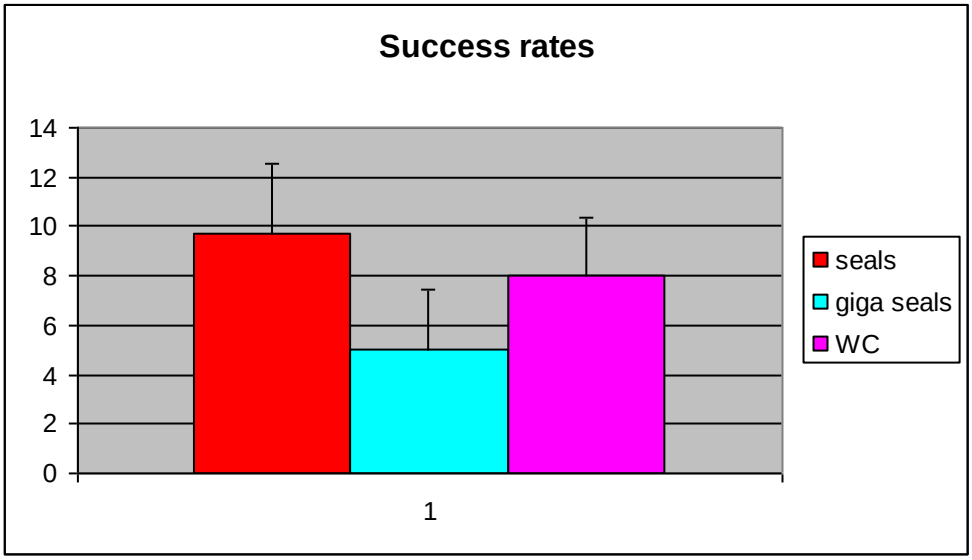

Figure 4. Average number of seals, true giga seals, and whole cells per QPlate 16. The cells were cultured for 24 hours and harvested according to the SOP made for Cor.At® cells. The data is based on measurements from 4 QPlate 16's.

References

Doetschman et al. 1985 J. Embryol. exp. Morph. 87, 27-45

Conclusion

The QPatch system is often used for ion channel compound screening in drug discovery. Typically recombinant cell lines e.g. HEK293, CHO, and RBL cells are used for ion channel screening as these cells can be acquired in high quantity, can be frozen in stocks, can be transfected with the ion channel of interest, are easy to handle and the cost production is (relatively) low. Differentiated stem cells, like Cor.At® cells, may be interesting for ion channel screening as they provide a more physiological relevant environment for the ion channel(s) under examination. The Cor.At® cells also have the advantage that they are produced in high amounts, are kept as frozen stocks, and can be thawed right before the experiments are to be conducted.

Here, three essential cardiac were recorded from mouse embryonic-stem cell derived Cor.At® cardiomyocytes with the QPatch system. It is possible that other essential cardiac currents like I_{erg} can also be recorded. It is not known if longer culture time will affect the quality of the recordings. The results show that the cells performed well in respect to the quality of the current recordings. The success rates for giga seals and whole cells were semi-good compared to a standard recombinant cell line used with QPatch.

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