# Long-term amiodarone exposure augments late sodium current via PI3K/Akt signaling

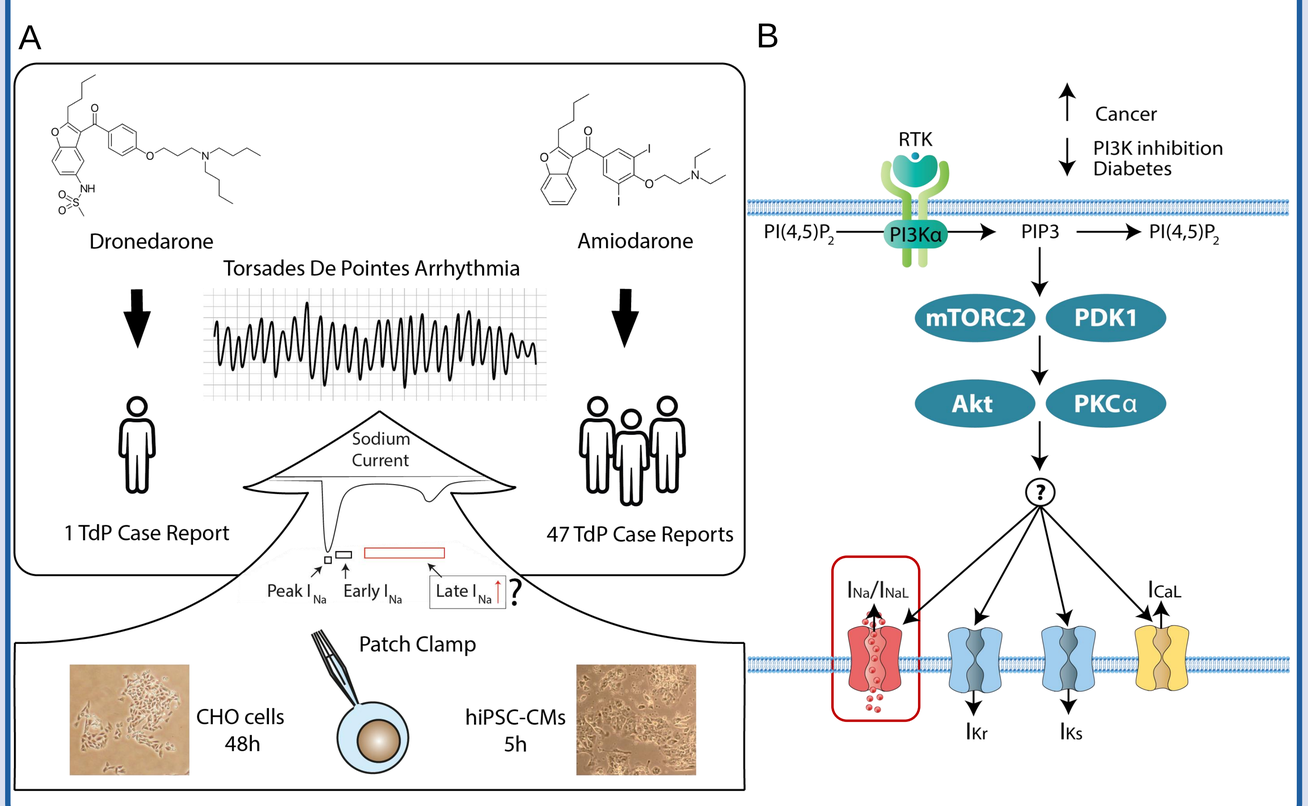
**Anna S. Savchenko, Sandrine R.M. Seyen, Roel L.H. Spätjens, Jordi Heijman, Paul G.A. Volders**

Department of Cardiology, Cardiovascular Research Institute Maastricht, Maastricht University Medical Center, the Netherlands

## Background

- Amiodarone is an effective antiarrhythmic drug (AAD) with distinct acute and long-term electrophysiological effects. Dronedarone was developed as a less-toxic alternative (Fig. 1A), but its differential acute and long-term effects compared to amiodarone remain largely unknown.
- We aimed to investigate the mechanism distinguishing distinct long-term electrophysiological effects between these drugs. As a potential mechanism, we focused on inhibition of PI3K signaling, which has been shown to affect multiple cardiac ion currents including the late sodium current ($I_{NaL}$; Fig. 1B).

