Long-Term Amiodarone Exposure Augments I_{NaL} via PI3K/Akt Inhibition and Remodels I_{Kr}

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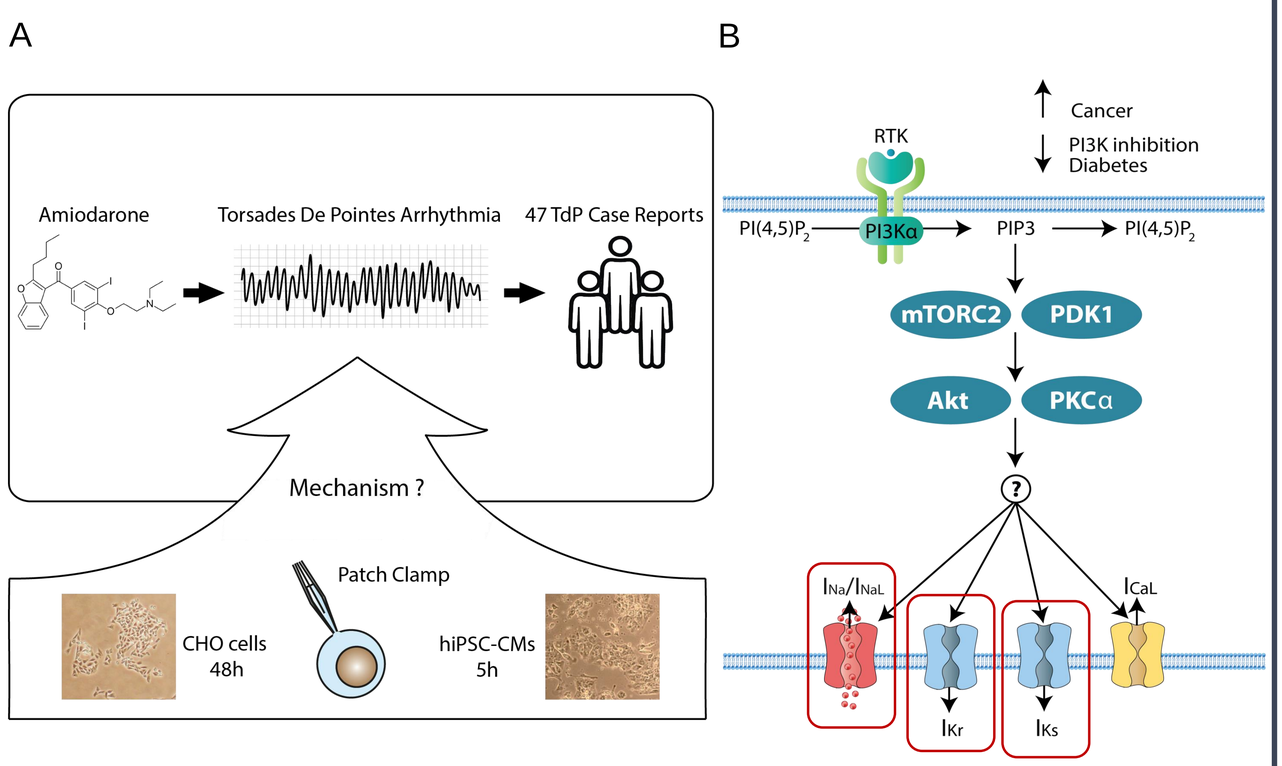
Background

- Amiodarone is an effective antiarrhythmic drug (AAD) widely used in clinics, yet with a tendency for drug-induced proarrhythmia in susceptible individuals. Differential acute and long-term electrophysiological effects of amiodarone remain incompletely understood and may affect proarrhythmia liability.
- Hence, we studied temporal effects of amiodarone on multiple cardiac ion channels. As a potential mechanism for the distinct acute and long-term effects, we proposed inhibition of the PI3K pathway, which is recognized to affect several cardiac ion currents, including late sodium current (I_{NaL}), rapid delayed-rectifier potassium current (I_{Kr}) and slow delayed-rectifier potassium current (I_{Ks}; Fig. 1B).

