Real-Time Simulation of I_{K1} in Cardiomyocytes Derived from Human Induced Pluripotent Stem Cells

Rosalie ME Meijer van Putten[1], Isabella Mengarelli[1], Kaomei Guan[2], Jan G Zegers[1], Antoni CG van Ginneken[1], Arie O Verkerk[1], Ronald Wilders[1]

[1]Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
[2]Georg-August-University of Göttingen, Göttingen, Germany

Abstract

Cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) are widely used in studying basic mechanisms of ventricular arrhythmias. However, their action potential profile—and thereby the profile of individual ionic currents active during that action potential—differs substantially from that of native human cardiomyocytes, which is largely due to an almost negligible expression of the inward rectifier potassium current (I_{K1}).

We attempted to 'normalize' the action potential profile of our hiPSC-CMs through real-time simulation of the lacking I_{K1} in the dynamic clamp configuration of the perforated patch clamp technique, which allows the injection of a voltage-dependent in silico I_{K1}. Without injection of I_{K1}, our hiPSC-CMs showed nodal-like spontaneous beating, but injection of an in silico I_{K1} unmasked their ventricular-like nature. Proarrhythmic action potential changes were observed upon real-time simulation of both loss-of-function and gain-of-function mutations in I_{K1}, as associated with Andersen-Tawil syndrome type 1 and short QT syndrome type 3, respectively.

We conclude that injection of in silico I_{K1} makes the hiPSC-CM a more reliable model for investigating mechanisms underlying ventricular arrhythmias.

1. Introduction

Cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs), building on the seminal work of Takahashi et al. [1], are a promising new tool in the research of cardiac ion channelopathies, i.e. disorders resulting from dysfunction of cardiac ion channels. Cell lines can be obtained from any individual, thus not only allowing research on the effects of channelopathies in their natural setting, but also on the role of genetic background. Over the past years, hiPSC-CMs have been used in a growing number of studies on channelopathies, as reviewed by Hoekstra et al. [2] and others (see [3] and references cited therein).

An essential problem with hiPSC-CMs is their immature phenotype. In general, hiPSC-CMs demonstrate spontaneous activity with a depolarized maximum diastolic potential (MDP), a low maximum upstroke velocity ($(dV/dt)_{max}$), a low action potential amplitude (APA), and a highly variable action potential duration [2]. If quiescent, their resting membrane potential (RMP) is depolarized in comparison with that of native working cardiomyocytes (CMs). The depolarized MDP or RMP of hiPSC-CMs results in inactivation and thus a lower functional availability of fast sodium channels, which may explain, at least in part, their low $(dV/dt)_{max}$. The functional availability of other ion channels may also be altered. Furthermore, there are ultrastructural differences between hiPSC-CMs and native CMs. In particular, hiPSC-CMs exhibit a poorly developed sarcoplasmic reticulum and a lack of T tubules [4].

A common observation in hiPSC-CMs is their virtual lack of I_{K1} [2], which readily explains their depolarized MDP or RMP. In the present study, we increased the expression level of I_{K1} in our hiPSC-CMs by adding *in silico* I_{K1} using a dynamic patch clamp approach. We made perforated patch clamp recordings from a series of hiPSC-CMs at physiological temperature, systematically varying the magnitude of I_{K1} and assessing the effects of several variants of I_{K1}.

2. Methods

2.1. Cardiomyocytes derived from hiPSCs

Skin punch biopsies were taken from adult healthy volunteers, as approved by the ethics committee of the University Medical Center of the Georg-August-University of Göttingen, and hiPSCs were generated from primary fibroblasts derived from these skin biopsies. Cultures of hiPSCs were differentiated to cardiomyocytes and single hiPSC-CMs were obtained by enzymatic dissociation [3].

