Chronic Treatment with PI3K Inhibitors Augments Late Cardiac Sodium Current Underlying QT-Prolonging Liability

An Acting Role for Akt

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Background

- An excessive increase in cardiac late sodium current (I_{NaL}) during the plateau phase of the ventricular action potential (AP) contributes to QT prolongation and can promote life-threatening Torsades de Pointes (TdP) arrhythmias (Fig. 1).
- Accumulating evidence indicates a role for pharmacological I_{NaL} augmentation in proarrhythmic drug-induced QT-interval lengthening, suggesting a need for additional preclinical drug-safety screening for the presence of excessive I_{NaL}.
- Chronic inhibition of the phosphoinositide 3-kinase (PI3K) signaling pathway by multiple drugs has been recognized to affect multiple ion currents including I_{NaL}. However, the exact downstream effectors remain unclear. Here, we addressed the hypothesis that the chronic inhibition of downstream Akt protein underlies I_{NaL} augmentation.

Figure 1: The role of I_{NaL} in arrhythmia

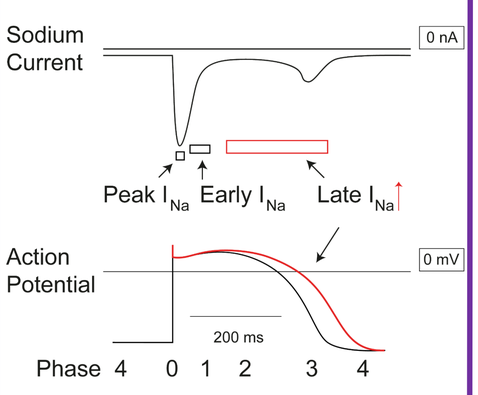
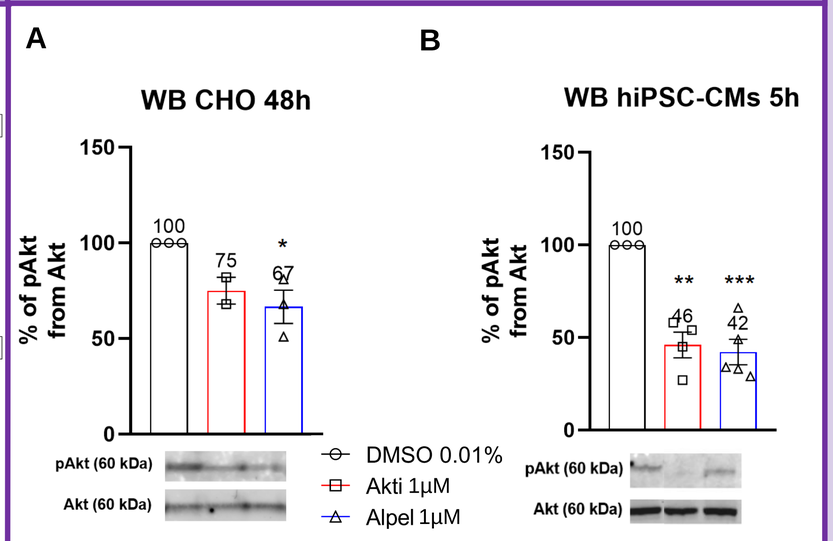