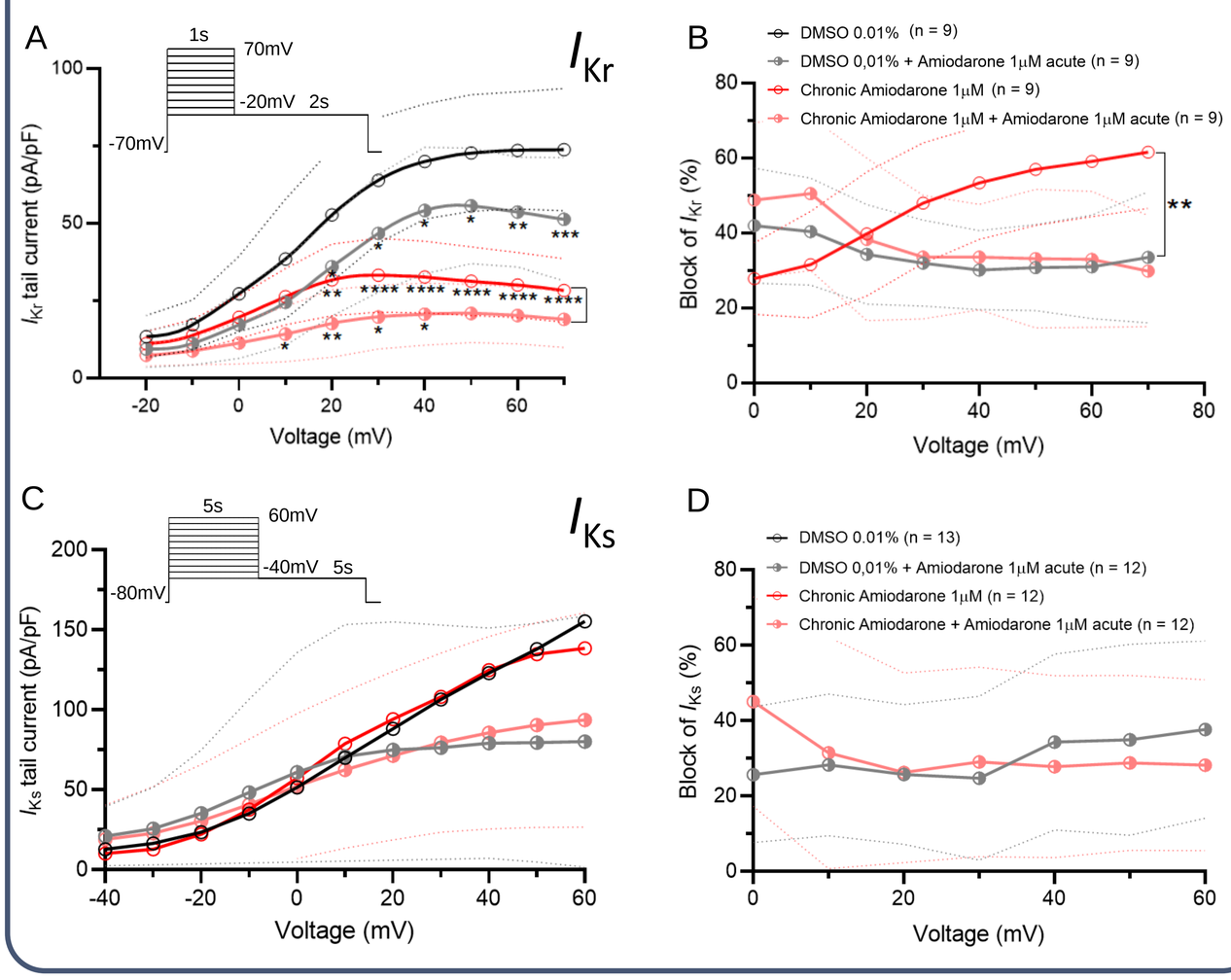
Figure 1: Differential long-term effects of amiodarone on ion channels can be mediated via PI3K signaling



Methods

- Membrane currents were measured using the whole-cell patch-clamp technique in Chinese Hamster Ovary (CHO) cells transiently transfected with GFP plasmids carrying: wild-type SCN5A; KCNH2; or KCNQ1/ KCNE1 / Yotiao, for I_{Na}, I_{Kr} and I_{Ks} measurements, respectively. Effects of amiodarone on I_{Na} were confirmed in human induced pluripotent stem-cell derived cardiomyocytes (hiPSC-CMs).
- I_{Kr}, I_{Ks}, peak I_{Na}, and tetrodotoxin (TTX)-sensitive I_{NaL} were measured at room temperature. Cells were incubated with different AADs or Akt inhibitor (Akti 1 μM) for 5 hours (hiPSC-CMs) or 48 hours (CHO) at 37°C. Phosphatidylinositol-3,4,5-triphosphate (PIP3) 1 μM was added intrapipette during I_{Na} experiments.