2.2. Electrophysiology

Action potential recordings were made through the amphotericin-B perforated patch clamp technique from single spontaneously active hiPSC-CMs with a membrane capacitance of 25 ± 4 pF (mean ± SEM, n = 9). All experiments were carried out at a temperature of 35–37°C [3].

In a separate series of experiments, the amplitude of the intrinsic I_{K1} of hiPSC-CMs was assessed using the voltage clamp mode of the whole cell patch clamp configuration. I_{K1} was recorded as the barium sensitive current at −100 mV, which was normalized for cell size through the membrane capacitance, which amounted to 33 ± 7 pF (mean ± SEM, n = 7) [3].

Dynamic clamp was used to supply hiPSC-CMs in current clamp mode with a controllable virtual I_{K1}. This computed I_{K1} was based on a fit to data from Kir2.1 channels expressed in HEK-293 cells by Dhamoon et al. [5]:

$$I_{K1} = A \times (V_m - E_K) / (1 + \exp(B \times (V_m + C))), \quad (1)$$

where the membrane potential V_m and potassium reversal potential E_K are in mV and I_{K1} is in pA/pF. The parameters A, B, and C amounted to 0.12979 (nS/pF), 0.093633 (mV^{-1}) and 72.0 (mV), respectively. With the E_K of −86.9 mV in our experimental setting, the outward I_{K1} current density then amounts to 1 pA/pF.

The thus computed I_{K1} was injected into a single hiPSC-CM through dynamic clamp. The current-voltage (I-V) relationship of the injected I_{K1} is illustrated in Figure 3 below.

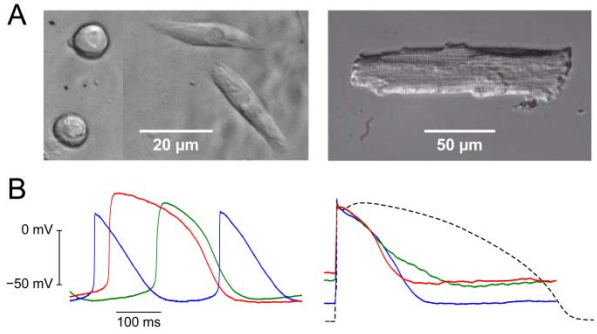

Figure 1. Morphological and electrophysiological phenotype of human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) and a typical native human ventricular myocyte (VM). (A) Phase-contrast micrographs of four hiPSC-CMs (left) and a typical human VM (right). Note different scale bars. (B) Action potentials of three different spontaneously active hiPSC-CMs (solid lines, left), three different intrinsically quiescent hiPSC-CMs upon 1 Hz stimulation (solid lines, right) and a typical VM isolated from a failing human heart upon 1 Hz stimulation (dashed line).

3. Results

3.1. Characteristics of hiPSC-CMs

Figure 1 illustrates the morphological and electrophysiological phenotype of our hiPSC-CMs in comparison with that of a typical native human ventricular myocyte (VM) [6]. Whereas native human VMs are elongated and show a clear and regular cross-striated pattern (Figure 1A, right), the hiPSC-CMs are relatively small and less well-organized (Figure 1A, left). In the same dish, their appearance can be circular, with a diameter of ≈10 µm, as well as somewhat elongated.

Action potentials of hiPSC-CMs may be widely different (Figure 1B). Some cells are visually beating and show pacemaker activity (Figure 1B, left), whereas others are intrinsically quiescent (Figure 1B, right). Their MDP or RMP is significantly depolarized compared to that of freshly isolated native VMs (Figure 1B, solid lines versus dashed line). Also, their $(dV/dt)_{max}$, APA, and APD at 90% repolarization (APD_{90}) are significantly smaller [3].

