Application Note

Channel: I_{Na}, I_{Ca}, I_K
Cells: ES cell-derived cardiomyocytes
Tools: Port-a-Patch®

Recordings of Action Potentials in Mouse ES Cell-Derived Cor.At® Cardiomyocytes on Nanion's Port-a-Patch®

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Introduction

To provide scientists in basic or applied cardiology and toxicology with a standardized and pure cardiac myocyte model with functional expression of all essential cardiac ion channels, Axiogenesis has developed Cor.At® cardiomyocytes. These mouse embryonic stem cell derived cardiomyocytes are ready to use and 99.9 % pure without contamination by other cell types. Using the Port-a-Patch®, Cor.At® cardiomyocytes have experimentally been shown to functionally express at least three essential cardiac currents I_{Na}, I_{Ca}, and I_K, and to exhibit typical cardiac action potentials.

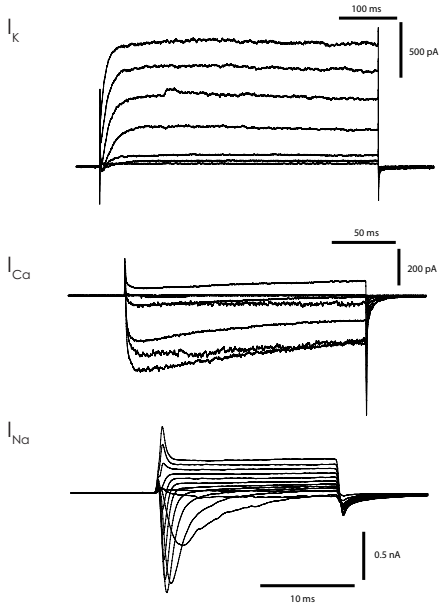

Figure 1:
Whole cell current as recorded in the voltage clamp mode reveal all three cardiomyocyte typical ion channels. K-channel (top), Ca-channel derived current (middle) and Na-current (bottom).

Results

Current responses in the voltage clamp mode of Cor.At® cardiomyocytes are shown in Figure 1. Figure 2 shows the corresponding current-voltage relationships.

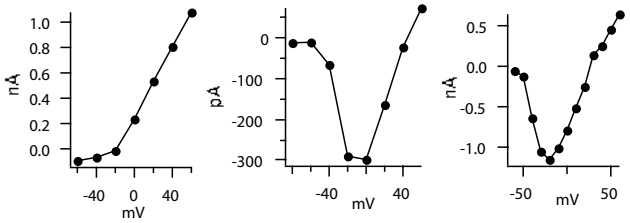

Figure 2:
Current-voltage relationship of the whole cell currents shown in Figure 1. K-outward current (left), Ca-channel derived current (middle) and Na-current (right).

When the configuration was switched to current clamp mode, the cells showed typical cardiac action potentials (Fig. 3).

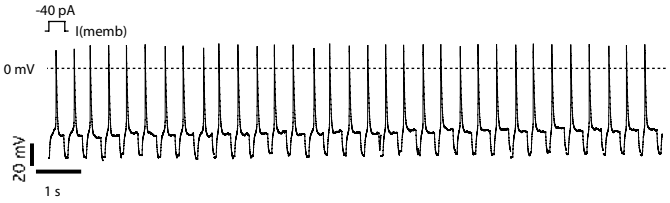

Figure 3:
Action potentials in Cor.At® cardiomyocytes. The action potentials were elicited by depolarisation from a holding current $I_{(memb)}$ to -40 pA for 350 ms with a sweep interval of 5 s.

Application Note

The Human Ether-a-go-go Related Gene hERG encodes a K-channel, which is considered responsible for repolarizing I_{Kr} current in the human cardiac action potential. The hERG blocker Dofetilide induced a prolongation of the action potential (Fig. 4 and Fig. 5 A), which gives a hint to the presence of the mouse analog of hERG-channels in Cor.At® cardiomyocytes. The effect proved to be reversible upon washout of the drug.