Figure 2: Confirmation of drug-induced PI3K/Akt inhibition



Methods

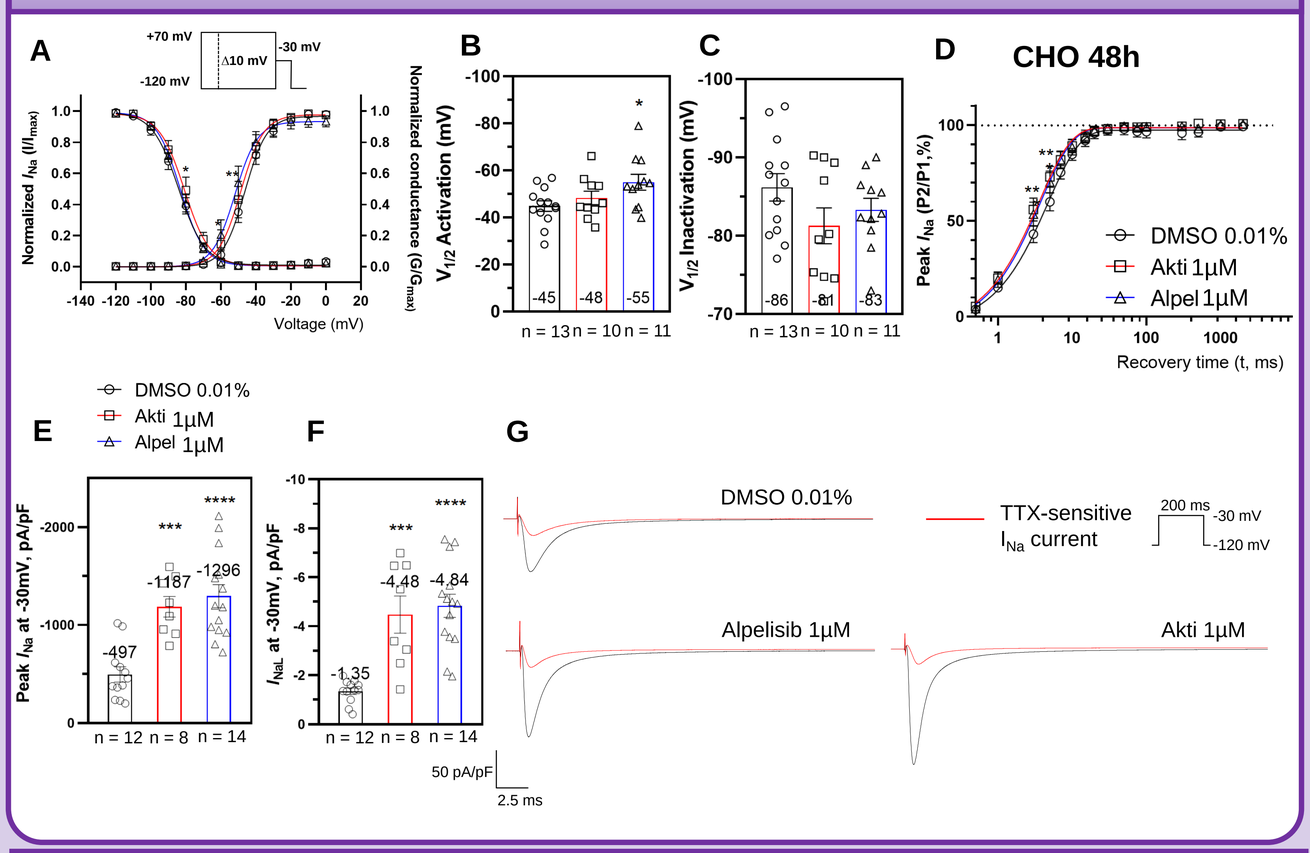
- Western Blotting analysis was used to confirm the inhibition of PI3K/Akt axis, by quantification of the amount of phosporylated (active) Akt (pAkt Ser473).
- Membrane currents were measured using the whole-cell patch-clamp technique in a heterologous expression model (Chinese Hamster Ovary [CHO] cells) transiently transfected with wild-type SCN5A-GFP-fused plasmid and in human induced pluripotent stem-cell derived cardiomyocytes (hiPSC-CMs[1]).
- I_{Na} and tetrodotoxin (TTX)-sensitive I_{NaL} currents were measured at room temperature. In hIPSC-CM 1µM nifedipine was used to block L-type Ca^{2+} channels.
- Cells were incubated for 5 or 48 hours with drugs directly inhibiting PI3Kα (alpelisib 1µM) or Akt (Akti 1µM) for mechanistic investigations of PI3K signaling.