Because we hypothesized that the depolarization of our hiPSC-CMs was caused by a lack of I_{K1}, we carried out voltage clamp experiments to determine the amplitude of I_{K1}. In only 2 out of 7 cells studied, a detectable I_{K1} was present, with an amplitude of 0.36 ± 0.14 pA/pF at −100 mV (Figure 2, rightmost bar). As illustrated in Figure 2, the amplitude of the native I_{K1} in isolated mammalian VMs is much larger, although widely different values have been reported [7–22]. Another difference between studies on I_{K1} in mammalian VMs concerns the I-V relationship. In some studies, it is reported that I_{K1} approaches zero at potentials >−20 mV, as in Figure 3 below, whereas a substantial current is reported in others.

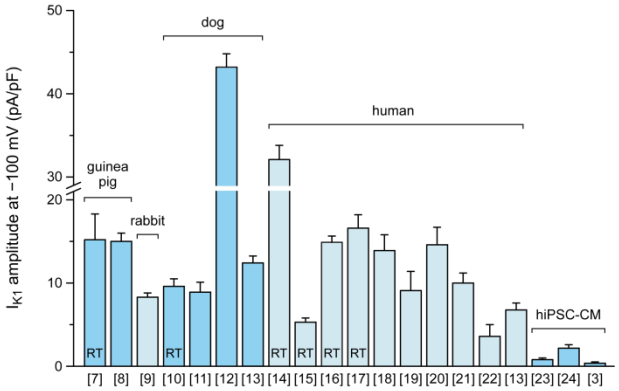

Figure 2. Amplitude of inward rectifier potassium current (I_{K1}) at a membrane potential of −100 mV in mammalian ventricular myocytes and human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs). Data are mean ± SEM obtained at room temperature (bars labeled 'RT') or at physiological or near-physiological temperature. Some data are estimated from graphs. Note axis break.

3.2. Injection of *in silico* I_{K1}

Because the native I_{K1} of our hiPSC-CMs appeared almost negligible in comparison with that of human VMs (Figure 2), we decided to supply our hiPSC-CMs with an 'I_{K1} boost' through dynamic clamp. To this end, we injected a current through the patch clamp pipette with the functional characteristics of the lacking I_{K1}, as diagrammed in Figure 3. The membrane potential V_m of the hiPSC-CM is continuously sampled and the V_m-dependent I_{K1} (Figure 3, bottom right) is computed and injected into the hiPSC-CM, together with any stimulus current. Both V_m and the injected current I_{in} are stored on the lab computer for offline analysis using custom software.

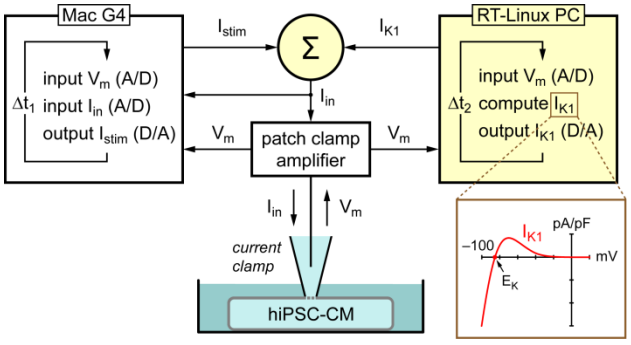

Figure 3. Experimental setup. The membrane potential (V_m) of a single human induced pluripotent stem cell derived cardiomyocyte (hiPSC-CM) is recorded using the perforated patch clamp technique in current clamp mode. The injected current (I_{in}) is the sum of a stimulus current (I_{stim}) and a virtual inward rectifier potassium current (I_{K1}), which is computed in real time, based on the recorded value of V_m ('dynamic clamp'). The stimulus protocol is run on an Apple Macintosh G4 lab computer (left), whereas a Real-Time Linux (RT-Linux) based PC is used for the continuous computation of I_{K1} (right). Sample rates are 4 and 25 kHz, respectively (Δt_1 = 0.25 ms and Δt_2 = 40 µs).