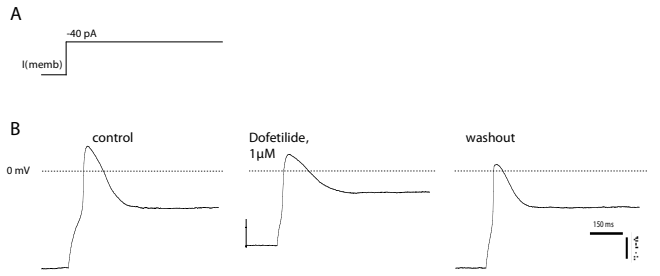

Figure 4:
A Current clamp protocol that was used for the stimulation of action potentials as seen in B. **B** Action potential in control solution (left), after the external application of 1 µM Dofetilide (middle) and after washout (right).

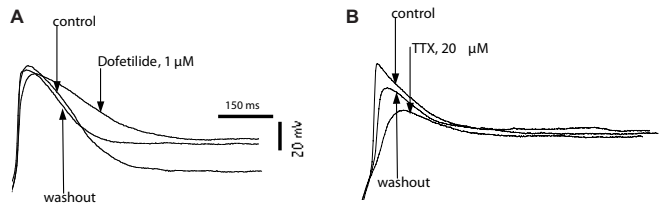

Figure 5:
A Overlay of the traces as seen in Fig. 4 B. Note the reversible prolongation of the action potential in the presence of the hERG blocker Dofetilide. **B** Application of the Na-channel blocker TTX (20 µM) leads to a reversible decrease of the maximum, as well as a slower depolarisation, of the action potential.

TTX is a Na-channel blocker and, after application of 20 µM to the external recording solution, the action potentials showed a decrease of the maximum as well as a slower depolarisation of the action potential.

Summary

Cor.At® cardiomyocytes display typical cardiac ion channel activity and action potentials. The hERG blocker Dofetilide induced a prolongation of the action potential, the Na-channel blocker TTX leads to a decrease of the action potential and to a slower depolarisation. Both effects were reversible upon washout of the drugs.

The results indicate that Cor.At® cardiomyocytes exhibit primary cell-like qualities, and that these electrophysiological properties can be observed with Nanion´s planar patch clamp technology. The technology allows recordings in the voltage clamp as well as in the current clamp mode. The measurements shown here were recorded with the one channel patch clamp device from Nanion, the Port-a-Patch®. This is the first demonstration of action potential recordings on a planar patch clamp system. Since the basic technology is the same also for the higher throughput devices from Nanion, we propose that these recordings could also be scaled up using the Patchliner®. Here, 8 channels in parallel could be recorded simultaneously.

Methods

Cells

Cor.At® cardiomyocytes (Axiogenesis AG, Cologne, Germany); www.axiogenesis.com, email: info@axiogenesis.com

Patch Clamp solutions

External solution for Ca-channel recordings: 145 mM TEA-Cl, 20 mM $BaCl_2$, 10 mM HEPES, 10 mM Glucose, pH 7.35. External solution for K- and Na-channel recordings: 140 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM D-Glucose monohydrate, 10 mM Hepes /NaOH pH 7.4. Internal solution: 50 mM KCl, 10 mM NaCl, 60 mM K-Fluoride, 20 mM EGTA, 10 mM Hepes /KOH, pH 7.2.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Port-a-Patch®. K-channel currents were elicited using 500 ms voltage steps from a holding potential of -80 mV to -60 mV up to +60 mV (20 mV increments, sweep interval 15 s) followed by a step back to the holding potential. Ca-channel currents were elicited using 200 ms voltage steps from a holding potential of -80 mV to -80 mV up to +60 mV (20 mV increments, sweep interval 5 s) followed by a step back to the holding potential. Na-currents were elicited using voltage steps from a holding potential of -80 mV to -60 mV up to +60 mV (10 mV increments, sweep interval 1 s) for 20 ms followed by a step back to the holding potential.

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