Late Sustained Sodium Current (INa,L) in Adult Human Primary Cardiomyocytes

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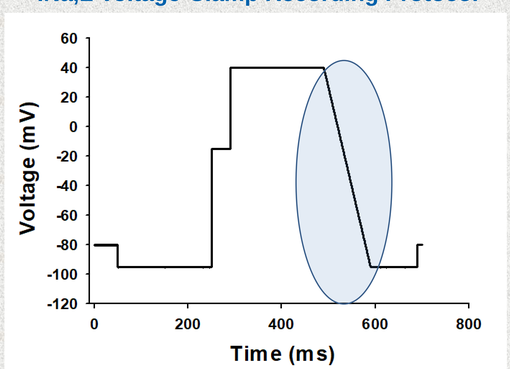
Introduction

The late sustained sodium current (INa,L), a depolarizing current that persists throughout the action potential (AP) plateau, contributes to the AP duration and maintains the intracellular homeostasis of Na^+. Increase or inhibition of INa,L is often associated with arrhythmogenicity or mitigation of pro-arrhythmia risk, respectively. Since INa,L was one of the selected channels for the CiPA (Comprehensive In Vitro Pro-arrhythmia Assay) initiative and drugs that block the hERG channel and inhibit INa,L are not associated with pro-arrhythmia in humans, identifying the effect of compounds on INa,L in human cardiomyocytes during preclinical development can aid in the determination of pro-arrhythmia risk for novel drugs.

Methods

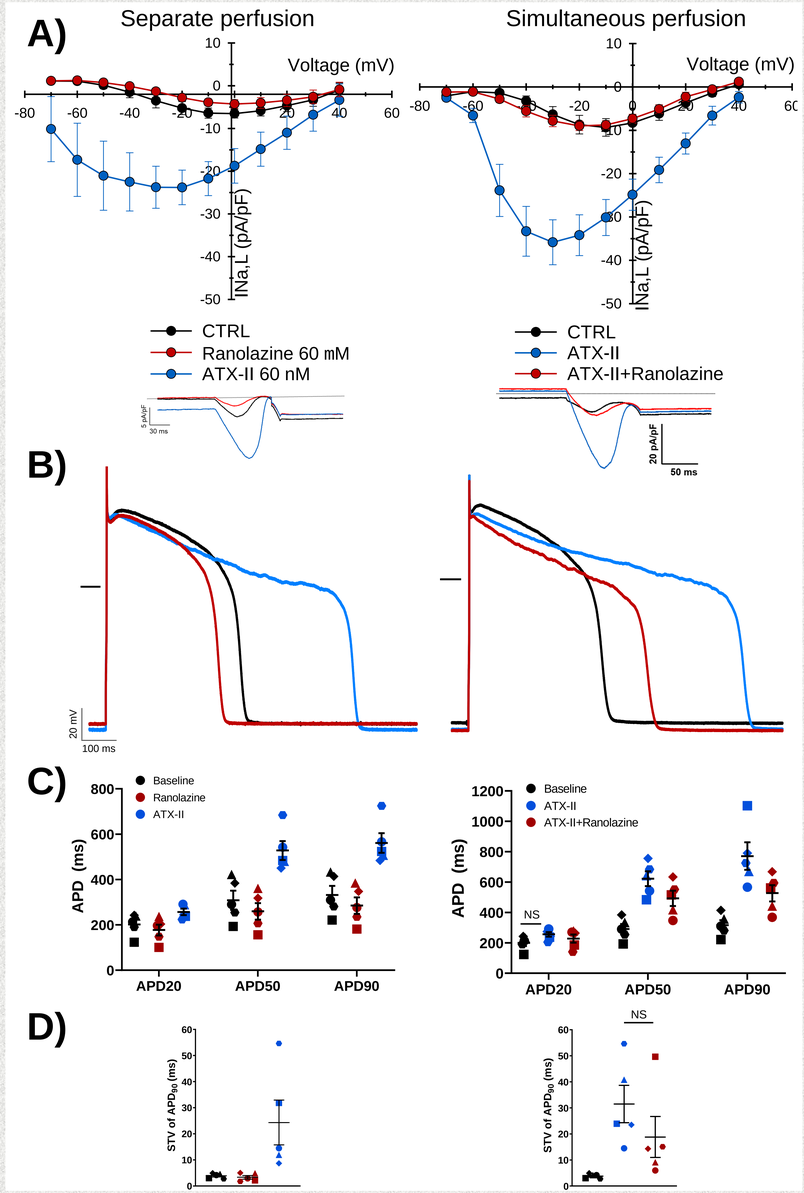
In order to better understand the properties of INa,L, we performed voltage-clamp and current-clamp recordings at physiological temperature (37°C), in whole-cell patch-clamp experiments using adult human primary ventricular cardiomyocytes isolated from ethically consented donor hearts. INa,L enhancer (ATX-II) and inhibitors (Ranolazine and GS967) were applied until a steady-state effect was achieved.

