Enhanced Expression and PKCδ-mediated Hyperphosphorylation underlie the Proarrhythmic 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 targets1.
- Upregulation of NCX1 in chronic AF (cAF) increases the transient inward current (I\textsubscript{t}) following spontaneous Ca\textsuperscript{2+}-release events, promoting atrial ectopic activity1.
- The molecular basis of NCX dysfunction in cAF patients is largely unknown and was the major focus of the present investigation.

SUMMARY & CONCLUSIONS

- We detected a higher frequency and amplitude of spontaneous Ca\textsuperscript{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 PKC\textalpha, PKC\delta, AMPK\alpha, PP1\alpha and PLM as components of the human atrial NCX1 macromolecular multiprotein complex.
- We could validate our NCX-PKC\delta co-IP results with the Duolink proximity ligation 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\textsubscript{t} in human atrial cardiomyocytes**

- Spontaneous I\textsubscript{t}
- Caffeine-induced I\textsubscript{t}

**Human atrial NCX1 complex**

- IB: NSB, Lys, NCX-IP
- PKCo, PKC\delta, AMPK\alpha, PP1\alpha, PLM

**PKCδ in human atrial lysates**

- NCX1 co-IP with PKCδ

**Cytosolic (Cyt) and Membrane (Mem) fractions**

- NCX1
- GAPDH

**PKCδ attached to NCX1**

- NCX1

**REFERENCES**

1. Temp et al., Enhanced sarcolemmal retention of Ca\textsuperscript{2+} by an increased Na/Ca-exchanger function underlie delayed afterdepolarizations in patients with chronic atrial fibrillation, Circulation 2012; 125(17):2059-70.
2. Luch et al., Abnormal calcium handling in atrial fibrillation is linked to up-regulation of adenine A2A receptors, Eur Heart J 2010; 31(16):1937-46.
3. Bala et al., Inhibition of the N:\Ca-exchanger NCX1, J Am Coll Cardiol 2007; 50(7):96-7.

METHODS

- Right atrial appendages were obtained from patients in sinus rhythm (Ct) 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.
- Proteins were isolated from atrial tissue homogenates and membrane fractions were obtained by centrifugation (5 min, 60 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, Smart) and G-Sepharose beads. Beads without primary antibody were used to detect non-specific binding (n.s.).
- For Duolink proximity ligation assay (PLA), fixed cells were incubated with primary Abs of proteins of interest. 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.