Enhanced Expression and PKCδ-mediated Hyperphosphorylation underlie the Proarrythmic Increase in NCX1 Activity in Patients with Chronic Atrial Fibrillation

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INTRODUCTION

- Recent work suggests dysregulation of the NCX1 macromolecular complex in atrial fibrillation (AF), making elements of the NCX1 complex potential novel antiarrhythmic targets[1].
- Upregulation of NCX1 in chronic AF (cAF) increases the transient-inward current (I_{ti}) following spontaneous Ca^{2+}-release events, promoting atrial ectopic activity[1].
- The molecular basis of NCX dysfunction in cAF patients is largely unknown and was the major focus of the present investigation.

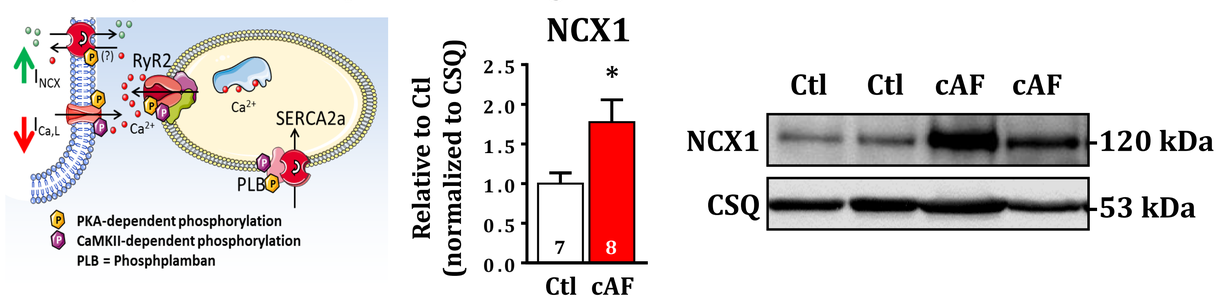


Fig. 1. Schematic representation of Ca^{2+}-handling in human atrial cardiomyocytes (left) and NCX1 protein expression in whole-tissue lysates in Ctl and cAF patients (right)[1].

SUMMARY & CONCLUSIONS

- **We detected a higher frequency and amplitude of spontaneous Ca^{2+}-mediated NCX currents in cAF, pointing to an increased NCX function.**
- **In membrane fractions of cAF patients we could detect an increase in the full-length functional 160 kDa and proteolytic 120 kDa NCX1 bands. The full-length NCX band represented >90% of total NCX1 protein in cAF.**
- **Using immunoprecipitation we discovered PKCα, PKCδ, AMPKα, PP1α and PLM as components of the human atrial NCX1 macromolecular multiprotein complex.**
- **We could validate our NCX-PKCδ co-IP results with the Duolink proximity lygase assay method in human cardiomyocytes only.**
- **Besides the increase of NCX1 proteins in cAF patients, a dysbalance between kinase and phosphatase activities in the atrial NCX1 complex may constitute a novel mechanism for the proarrhythmic NCX dysfunction during clinical AF.**

