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 phases (Pourrier et al, 2014). Na channel late openings allow influx of Na^+ that creates a small, "late current" (late I_{Na} or $I_{Na,L}$) that persists throughout the action potential plateau and repolarization. In a variety of pathophysiological settings (inherited and acquired), the number of Na^+ channel late openings and thus the amplitude of $I_{Na,L}$ 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 after depolarizations and triggered activity. In physiologic conditions, block of $I_{Na,L}$ can have a protective effect by counteracting the effect of I_{hERG} inhibition (Orth et al, 2006). As a result, drugs inhibiting both $I_{Na,L}$ and I_{hERG} are not considered pro-arrhythmic. The importance of testing the effects of compounds beyond hERG is now widely recognized. And $I_{Na,L}$ is now accepted as part of the cardiac ionic current panel for drug testing. More recently, it has been proposed to use human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) 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 this model. At present, it is unclear whether or not $I_{Na,L}$ is present in commercially available hiPSC-CM. Thus, the goal of this study is to record $I_{Na,L}$ from Cor.4U® cells (Axiogenesis AG), to determine its pharmacologic properties as well as its importance in shaping the action potential morphology.

METHODS

Late I_{Na} recording: Cor.4U® 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. $I_{Na,L}$ 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 CsCl, 2.8 Na acetate, 10 HEPES, 10 Glucose, 1 MgCl2, 1 $CaCl_2$, pH7.4 with NaOH, 20 uM nifedipine. Electrodes had resistances of 2-3.5 MΩ when filled with control filling solution, in mM: 120 CsF, 20 CsCl, 1 Na2ATP, 5 HEPES, 10 TEA-Cl, 10 EGTA, pH was adjusted to 7.3 with CsOH. Whole cell current recording and analysis were made using Axopatch 200B amplifier and pClamp10 software.

Action potential study, Cor.4U® cardiomyocytes were thawed and placed on glass coverslips precoated with 0.1% gelatin; This was defined as culture day one. After 6 to 17 days in culture, the cardiac cells adhering to the coverslips were placed in a Warner perfusion chamber and maintained at 34.6°C by use of an in-line heater (AutoMate Scientific) with a perfusion rate of 1.2ml/min. The external perfusate solution was composed of (in mM): 150 NaCL, 5.4 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 15 glucose, 15 HEPES, 1 Na-pyruvate. pH was adjusted to 7.4 with NaOH. The pipette solution contained (in mM): 150 KCl, 5 NaCl, 2 $CaCl_2$, 5 $MgCl_2$, 5 EGTA, 10 HEPES, pH was adjusted to 7.2 with KOH. The tip of the recording pipette was filled with normal internal solution. The rest of the electrode was backfilled with internal solution containing 25-75 ug/ml gramicidin. 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% DMSO stock to the final concentrations shown for ATXII.

HYPOTHESIS

We tested the hypothesis that:

- $I_{Na,L}$ is present in Cor.4U® cardiomyocytes.
- $I_{Na,L}$ recorded from Cor.4U® cells is inhibited by Na channel inhibitors such as TTX, ranolazine and flecainide.
- Enhancement of late I_{Na} by ATXII results in prolongation of the action potential recorded from Cor.4U® cells.

RESULTS

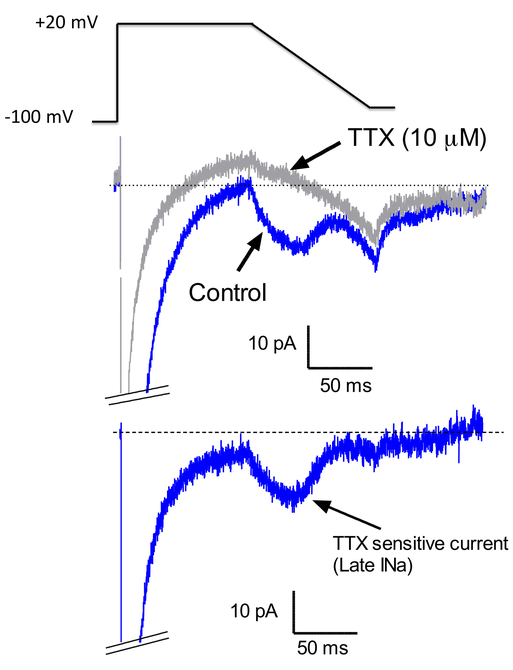


Figure 1: Recording of TTX-sensitive $I_{Na,L}$ from Cor.4U® cardiomyocytes. Top panel: Cells were subjected to the step/ramp voltage protocol shown on top of the current records in order to uncover the late sodium current (non-equilibrium) active during repolarization. Current was recorded in the steady-state in control (pre-drug) followed by wash in of 10 uM TTX. Bottom panel: The TTX-sensitive portion of the current trace 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 cut off. At high gain during the plateau phase of the protocol, a sustained current was present. As the voltage was ramped back to -100 mV, a late inward current ($I_{Na,L}$) developed.

