Enhanced Sarcoplasmic Reticulum Ca^{2+}-Leak and Increased Na^{+}-Ca^{2+}-Exchanger Function Underlie Delayed Afterdepolarizations in Patients with Chronic Atrial Fibrillation

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SUPPLEMENTAL MATERIAL

Methods

Measurement of Intracellular [Ca^{2+}] and Patch-clamp Experiments

Membrane currents and potentials were measured at 37°C in whole-cell ruptured-patch configuration using voltage-clamp and current-clamp techniques with simultaneous intracellular [Ca^{2+}] measurement. pClamp-Software (V10.2, Molecular Devices, Sunnyvale, CA) was used for data acquisition and analysis.

Intracellular [Ca^{2+}] was quantified using Fluo-3-acetoxymethyl ester (Fluo-3 AM; Invitrogen, Carlsbad, CA; 10 µmol/L, 10 min loading and 30 min de-esterification).[1] In addition Fluo-3 was included into the electrode solution containing (in mmol/L): EGTA 0.02, Fluo-3 0.1 (Invitrogen), GTP-Tris 0.1, HEPES 10, K-aspartate 92, KCl 48, Mg-ATP 1, Na_2-ATP 4; pH=7.2.

Borosilicate glass microelectrodes had tip resistances of 2-5 MΩ when filled with pipette solution. Seal-resistances were 4-8 GΩ. For voltage-clamp experiments series resistance and cell capacitance were compensated.

During experiments myocytes were superfused at 37°C with a bath solution containing (in mmol/L): $CaCl_2$ 2, glucose 10, HEPES 10, KCl 4, $MgCl_2$ 1, NaCl 140, probenecid 2; pH=7.4. For voltage-clamp experiments, K^+ currents were blocked by adding 4-aminopyridine (5 mmol/L) and $BaCl_2$ (0.1 mmol/L) to the bath solution.

L-type Ca^{2+}-current ($I_{Ca,L}$) and corresponding triggered $[Ca^{2+}]_i$-transients (CaTs) were recorded simultaneously, using a holding potential of -80 mV and a 100-ms ramp-pulse to -40 mV to inactivate the fast Na^+-current followed by a 100-ms test-pulse to +10 mV at 0.5 Hz. Action potentials were stimulated at 0.5 Hz using 1 ms current pulses of 1.2x threshold strength. Caffeine (10 mmol/L) was used for quantification of SR Ca^{2+}-content.[2] Isoprenaline (1 µmol/L) and the phosphodiesterase-inhibitor 3-isobutyl-1-methylxanthine (IBMX, 10 µmol/L) were used to increase cAMP-formation.

Current-Clamp Recordings in Perforated-patch Configuration

In Online-Figures 6 and 7 the amphotericin-perforated-patch technique was used to avoid dialysis of cytosolic components and concomitant changes in Ca^{2+} handling properties. The composition of the pipette solution was (mmol/L): EGTA 0.02, GTP-Tris 0.1, HEPES 10, K-aspartate 92, KCl 48, Mg-ATP 1, Na_2-ATP 4; pH=7.2. On experiment days amphotericin (Sigma-Aldrich, St. Louis, MO) was added to the pipette solution at a final concentration of 240 µg/mL from a 60 mg/mL DMSO stock solution. Intracellular $[Ca^{2+}]$ was quantified using Fluo-3-acetoxymethyl ester (Fluo-3 AM; 10 µmol/L, 10 min loading and 30 min de-esterification).[1] During experiments myocytes were superfused at 37°C with a bath solution containing (in mmol/L): $CaCl_2$ 2, glucose 10, HEPES 10, KCl 4, $MgCl_2$ 1, NaCl 140, probenecid 2; pH=7.4. Action potentials were stimulated in current-clamp configuration at 0.5 Hz using 1 ms current pulses of 1.2x threshold strength. No series resistance compensation was employed.

Quantification of Diastolic SR Ca^{2+} Leak with Tetracaine

SR Ca^{2+}-leak in intact myocytes was measured according to Shannon et al.[3] using Na^+- and Ca^{2+}-free bath solution (in mmol/L: 4-aminopyridine 5, $BaCl_2$ 0.1, EGTA 10, glucose 10, HEPES 10, KCl 4, $LiCl_2$ 140, Mg_2Cl 1, probenecid 2) and tetracaine (1 mmol/L). KN-93 (1 µmol/L; EMD Chemicals, Gibbstown, NJ) and H-89 (1 µmol/L EMD Chemicals) were used as blockers of CaMKII and PKA, respectively. The inactive KN-93 analogue KN-92 (1 µmol/L; EMD Chemicals) was used as negative control.