RESULTS

I_{ti} in human atrial cardiomyocytes

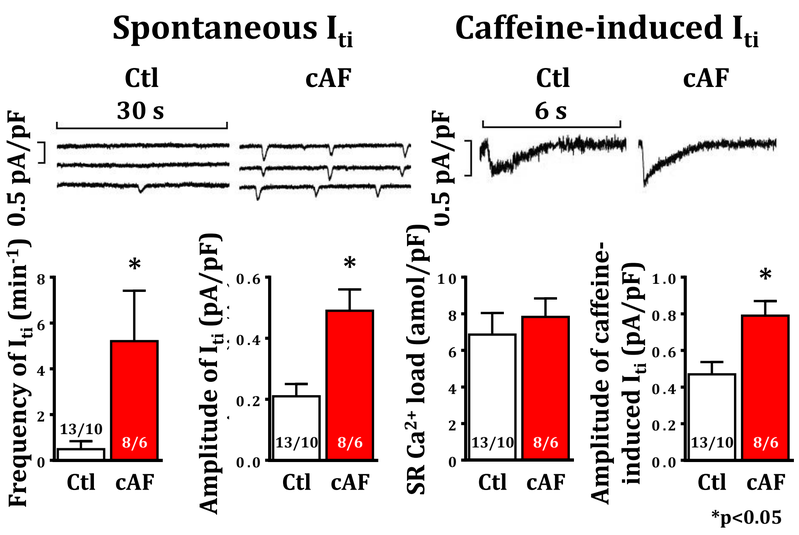


Fig. 2. Perforated-patch voltage-clamp recordings of I_{ti} currents. Representative traces of transient inward currents (I_{ti}) elicited by spontaneous SR Ca^{2+}-release events **(top, left)** and caffeine-induced SR Ca^{2+} release **(top, right)**. Mean values of frequency and amplitude of spontaneous I_{ti}, **(bottom, left)** and SR Ca^{2+} load (integrated caffeine-induced I_{ti}) and amplitude of caffeine-induced I_{ti} **(bottom, left)**.

NCX1 in human atrial membrane fractions

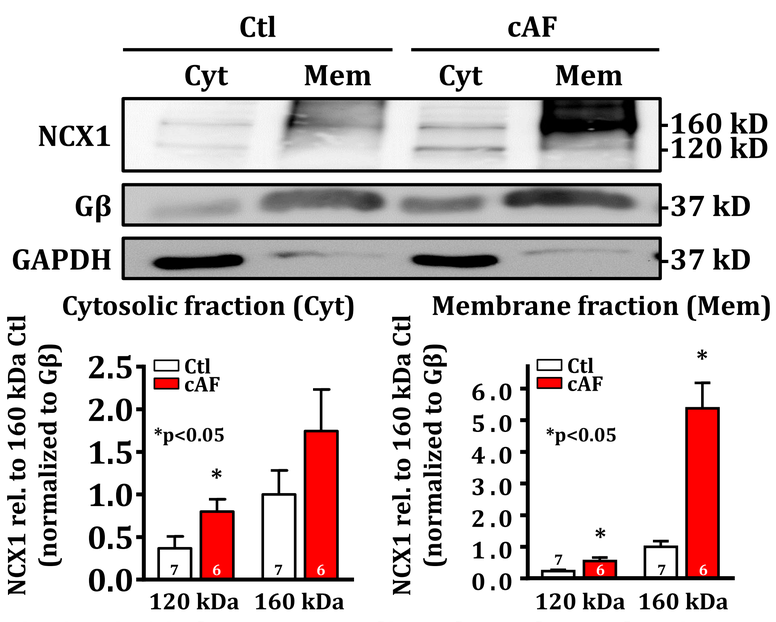
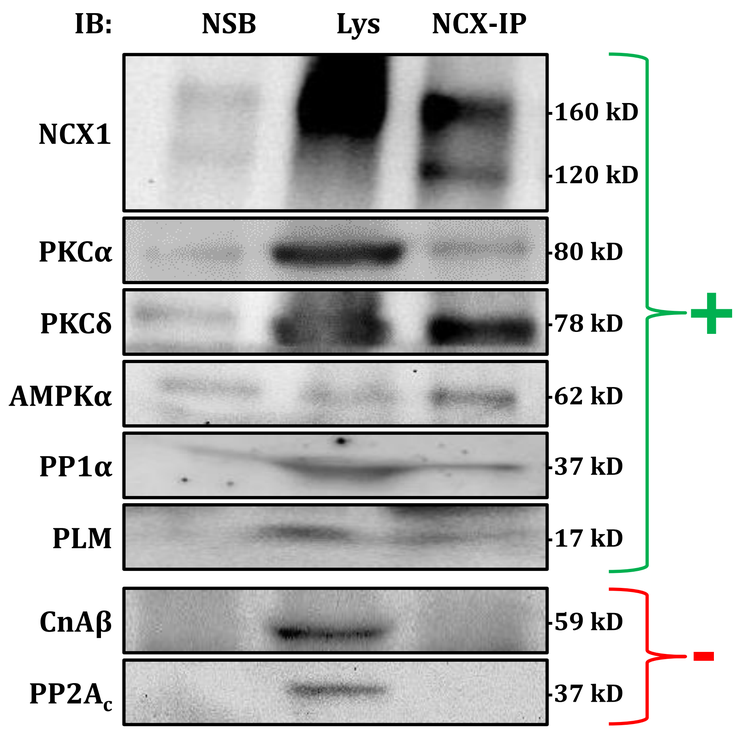
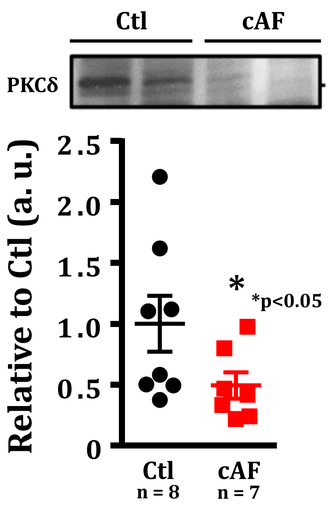