INa,L Voltage-Clamp Recording Protocol



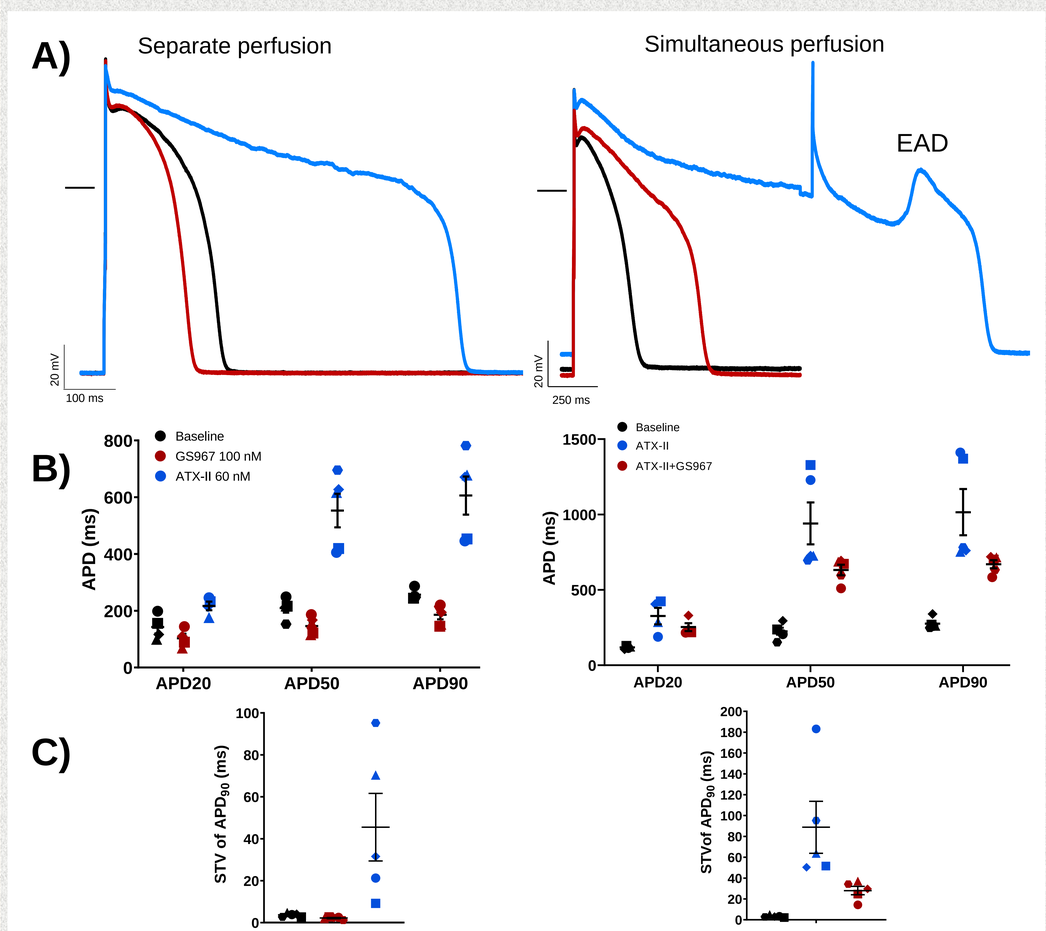
INa,L protocol was elicited every 10 s from a holding potential of -80 mV. INa,L current was measured during the step-ramp, which goes from 40 mV to -95 mV over a duration of 100 ms.

Ranolazine Inhibits the ATX-II Stimulation of INa,L



A. Representative recordings of INa,L and current-voltage relationships (n=6 cells/group). **B.** Representative AP recordings **C.** AP duration (APD) at 20%, 50% and 90% repolarization (n=6 cells/group). **D.** Short term variability (STV) of APD_{90}. Ranolazine (60 µM Voltage-clamp; 10 µM Current-clamp) and ATX-II (60 nM) were independently perfused (left) or simultaneously perfused (right). Data are statistically different p<0.05, unless specified otherwise (NS) when effects are compared to Baseline in the separate perfusion group and when compared to ATX-II in the simultaneous perfusion group. Solid black line: 0 mV level.

GS967 Blocks the ATX-II Stimulation of INa,L



A. Representative AP recordings **B.** AP duration (APD) at 20%, 50% and 90% repolarization (n=5 cells/group). **C.** Short term variability (STV) of APD_{90}. GS967 (300 nM) and ATX-II (60 nM) were independently perfused (left) or simultaneously perfused (right). Data are statistically different p<0.05 when effects are compared to Baseline in the separate perfusion group and when compared to ATX-II in the simultaneous perfusion group. Solid black line: 0 mV level. EAD: Early afterdepolarization.

Summary

1. Adult human primary ventricular cardiomyocytes express functional INa,L and can differentiate facilitators (ATX-II) from inhibitors (Ranolazine and GS967).
2. ATX-II prolongs APD, causes STV and induces EAD, a marker of pro-arrhythmia. Meanwhile, Ranolazine and GS967 reduce APD and reverse ATX-II-induced effects.
3. Ranolazine and GS967 reduce ATX-II-induced incidence of aftercontraction and contraction failure, makers of abnormal relaxation and pro-arrhythmia, in contractility experiments (data not shown).
4. Human cardiomyocytes provide valuable assessment of novel INa,L modulators to prevent the occurrence of pro-arrhythmia or development of arhythmia.