

Channel:

I_{Na} , I_{Ca} , I_K

Cells:

ES cell-derived
cardiomyocytes

Tools:

Patchliner®

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

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Introduction

Axiogenesis is a provider of pure mouse embryonic stem cell-derived cardiomyocytes (Cor.At® (1)). These cardiomyocytes have been evaluated with Nanion's automated patch clamp platforms the Port- α -Patch® (2) and the Patchliner®. The aim of this study was to show that the Patchliner®, Nanion's planar patch clamp device for increased throughput, can be used for studies investigating compounds which exhibit chronotropic or arrhythmic effects. In the voltage clamp mode voltage-dependent Na^+ -, Ca^{2+} - and K^+ -channel currents could be recorded. As expected, action potentials could be elicited in the current clamp mode. Effects of compounds on action potentials have been successfully demonstrated.

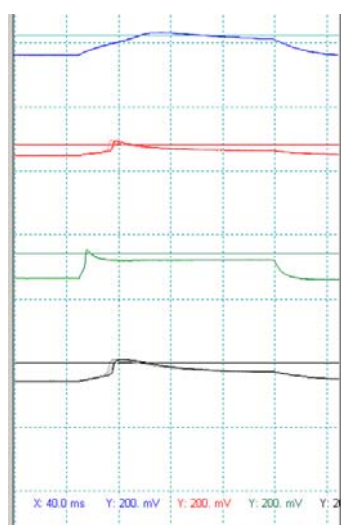


Figure 1: Action potentials in Cor.At® cardiomyocytes. Screenshot of recordings performed on a Patchliner® Quattro (four recording channels).

Results

Action potentials recorded in the current clamp mode of Cor.At® cardiomyocytes were sensitive to the ion channel modulators Quinidine and Lidocaine (Fig. 2).

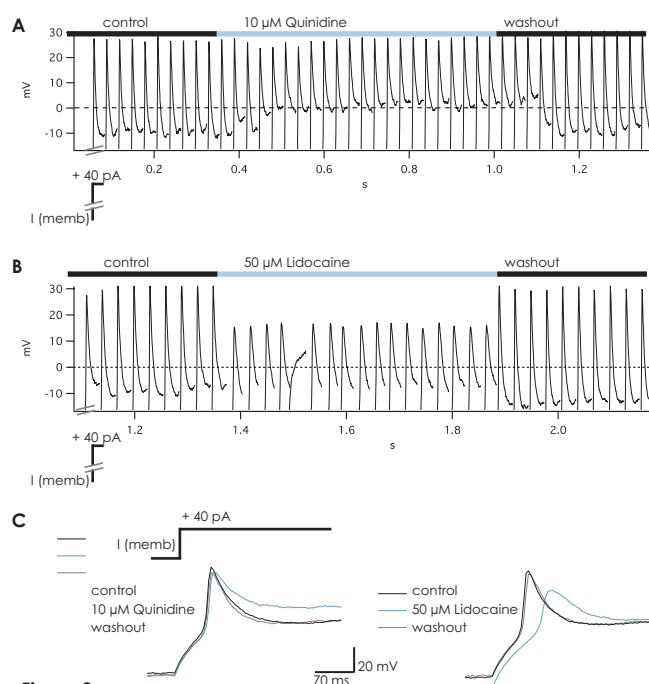


Figure 2: Action potentials from Cor.At® cardiomyocytes. The action potentials were elicited by depolarisation from a holding current $I_{(memb)}$ to +40 pA for 150 ms with a sweep interval of 10 s. **A** Action potentials in control conditions, in the presence of 10 μ M Quinidine and after washout. **B** Action potentials in control conditions, in the presence of the Na^+ -channel blocker Lidocaine (50 μ M) and after washout. Same cell as in A. **C** Overlay of three traces from the recordings shown in A and B.

Application Note

When this cell (shown in Fig. 2) was switched to voltage clamp mode, Na⁺-, Ca²⁺- and K⁺-channel currents (Fig. 3) could be recorded.

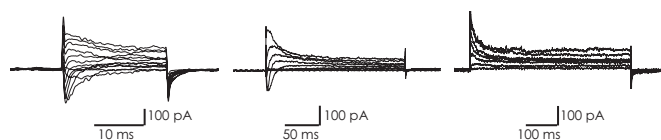


Figure 3: Na⁺-channel (left), Ca²⁺-channel (middle) and K⁺-channel currents (right).

The Human Ether-a-go-go Related Gene (hERG) encodes a K⁺-channel, which is responsible for the repolarising I_{Kr} current in the human cardiac action potential. The hERG blocker Cisapride induced a change of the repolarisation (Fig. 4), which indicates functionality of the mouse analogue of hERG-channels in Cor.At[®] cardiomyocytes. The effect was non-reversible upon washout.

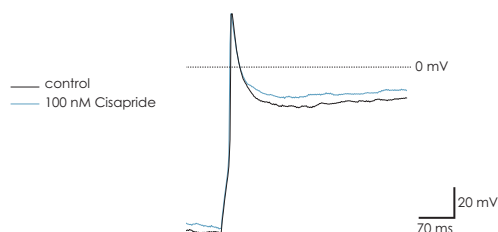


Figure 4: Action potentials in control solution and after the external application of 100 nM Cisapride.

Methods

Cells

Mouse embryonic stem cell-derived cardiomyocytes, ready to use and 99.9 % pure without contamination by other cell types (1).

Patch Clamp Solutions

External solution for Ca²⁺-channel recordings: 80 mM NaCl, 3 mM KCl, 10 mM MgCl₂, 35 mM CaCl₂, 10 mM HEPES (Na⁺-salt)/HCl, pH 7.4. External solution voltage clamp and current clamp recordings: 140 mM NaCl, 4 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 5 mM D-Glucose monohydrate, 10 mM HEPES /NaOH pH 7.4. Internal solution: 50 mM KCl, 10 mM NaCl, 60 mM KF, 20 mM EGTA, 10 mM HEPES /KOH, pH 7.2.

Cor.At[®]

Summary

Cor.At[®] cardiomyocytes display typical cardiac ion channel activity and action potentials. Both the hERG blockers Quinidine and Cisapride, and the Na⁺-channel blocker Lidocaine, modulated the action potentials. The Quinidine and Lidocaine effects were reversible, the Cisapride effects were non-reversible.

The results demonstrate the presence of an array of ion channels in Cor.At[®] cardiomyocytes which in conjunction are capable of generating action potentials. Hence these stem cell-derived cardiomyocytes are a suitable alternative to primary cardiomyocytes in drug screening and safety testing.

In addition, these experiments demonstrate for the first time the suitability of a higher throughput planar patch clamp system, i.e. Nanion's Patchliner[®], for recording action potentials. This is possible because of the flexibility of Nanion's patch clamp systems allowing for a multitude of different experiments to be performed in both voltage and current clamp modes.

Cells expressing multiple ion channels and therefore able to elicit action potentials, as opposed to cell lines over-expressing a single ion channel subtype, better represent the physiological system. Thus, the Patchliner[®] in combination with Cor.At[®] cardiomyocytes form a powerful tool for ion channel research, drug screening and safety testing.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner[®]. Protocol for ion channel recordings see (2).

References

- (1) Cor.At[®] cardiomyocytes from Axiogenesis AG, Cologne, Germany; www.axiogenesis.com, email: info@axiogenesis.com.
- (2) Recordings of Action Potentials in Cor.At cells on Nanion's Port-a-Patch[®]. www.nanion.de