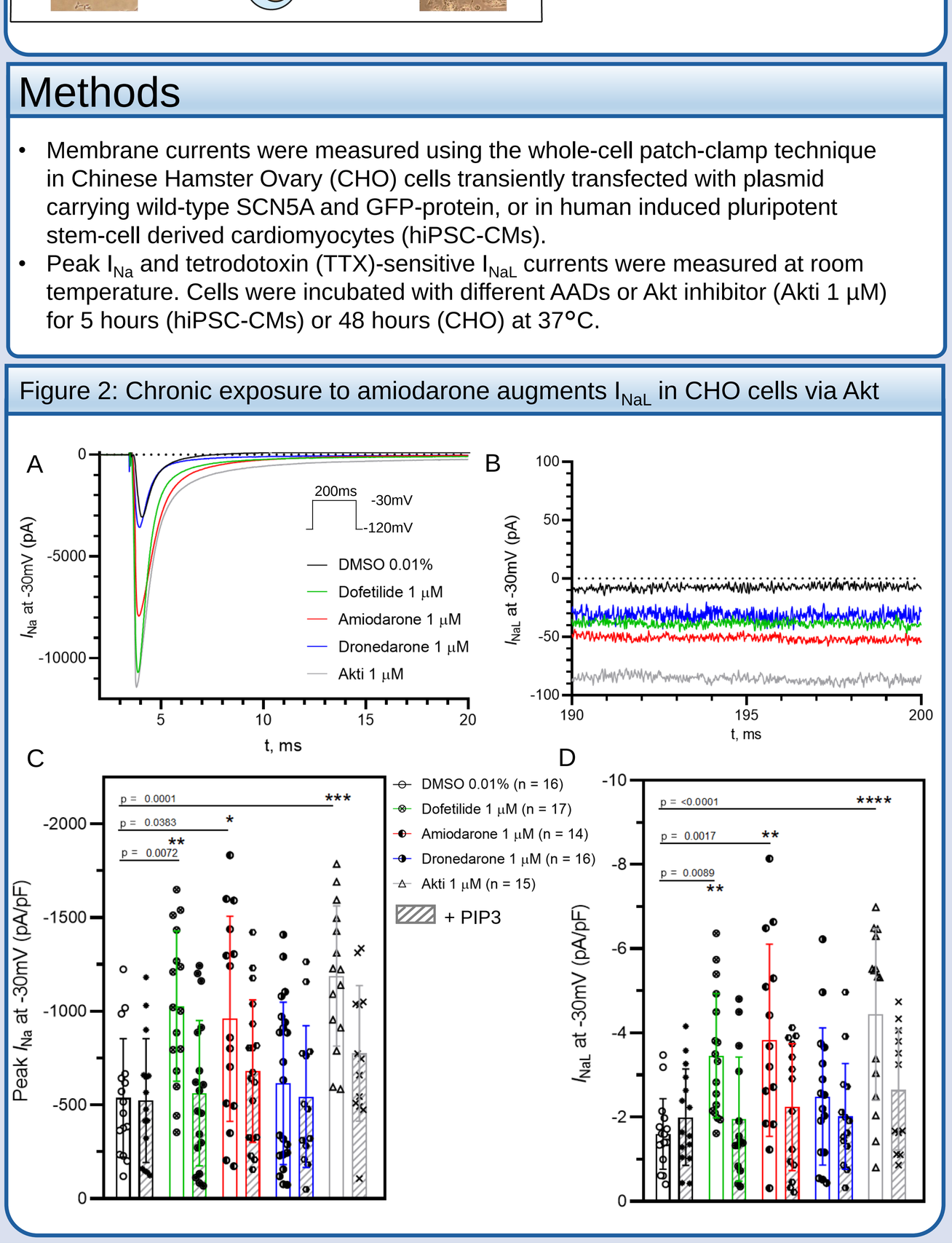
Figure 1: Differential effects of amiodarone versus dronedarone on ion channels via effects on PI3K signaling




## Methods

- Membrane currents were measured using the whole-cell patch-clamp technique in Chinese Hamster Ovary (CHO) cells transiently transfected with plasmid carrying wild-type SCN5A and GFP-protein, or in human induced pluripotent stem-cell derived cardiomyocytes (hiPSC-CMs).
- Peak $I_{Na}$ and tetrodotoxin (TTX)-sensitive $I_{NaL}$ currents 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.