Figure 2: Acute and chronic effects of amiodarone on main repolarizing currents



Results

- In CHO cells, acute amiodarone treatment resulted in ~30% I_{Kr} channel block whether combined with control or chronic treatment (Fig. 2A, B).
- Interestingly, long-term amiodarone exposure (48 h) reduced I_{Kr} even upon drug wash out resulting in a 30%-60% current reduction (Fig. 2A, B).
- In contrast to I_{Kr}, I_{Ks} recordings showed only acute block of ~30% by amiodarone, without any chronic effect (Fig. 2C, D).
- Long-term treatment of CHO cells with dofetilide, known to augment I_{NaL} in a PI3K-dependent manner, with Akt inhibitor and with amiodarone, augmented both peak I_{Na} (Fig. 3A, C) and I_{NaL} (Fig. 3B, D) currents. This increase was abolished by the pathway activation with PIP3 (Fig. 1B; Fig. 3C,D).
- Dronedarone had no significant effect on peak I_{Na} or I_{NaL} and showed no significant changes in the presence of PIP3.
- In hiPSC-CMs, augmentation of I_{Na} and I_{NaL} currents by amiodarone was confirmed after 5 hours of exposure (Fig. 4A-D).

Figure 3: Chronic amiodarone unlike dronedarone augments I_{NaL} via PI3K/Akt

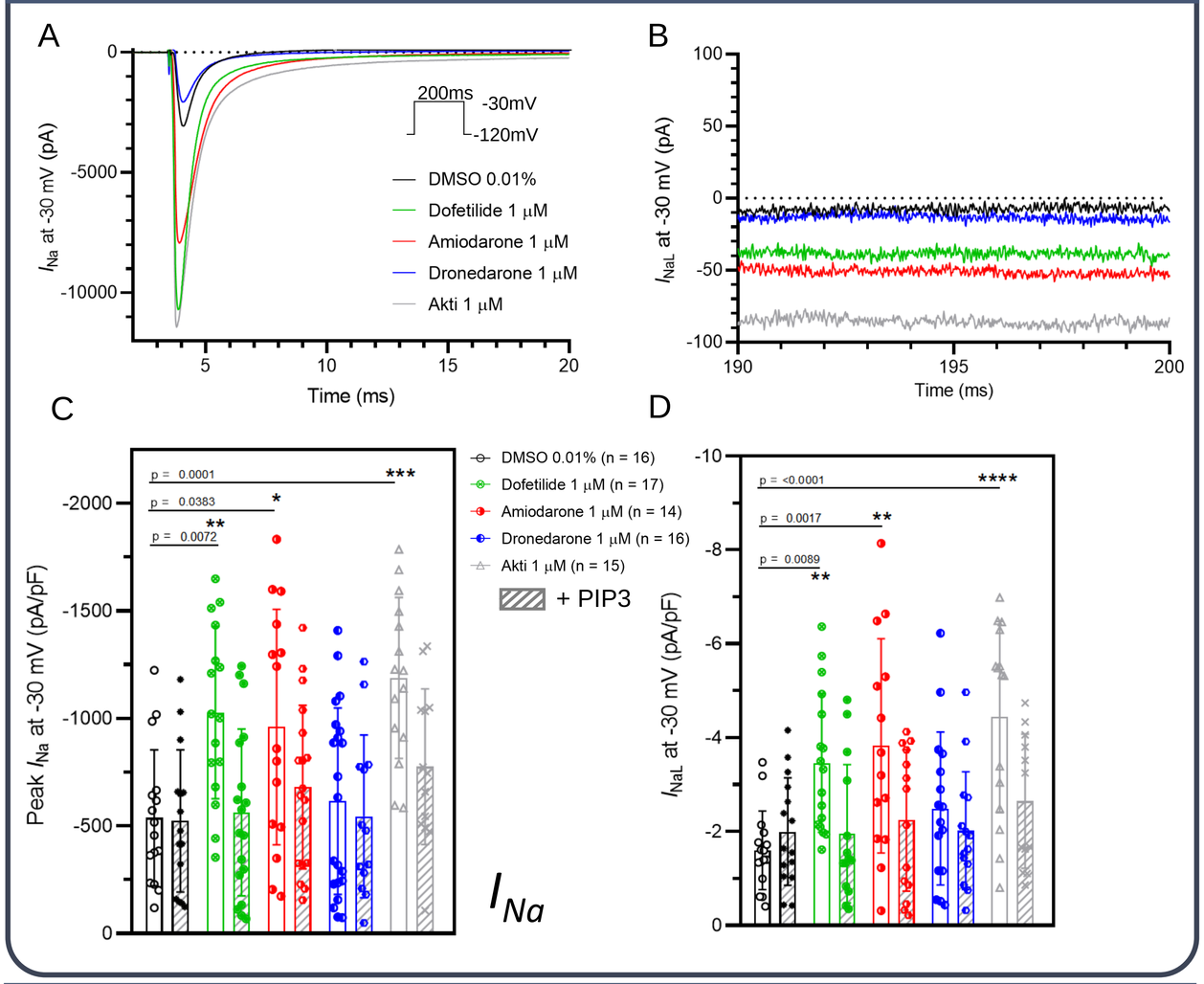
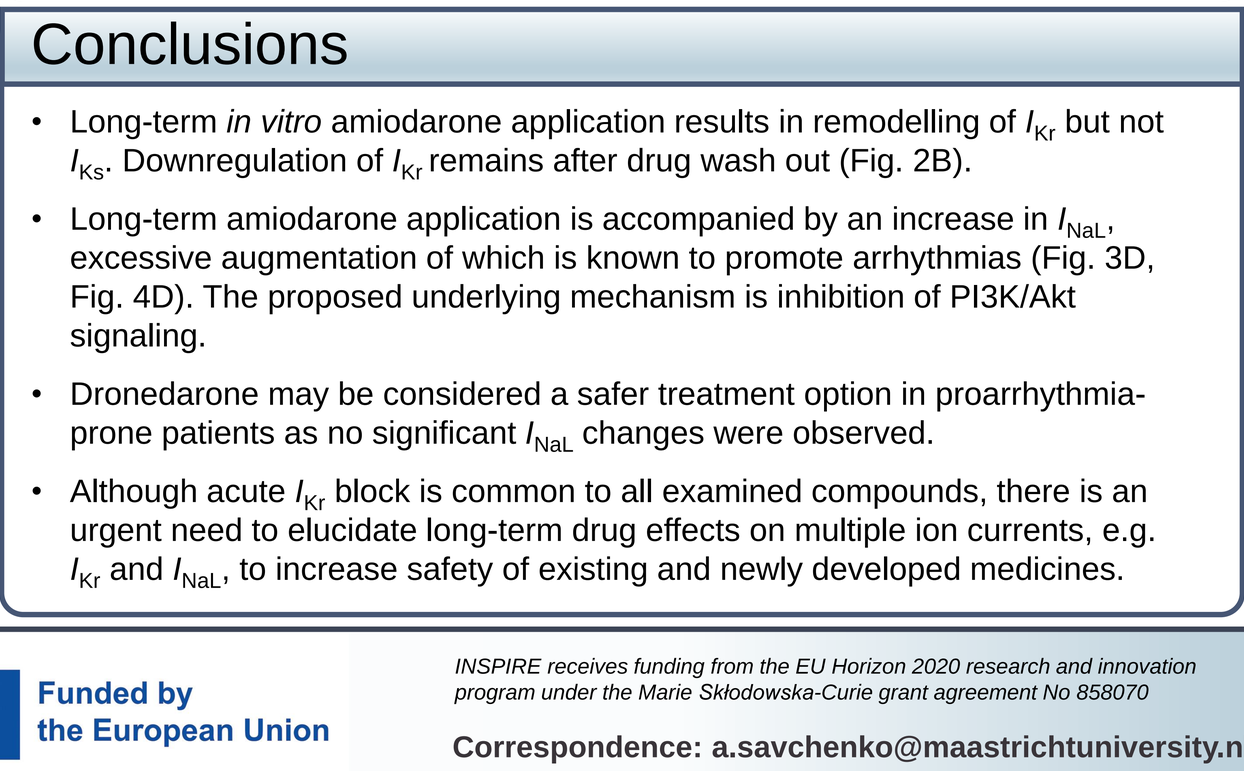


Figure 4: In hiPSC-CMs amiodarone also increases I_{Na} and I_{NaL}



Conclusions

- Long-term *in vitro* amiodarone application results in remodelling of I_{Kr} but not I_{Ks}. Downregulation of I_{Kr} remains after drug wash out (Fig. 2B).
- Long-term amiodarone application is accompanied by an increase in I_{NaL}, excessive augmentation of which is known to promote arrhythmias (Fig. 3D, Fig. 4D). The proposed underlying mechanism is inhibition of PI3K/Akt signaling.
- Dronedarone may be considered a safer treatment option in proarrhythmia-prone patients as no significant I_{NaL} changes were observed.
- Although acute I_{Kr} block is common to all examined compounds, there is an urgent need to elucidate long-term drug effects on multiple ion currents, e.g. I_{Kr} and I_{NaL}, to increase safety of existing and newly developed medicines.

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