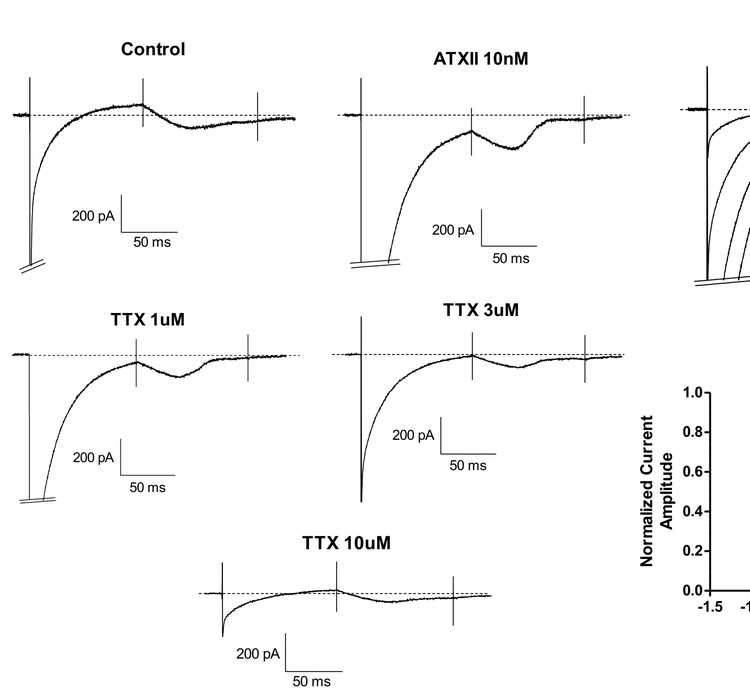


Figure 2: Concentration-dependent effects of TTX on $I_{Na,L}$ recorded from Cor.4U® cells: Cells were subjected to the same step/ramp voltage protocol shown in Figure 1. Current was recorded in the steady-state in control (pre-drug) followed by wash in of 10 nM ATXII to specifically enhance late I_{Na}. Escalating concentrations of TTX (1 to 10 uM in this example) were then added to the superfusate. Current traces shown here result from the average of 10 to 15 traces.

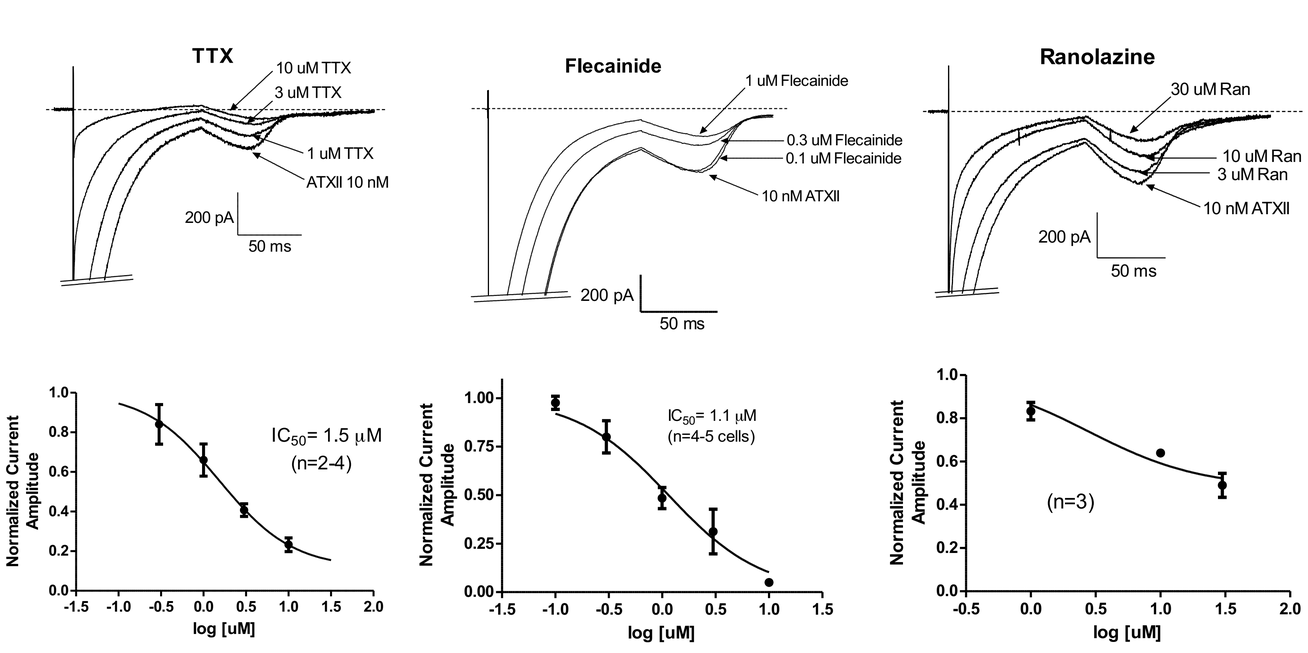
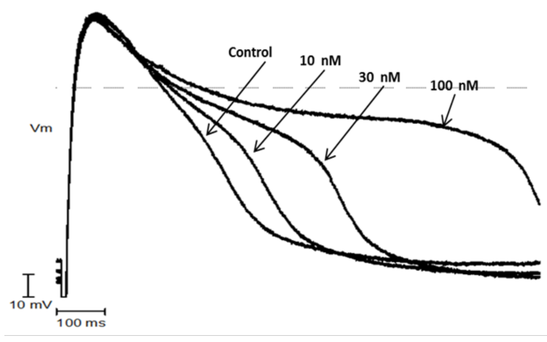


