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

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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 ($\left(\frac{dV}{dt}\right)_{\text{max}}$), 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 $\left(\frac{dV}{dt}\right)_{\text{max}}$. 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 \pm 4 \text{ pF (mean} \pm \text{ 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 $−100 \text{ mV}$, which was normalized for cell size through the membrane capacitance, which amounted to $33 \pm 7 \text{ pF (mean} \pm \text{ SEM, n = 7)}$ [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]:

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

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 $−86.9 \text{ mV}$ 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-M through dynamic clamp. The current-voltage (I-V) relationship of the injected $I_{K1}$ is illustrated in Figure 3 below.

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 $≈10 \mu\text{m}$, 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 $(dV/dt)_{\text{max}}$, 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 \pm 0.14 \text{ pA/pF at } −100 \text{ 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 $>−20 \text{ mV},$ as in Figure 3 below, whereas a substantial current is reported in others.

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).

Figure 2. Amplitude of inward rectifier potassium current ($I_{K1}$) at a membrane potential of $−100 \text{ mV}$ in mammalian ventricular myocytes and human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs). Data are mean $\pm$ 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 IK1

Because the native IK1 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 ‘IK1 boost’ through dynamic clamp. To this end, we injected a current through the patch clamp pipette with the functional characteristics of the lacking IK1, as diagrammed in Figure 3. The membrane potential Vm of the hiPSC-CM is continuously sampled and the Vm-dependent IK1 (Figure 3, bottom right) is computed and injected into the hiPSC-CM, together with any stimulus current. Both Vm and the injected current Im are stored on the lab computer for offline analysis using custom software.

Figure 3. Experimental setup. The membrane potential (Vm) 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 (Im) is the sum of a stimulus current (Istim) and a virtual inward rectifier potassium current (IK1), which is computed in real time, based on the recorded value of Vm ('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 IK1 (right). Sample rates are 4 and 25 kHz, respectively (∆t1 = 0.25 ms and ∆t2 = 40 µs).

3.3. Changes in hiPSC-CM phenotype

We injected an in silico IK1 into nine different hiPSC-CMs and assessed the effects on the action potential. The amplitude of the injected IK1 was scaled according to its peak outward current density, which was set to values of 0 (no IK1 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 IK1 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 IK1 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 APD90 (Figure 4F).

Figure 4. (A) Action potential of a single hiPSC-CM upon injection of simulated IK1, 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 APD90 of 9 hiPSC-CMs at IK1 peak outward amplitudes of 0–10 pA/pF. *P<0.05.

4. Conclusion

We conclude that the injection of an in silico IK1 through dynamic clamp makes the hiPSC-CM a more...
reliable model for investigating mechanisms underlying ventricular arrhythmias.

References


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