Fig. 3. NCX1 in human cytosolic and membrane fractions. WBs show the NCX1 protein expression (160 kDa and 120 kDa bands) in Ctl and cAF patients. Gβ and GAPDH were used as membrane and cytosolic marker proteins **(top)**. Mean values show the cAF-related increase of NCX1 bands in both fractions **(bottom)**.

Human atrial NCX1 complex



PKCδ in human atrial lysates



NCX1 co-IP with PKCδ

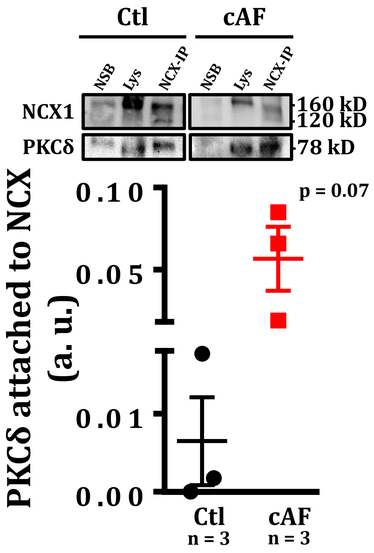


Fig. 4. Immunoprecipitation (IP) of NCX1 in human atrial tissue. Immunoblots (IB) of NCX1, PKCα, PKCδ, AMPKα, PP1α, and phospholemman (PLM), calcineurin Aβ (CnAβ) and protein phosphatase 2A, catalytic ($PP2A_c$). Lys, lysate; NSB, non-specific binding (**top**). Quantification of PKCδ protein expression level in tissue homogenates of Ctl and cAF patients **(bottom, left)**. Quantification of NCX1 and PKCδ co-IP in samples of Ctl and cAF patients **(bottom, right)**.