Figure 3: Concentration-dependent effects of TTX, flecainide and ranolazine on $I_{Na,L}$ recorded from Cor.4U® cells: Cells were subjected to the same step/ramp voltage protocol shown in Figure 1. Current was recorded in the steady-state in control (pre-drug) followed by wash in of 10 nM ATXII to specifically enhance $I_{Na,L}$. Escalating concentrations of TTX, flecainide or ranolazine were then added to the superfusate. An overlap of current traces is shown to better visualize the concentration dependent effect of each agent on $I_{Na,L}$ Bottom panels: Dose-response relationships. Data are represented as mean ± SEM. The IC_{50} was obtained from the Hill equation: $f = 1/(1+ (IC_{50}/[D]^n))$, where f is the fractional current $f = I_{drug}/I_{control}$) at drug concentration [D].



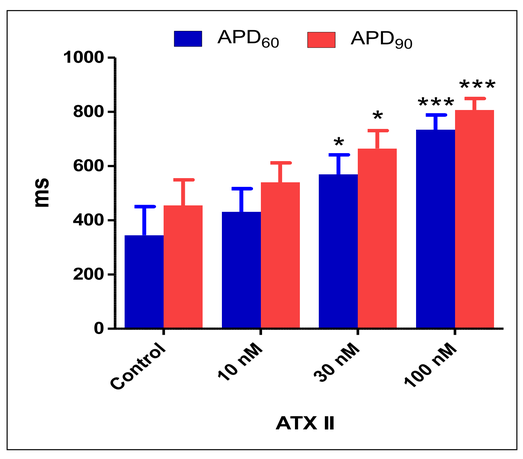


Figure 4 : Concentration-dependent effects of ATXII on action potential duration. Left panel: Cor.4U® cardiomyocytes were paced at 1Hz during a four minute baseline period, as well as during exposure to increasing concentrations of ATXII which was administered at 4 minute intervals. AP parameters, including duration (APD_{60} & APD_{90}), resting membrane potential, action potential rate of rise (Vmax), and action potential amplitude, were routinely measured throughout the entire experiment. In this example, ATXII prolonged the action potential in a concentration dependent manner. Right panel: APD_{60} and APD_{90} data are presented as mean ± standard deviation. Statistical significance was determined with the one way repeated measures (ANOVA), followed by Dunnett's Multiple Comparison test. *p<0.05, ***p<0.001 vs. control.

ATXII (n=4)	Control	10 nM	30 nM	100 nM
RMP (mV)	-79 ± 3	-80 ± 5	-82 ± 6	-77 ± 6
APD_{60} (msec)	345 ± 106	431 ± 86	569 ± 73*	918 ± 165***
APD_{60} (% baseline)	0	29 ± 19	73 ± 38	179 ± 76
APD_{90} (msec)	455 ± 94	540 ± 72	665 ± 66*	938 ± 123***
APD_{90} (% baseline)	0	20 ± 11	49 ± 19	110 ± 31
Rise-rate (V/s)	42 ± 10	36 ± 2	49 ± 22	52 ± 27
Peak (mV)	103 ± 9	104 ± 10	103 ± 9	107 ± 5

Table 1: Summary of data obtained from action potential recordings in the presence of ATXII. *p≤0.05 vs. control, ***p≤0.001 vs. control.
Data are expressed as mean ± standard deviation (X ± SD). Tissue bath temperature was 34.5 ± 0.1 degrees Centigrade.
RMP: Resting membrane potential prior to stimulation; APD_{60}: Action potential duration at 60% repolarization; APD_{90}: Action potential duration at 90% repolarization; Rate of rise: Rate of rise of the action potential when stimulated; AP peak: maximum amplitude of action potential from threshold.

CONCLUSION

These data demonstrate that late I_{Na} is present in human induced pluripotent stem cell-derived cardiomyocytes (Cor.4U® cells). Similar to ATXII-enhanced $I_{Na,L}$ currents carried by Nav1.5 channels, ATXII-enhanced $I_{Na,L}$ recorded from hiPSC-CM is inhibited by micromolar concentrations of sodium channel inhibitors such as TTX, ranolazine and flecainide. As expected, ATXII-Enhanced $I_{Na,L}$ results in prolongation of the action potential, indicating that $I_{Na,L}$ contributes to action potential repolarization in these cells. It is predicted that mixed ion channel inhibitors (e.g. mixed Na and K channel inhibitors) do have limited APD prolonging potential due to the balancing effect of inhibiting both inward (Na^+) and outward (K^+) currents. Thus 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.

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