The late sodium current participates in repolarization of hiPSC-derived cardiac myocytes.

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INTRODUCTION

The voltage-gated sodium channel Nav1.5 is highly expressed in cardiac myocytes. Depolarization causes these channels to open briefly, allowing a large entry of Na+ ions that peaks within ~2.3 ms and further depolarizes the cell to generate the upstroke of the cardiac action potential (phase 0). After opening, most channels quickly inactivate to prevent further movement of Na+ and remain inactivated throughout the duration of the action potential. However, some channels continue to conduct, or even reactivate, at relatively positive membrane potentials during the plateau and repolarization phase (Pourrier et al. 2014). Na channel late openings allow influx of Na+ that creates a small, "late" current [late I Na or I NaL] that persists throughout the action potential plateau and repolarization. In a variety of pathophysiological settings (inherited and acquired), the number of Na channel late channel openings and thus the amplitude of the late I NaL is significantly increased, resulting in slowed repolarization and prolonged action potential duration (Zaza et al. 2008). Under these conditions, prolonged action potential duration can lead to afterdepolarizations and triggered activity. In physiological conditions, block of I NaL can have a protective effect by countering the effect of I NaL inhibition (Orth et al. 2006). As a result, drugs, inhibiting both I Na and I NaL are not considered pro-arrhythmic. The importance of testing the effects of compounds beyond NEBS is widely recognized and I NaL is now accepted as part of the cardiac ion current panel for drug testing. More recently, it has been proposed to use human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to assess the pro-arrhythmic liability of novel compounds in safety studies. In this regard, it is important to demonstrate that all the ionic currents contributing to the human action potential are present in the model. It is unclear whether or not I NaL is present in commercially available hiPSC-CMs. Thus, the goal of this study is to record I NaL, from hiPSC-CMs, to determine its pharmacological properties as well as its importance in shaping the action potential morphology.

METHODS

Late I NaL recording: Cor-A® cardiomyocytes were thawed and placed in a single well of a 6 well plate precoated with 0.1% gelatin. Cells were maintained for ~10 days before being trypsinized. Single cells were added on glass coverslips precoated with 0.2% gelatin and maintained for about a week. I NaL was recorded from single cells using a step/ramp voltage protocol (See Figure 1). Cells were superfused in the control bath solution containing (in mM): 135 NaCl, 5 ClO4, 2.8 Na acetate, 10 HEPEs, 10 glucose, 1 MgCl2, 1 CaCl2, pH 7.4 with 95% N2/5% CO2. Electrophysiological traces were recorded using 2.5 V/s when filling with 75-75 um/gl granidric. Permeabilization of the patch and access to the internal milieu of the heart cell typically required less than 15 minutes exposure for good access. Drug containing solution was made from the external bath solution by serial dilution of a 100% OSMO stock to the final concentrations shown for AXXtII.

HYPOTHESIS

We tested the hypothesis that:

I NaL is present in Cor-A® cardiomyocytes.

- Late I NaL was recorded from Na channel inhibitors such as TTX, ranolazine and flecainide.
- Enhancement of late I NaL by ATXII results in prolongation of the action potential recorded from Cor-A® cells.

RESULTS

Figure 1: Recording of TTX-sensitive I NaL from Cor-A® cardiomyocytes. Tip zero currents were subjected to the step/ramp voltage protocol shown on top of the current records in order to uncover the late sodium current (time-equivalent) active during depolarization. Current was recorded in the steady state in control (black) and followed by additions of 300 nM TTX (red) and 300 nM TTX + 3 uM TTX (green). TTX blocked the current (red trace) and the late I NaL was obtained by subtracting the currents recorded in the presence of TTX from those in control. The initial peak sodium current arising from the step depolarization is not affected. At high gain during the initial phase of the protocol, a sustained current was present. The current was blocked back to 0.1% only a late inward current (I NaL) developed. (Figure adapted from M. Castrillon, J. Luerman, D. Fedida, P. Belardinelli, unpublished).

Figure 2: Concentration-dependent effects of TTX on I NaL recorded from Cor-A® cells. Cells were subjected to the same step/ramp voltage protocol shown in Figure 1. Current was recorded in the steady state in control (black) and followed by wash-out of I NaL currents with 300 nM TTX. The late I NaL was obtained by subtracting the I NaL recorded in control from I NaL recorded in the presence of TTX. Control traces shown here result from the averaged 20 ± 5 traces.

Figure 3: Concentration-dependent effects of ATXII on I NaL recorded from Cor-A® cells. Cells were subjected to the same step/ramp voltage protocol shown in Figure 2. Current was recorded in the steady state in control (black) followed by wash-out of ATXII. The late I NaL was obtained by subtracting the I NaL recorded in control from I NaL recorded in the presence of ATXII. Control traces shown here result from the averaged 20 ± 5 traces.

Figure 4: Concentration-dependent effects of ATXII on action potential repolarization. Current was recorded in the steady state in control (black) followed by wash-out of ATXII. I NaL was obtained by subtracting the I NaL recorded in control from I NaL recorded in the presence of ATXII. The late I NaL was obtained by subtracting the I NaL recorded in control from I NaL recorded in the presence of ATXII. The half maximal effect of ATXII on I NaL was obtained from the Hill equation. (Hill equation): Y = 100 * (I/I0), where I is the fractional change in I NaL, I0 is the control I NaL, Y is the response. Hill coefficient (K) was 1.0 ± 0.8 (n=4-5 cells). Table 1 summarizes data obtained from action potential repolarization in the presence of AXXtII (± vs. control).

Table 1: Summary of data obtained from action potential repolarization in the presence of AXXtII (± vs. control).

CONCLUSION

These data demonstrate that late I NaL is present in human induced pluripotent stem cell-derived cardiomyocytes (Cor-A® cells). Similar to ATXII-enhanced I NaL currents carried by Nav1.5 channels, ATXII-enhanced I NaL recorded from hiPSC-CM is inhibited by micromolar concentrations of sodium channel blockers such as TTX, ranolazine and flecainide. As expected, ATXII-enhanced I NaL results in prolongation of the action potential, indicating that I NaL is likely contributing to arrhythmia in human. Both in vitro and in vivo, Electrophysiological traces of 2.5 V/s when filling with control only show the balancing effect of inhibiting both inward (INa) and outward (IK1) currents. This work, in addition to others, confirms the value of using hiPSC-CM to characterize the electrophysiological properties of test compounds and assess their potential pro-arrhythmic liability.

REFERENCES