RyR2 Single-channel Recordings

Single-channel recordings were obtained under voltage-clamp conditions at 0 mV, as previously described.[4] Atrial SR membrane-preparations were incorporated into lipid-bilayer membranes comprised of a mixture of phosphatidylethanolamine and phosphatidylserine at a ratio of 3:1 (Avanti Polar Lipids, Alabaster, AL) dissolved in n-decane (25 mg/ml). Bilayers were formed across a 150 µm aperture of a polystyrene cuvette. The *cis* and *trans* chambers correspond to the cytosolic and the luminal sides of the SR, respectively. The *trans* chamber contained (in mmol/L) HEPES 250, KCl 50 and $Ca(OH)_2$ 53. The *cis* chamber contained (in mmol/L) HEPES 250, Tris-base 125, KCl 50, EGTA 1, $CaCl_2$ 0.5, pH=7.35. Ca^{2+}-activation curves were generated by varying $[Ca^{2+}]$ in the *cis* compartment. Ryanodine (5 µmol/L) was applied to the *cis* chamber to confirm identity of RyR2 channels at the end of each experiment. KN-93 (10 µmol/L, EMD Chemicals), KN-92 (10 µmol/L, EMD Chemicals), H-89 (10 µmol/L, Sigma-Aldrich), and PKI (10 µmol/L, EMD Chemicals) were applied to the *cis* chamber and mixed with the buffer for 5 min before channel tracings were recorded. Data were collected using Digidata 1322A (Molecular Devices, Sunnyvale, CA) and Warner Bilayer Clamp Amplifier BC-535 (Warner Instruments, Hamden, CT) under voltage-clamp conditions. Cytosolic free $[Ca^{2+}]$ was calculated with WinMax32. Data were analyzed

from digitized current recordings using pCLAMP-9.2 software (Molecular Devices). Clampfit-9.2 was used to create amplitude histograms from one representative single-channel trace (10 kHz sampling rate, 0.2 pA bin-width) from a Ctl and a cAF patient, respectively.

Ca^{2+}-spark Measurements in Atrial Myocytes From S2814D Knock-in Mice

Animal studies were performed according to protocols approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine. Mouse atrial myocytes were isolated as previously described.[5] The heart was removed and blood rinsed out with 0-Ca^{2+} Tyrode solution (in mmol/L: NaCl 137, KCl 5.4, $MgCl_2$ 1, HEPES 5, glucose 10, NaOH 3, pH=7.4). Hearts were cannulated through the aorta and perfused on a Langendorff apparatus with 0-Ca^{2+} Tyrode for 3-5 minutes at 37°C, followed by 0-Ca^{2+} Tyrode solution containing 20 µg/mL Liberase (Roche, Indianapolis, IN) for 10-15 minutes at 37°C. After digestion, hearts were perfused with 5 mL KB solution (in mmol/L: KCl 90, K_2HPO_4 30, $MgSO_4$ 5, pyruvic acid 5, β-hydroxybutyric acid 5, creatine 5, taurine, glucose 10, EGTA 0.5, HEPES 5, pH=7.2). Both left and right atrium were minced in KB solution and gently agitated, then filtered through a 210 µm polyethylene mesh. Atrial myocytes were stored in KB solution at room temperature before use.

Only rod-shaped myocytes showing clear striations were studied. Atrial myocytes were loaded with 2 µmol/L Fluo-4-AM (Invitrogen, Carlsbad, CA) in normal Tyrode solution containing 1.8 mmol/L Ca^{2+} for 30 minutes at room temperature. Cells were then washed with dye-free Tyrode solution for 15 minutes for de-esterification and transferred to a chamber equipped with a pair of parallel platinum electrodes. The chamber was placed on a LSM510 confocal microscope (Carl Zeiss, Thornwood, NY). Fluorescence images were recorded in line-scan mode with 1024 pixels per line at 500 Hz. Once steady-state Ca^{2+}-transients during 1 Hz-pacing (20 V) were observed, pacing

was stopped for 45 seconds and Ca^{2+} sparks were counted. KN-93 (10 µmol/L, EMD Chemicals) was applied to inhibit CaMKII. Steady-state SR Ca^{2+}-content was assessed by rapid application of 10 mmol/L caffeine.