Figure 2: Chronic exposure to amiodarone augments $I_{NaL}$ in CHO cells via Akt




## Results

- Long-term inhibition of Akt (48 h) in CHO cells augmented both peak $I_{Na}$ (Fig. 2A, C) and $I_{NaL}$ (Fig. 2B, D). The latter was fully abolished by PI3K pathway activation with intrapipette phosphatidylinositol (3,4,5)-trisphosphate (PIP3; Fig. 1B; Fig. 2C, D).
- Dofetilide and amiodarone increased peak $I_{Na}$, by 1.9 and 1.8 fold (Fig. 2C), respectively, and $I_{NaL}$ by 2.2 and 2.4 fold (Fig. 2D), compared to control, albeit, to a lesser degree than Akti. This $I_{Na}/I_{NaL}$ increase was fully abolished by PIP3 (Fig. 2C, D).
- Dronedarone had no significant effect on peak $I_{Na}$, nor $I_{NaL}$ and showed no significant changes with PIP3 present.
- In CHO cells, all tested drugs, except for dronedarone, increased current density in I-V relationship (Fig. 3A). By contrast, dronedarone, dofetilide and Akti induced faster channel reactivation (Fig. 3B), whereas amiodarone had opposite effects, increasing refractoriness. A rightward shift in voltage dependence of inactivation was observed for all drugs (Fig. 3C), whereas voltage-dependent activation was only significantly altered for dofetilide (Fig. 3D).
- In hiPSC-CMs, amiodarone also increased peak $I_{Na}$ and $I_{NaL}$ currents (Fig. 4A-C) after 5 hours of exposure.

Figure 3: Chronic dronedarone does not increase $I_{Na}$ unlike amiodarone in CHO