[1] *Ncytes®, Ncardia, Leiden (NL)*

Results

- Chronic inhibition (>5 hours) of either PI3K or Akt results in decreased PI3K pathway activity in CHO (Fig. 2A) and hiPSC-CMs (Fig. 2B).
- In CHO cells, chronic PI3K inhibition leads to negative shift in the voltage dependence of steady-state activation (Fig. 3A, B), Akt inhibition shows a similar yet non-significant trend (Fig. 3A, B). Both drugs accelerate channel kinetics, shortening the NaV1.5 channel recovery from inactivation (Fig. 3D).
- Inhibition of PI3K and downstream Akt promotes increased peak I_{Na} in both CHO (Fig. 3E, G) and hiPSC-CMs (Fig. 4A, C).
- Chronic PI3K/Akt inhibition induces increased total I_{NaL} in both cell types (Fig. 3F, Fig. 4B).
- The effect of Akt inhibition on peak and late I_{Na} is prevented by intrapipette addition of phosphatidylinositol (3,4,5)-trisphosphate (PIP3, Fig. 4A, B).

Figure 3: Effect of chronic Akt inhibition on NaV1.5 channel in CHO

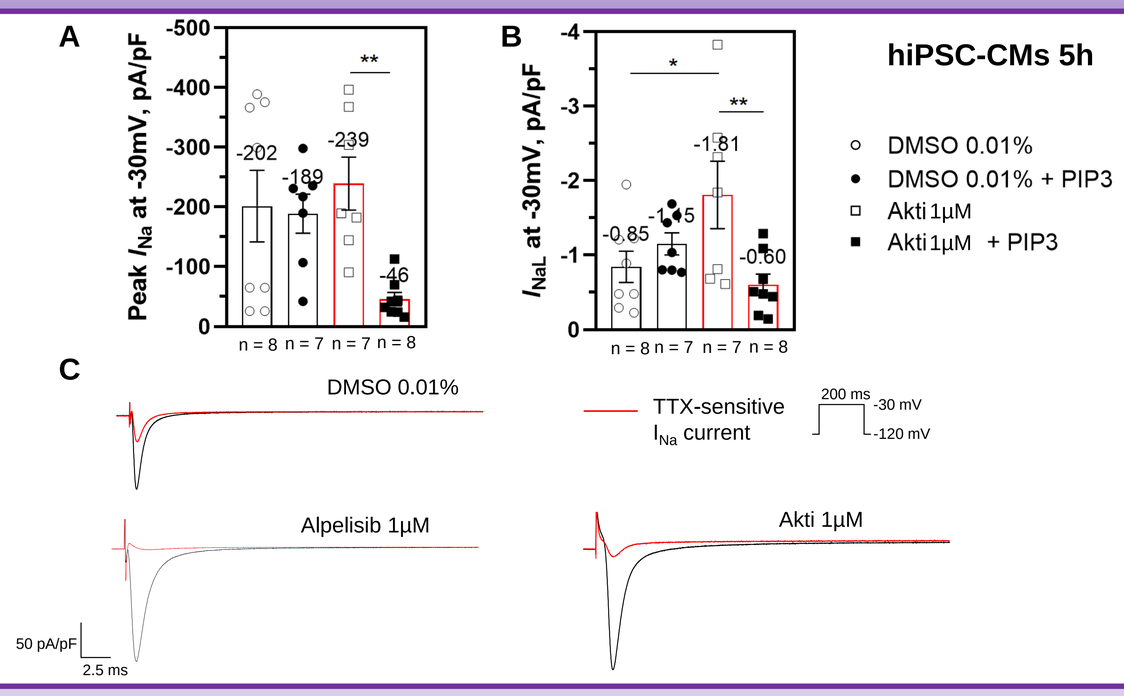


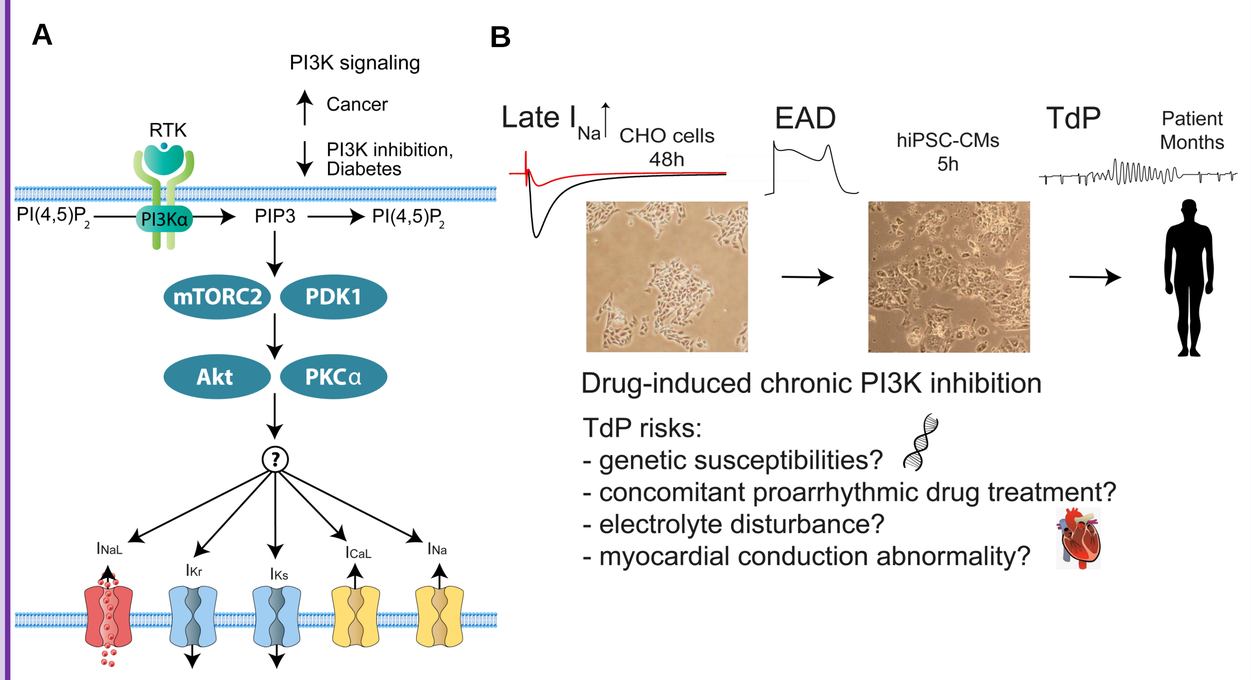
Conclusions

- Inhibition of PI3K/Akt signalling (Fig. 5A) regulates ion-channel function, particularly of NaV1.5 already after 5 hours of exposure, leading to changes in kinetics and amplitude of peak and late I_{Na}.
- One significant change is the proarrhythmic increase in I_{NaL}, prolonging the repolarization phase of the AP. This can potentially lead to early afterdepolarizations (EAD; Fig. 5B), which may degenerate into TdP arrhythmia in the presence of concomitant cardiovascular risks and morbidities in patients.
- This I_{NaL} increase is mediated by PI3K signaling. Moreover, our work indicates that Akt (Fig. 5A) is the main downstream effector of peak and late I_{Na} regulation. Thus, modulation of PI3K/Akt signalling should be considered in drug-incuded proarrhythmia screening.

Figure 5: Conceptual overview of the potential role of PI3K/Akt inhibition in cardiac arrhythmogenesis

Figure 4: Confirmation of I_{NaL} augmentation induced by Akt inhibition in hiPSC-CMs





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