Working hypothesis

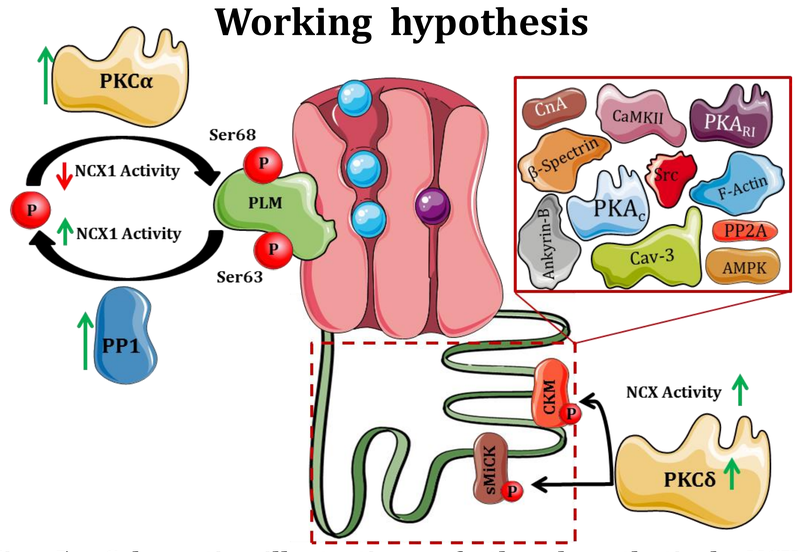


Fig 5. Schematic illustration of the hypothetical NCX1 macromolecular complex in atria[3-8]. Src: Sarcoma kinase; **PKC**: Protein kinase C; **CnA:** Calcineurin A; **Cav-3:** Caveolin 3; **PP2A:** Protein phosphatase 2A; **PP1:** Protein phosphatase 1; **PLM:** Phospholemman; **CaMKII:** Ca^{2+}/calmodulin-dependent protein kinase II; **PKA_{RI}:** type-I regulatory subunit of protein kinase A**; PKA_c:** catalytic subunit of protein kinase A; **CKM:** Creatine kinase (muscle type); **sMiCK:** S-type creatine kinase (mitochondrial).

Duolink of NCX1 & PKCδ in Ctl human

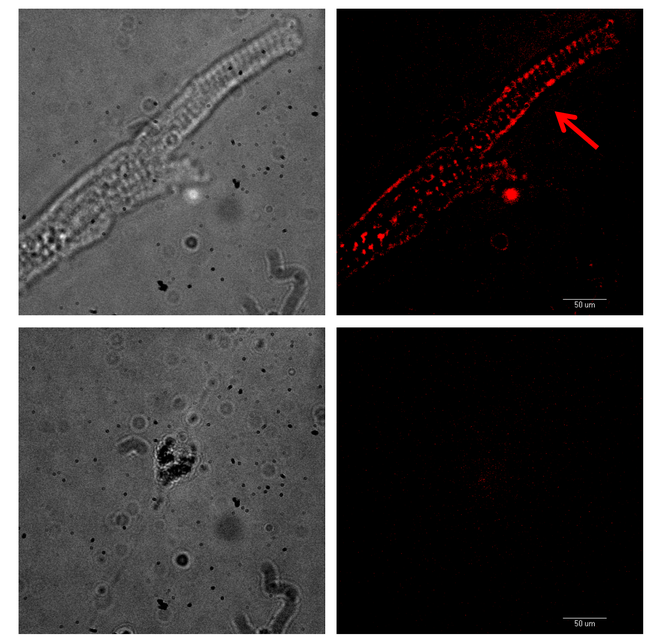


Fig. 6. Confocal microscopy image of protein-protein interactions between NCX1 and PKCδ using PLA. Human atrial cardiomyocyte **(top)** and fibroblast **(bottom)** were stained with specific anti-NCX1 and anti-PKCδ antibodies. Red dots represent positive protein-protein interaction.

METHODS

- Right atrial appendages were obtained from patients in sinus rhythm (Ctl) or with chronic atrial fibrillation (cAF) (>6 months) undergoing open heart surgery.
- Membrane current measurements in isolated human atrial cardiomyocytes were performed as previously described[2].
- Proteins were isolated from atrial tissue homogenates and membrane fractions were obtained by centrifugation (5 min, 600 g; 1 h, 100.000 g), whereby the resulting pellet was re-suspended and the supernatant served as cytosolic fraction. Protein expression was detected using Western blot (WB).
- Immunoprecipitations were performed in whole-tissue homogenates using specific antibody (NCX1, Swant) and G-sepharose beads. Beads without primary antibody were used to detect non-specific binding (n.s.b.).
- For DuoLink proximity ligase assay (PLA), fixed cells were incubated with primary ABs of proteins of interests. DNA-tagged Abs and a ligase were added to connect both DNA-tags to a circle-DNA. After amplification of the circle-DNA the visualization of the product occurred with labeled probes and a confocal microscope.

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