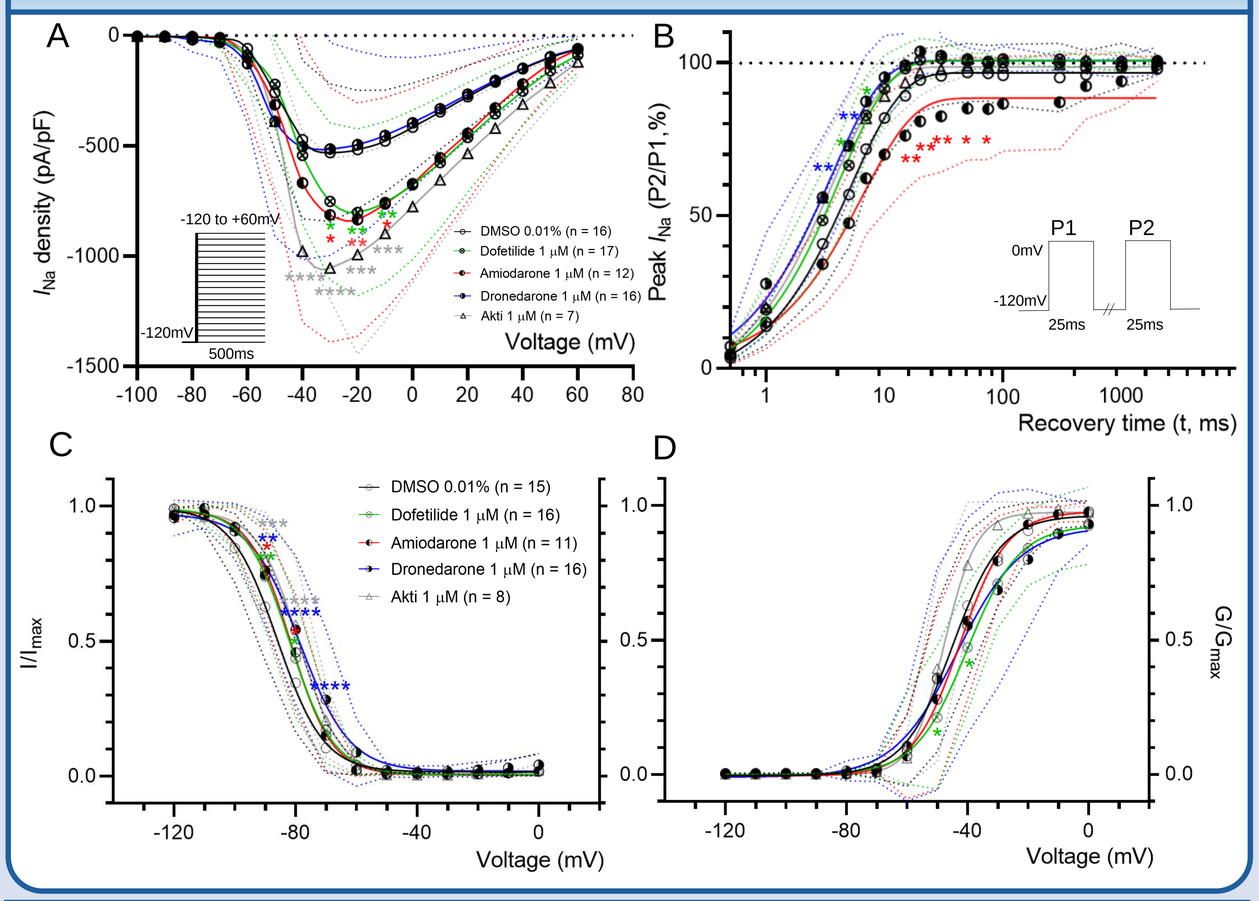
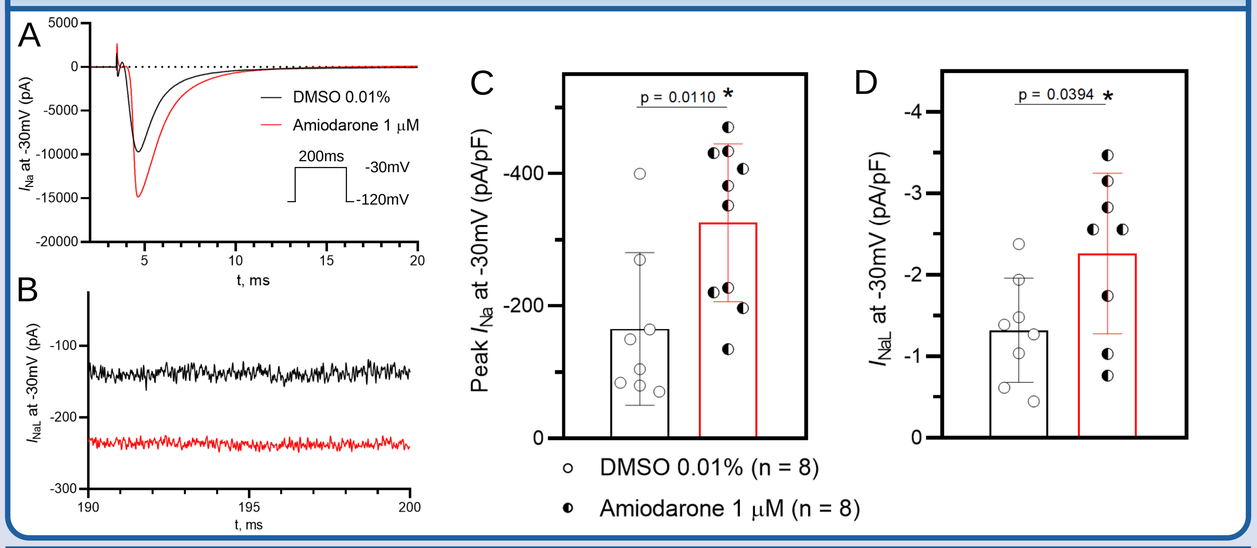



Figure 4: In hiPSC-CMs amiodarone increases $I_{Na}$ and $I_{NaL}$ after 5h treatment




## Conclusions

- Inhibition of PI3K/Akt signaling contributes to the increase in $I_{NaL}$ induced by long-term treatment with amiodarone or dofetilide (Fig. 2B, D).
- Dronedarone did not augment $I_{NaL}$ and might therefore be considered a safer treatment option in proarrhythmia-susceptible patients.
- The differential long-term effects of amiodarone and dronedarone on $I_{NaL}$ could contribute to the higher TdP rates with amiodarone (Fig. 1A).
- Although acute $I_{Kr}$ block is common for all examined compounds, it is necessary to elucidate long-term drug effects on other ion currents, as observed with $I_{NaL}$ in this and previous studies.

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**Correspondence: a.savchenko@maastrichtuniversity.nl**