3.3. Changes in hiPSC-CM phenotype

We injected an *in silico* I_{K1} into nine different hiPSC-CMs and assessed the effects on the action potential. The amplitude of the injected I_{K1} was scaled according to its peak outward current density, which was set to values of 0 (no I_{K1} injected), 1, 2, 4, 6, 8, or 10 pA/pF. Figure 4A shows the native action potential of a hiPSC-CM stimulated at a frequency of 1 Hz (black trace) and its action potential upon injection of I_{K1} with a peak outward density of 1–10 pA/pF (other traces). The injected current is shown in Figure 4B.

As becomes apparent from Figure 4, A and B, and is further substantiated by Figure 4, C–E, the injection of a sufficiently large I_{K1} results in a stable RMP near −80 mV as well as a significant increase in both $(dV/dt)_{max}$ and APA. Furthermore, the action potential shows a more pronounced final repolarization phase (Figure 4A), which is accompanied by a negative trend in APD_{90} (Figure 4F).

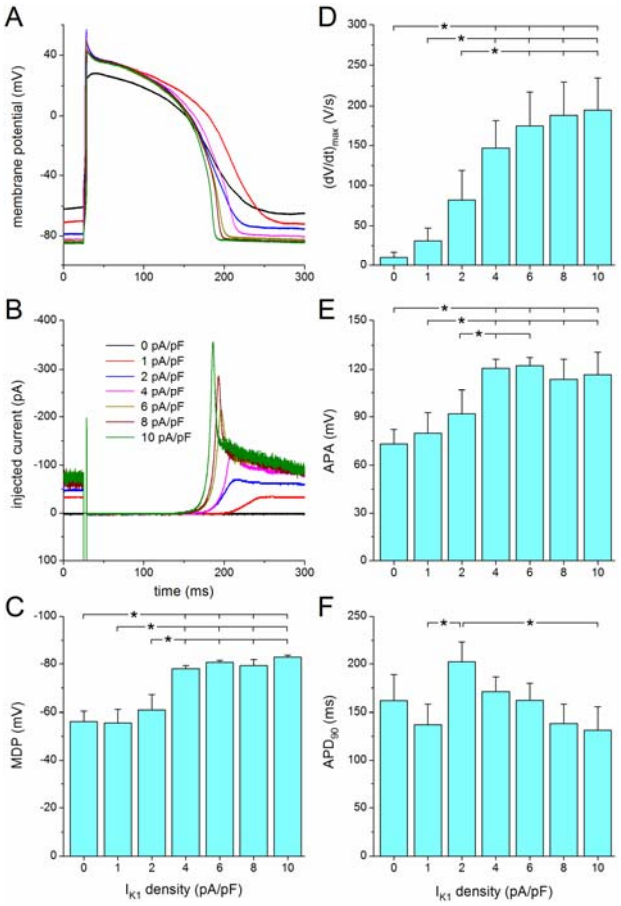

Figure 4. (A) Action potential of a single hiPSC-CM upon injection of simulated I_{K1}, with its peak outward amplitude scaled to 0–10 pA/pF, as indicated. (B) Corresponding dynamic clamp current injected into the cell. (C–F) Action potential parameters MDP, $(dV/dt)_{max}$, APA, and APD_{90} of 9 hiPSC-CMs at I_{K1} peak outward amplitudes of 0–10 pA/pF. **P*<0.05.

Similar experiments revealed proarrhythmic action potential changes upon real-time simulation of both loss-of-function and gain-of-function mutations in I_{K1}, as associated with Andersen-Tawil syndrome type 1 and short QT syndrome type 3, respectively [3].

4. Conclusion

We conclude that the injection of an *in silico* I_{K1} through dynamic clamp makes the hiPSC-CM a more

reliable model for investigating mechanisms underlying ventricular arrhythmias.

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Address for correspondence:

Ronald Wilders, PhD
Department of Anatomy, Embryology and Physiology
Academic Medical Center, University of Amsterdam
Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands
E-mail: r.wilders@amc.uva.nl