Immunoblot Analysis

The protein levels of calmodulin (1:1000; abcam, Cambridge, UK), total CaMKIIδ (1:200; Santa Cruz Biotechnology, Santa Cruz, CA), Thr287 and Thr306/Thr307 phosphorylated CaMKIIδ (1:5000; Promega, Madison, WI and 1:2500; biomol, Hamburg, Germany, respectively), calsequestrin (CSQ, 1:2500; Dianova, Hamburg, Germany), GAPDH (1:100000; HyTest, Turku, Finland), junctin and triadin (JNC, 1:100; TRD, 1:1000; kind gifts from Dr. Uwe Kirchhefer, Münster, Germany), NCX1 (1:500; Fitzgerald, Concord, MA); catalytic PKA-subunit (PKAc, 1:1000; BD Biosciences, Franklin Lakes, NJ); regulatory $PKA_{II\alpha}$-subunit ($PKA_{II\alpha}$, 1:500, Santa Cruz Biotechnology), total RyR2 (1:3000, Affinity BioReagents, Golden, CO), Ser2808 and Ser2814 phosphorylated RyR2 (1:3000 and 1:1000, respectively)[6,7] were quantified by Western blotting as described.[8,9] The RyR2-Ser2808 and RyR2-Ser2814 phosphoepitope-specific antibodies were custom generated using the peptide C-RTRRI-(pS)-QTSQV corresponding to the PKA phosphorylation site region at Ser2808 on RyR2 and peptide CSQTSQV-(pS)-VD corresponding to CaMKII phosphorylated RyR2 at Ser2814. Appropriate peroxidase-conjugated goat anti-rabbit (Sigma-Aldrich), goat anti-mouse (Sigma-Aldrich) and donkey anti-goat (Santa-Cruz) were used as secondary antibodies and visualized by chemifluorescense (GE Healthcare, Chalifont St. Giles, UK). Quantity One Software (Bio-Rad, Hercules, CA) was used for quantification.[8] Protein expression was normalized to CSQ and GAPDH, respectively, which were unchanged in cAF compared to SR samples.

Reverse Transcription and Quantitative Real-time PCR

Total RNA was isolated from human heart tissue samples using a RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. cDNA was synthesized with RevertAid First-Strand cDNA synthesis kit (Fermentas, St. Leon-Rot, Germany). The reaction mix consisted of 10 U/µL of reverse transcriptase, 1 U/µL of RNase inhibitor, 1 mmol/L dNTP, 5 µmol/L random primers, and 0.2 µg RNA in 20 µL total volume. The reaction mixture was incubated at 25°C for 10 min and then at 42°C for 60 min. Finally, the mixture was heated at 70°C for 5 min. Real-time PCR was performed by using 2X Taqman Universal PCR mix (Applied Biosystems, Foster City, CA) with commercial primers (NCX1: Hs01062258_m1; CaMKIIδ_B: Hs00945363_m1; CaMKIIδ_C: Hs00949946_m1; HPRT1: Hs01003267_m1; Applied Biosystems, Foster City, CA) on an ABI7500 Fast real-time PCR system for 20 sec at 95°C, followed by 40 cycles for 3 sec at 95°C and 30 sec at 60°C. Relative amount of target gene in each sample was calculated by the PCR system using a series 1:2 dilution of sample pool as standard curve. Mean value of relative amount for each gene was calculated from duplicate measure of each sample and normalization is performed to get ratio of mean value of the target gene to that of housekeeping gene in corresponding sample.

cAMP Assay

The cAMP content of frozen atria was determined as described before.[10] In brief, after homogenization of the tissue in the presence of 5% trichloroacetic acid (TCA) and 1 mmol/L 3-isobutyl-1-methylxanthine (IBMX), the insoluble debris pellets were removed by centrifugation at 1.500 x g for 10 min. Thereafter, the remaining TCA was extracted from the supernatant by using water-saturated ether. Twenty-five µl of the supernatant were then used for the competitive cAMP enzyme immunoassay (Cayman Chemical Company, Ann Arbor, MI) according to the manufacturer's recommendations. Protein

concentrations were determined by the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL) after neutralization of the protein containing debris pellets with 0.1 mol/L NaOH.

Ca^{2+} Transport via SERCA, NCX and Plasmalemmal Ca^{2+} ATPase (PMCA)

During decay of $I_{Ca,L}$-induced Ca^{2+}-transient (CaT) the Ca^{2+} is extruded from the cytosol by Ca^{2+} reuptake into the SR mediated via SR Ca^{2+}-ATPase (SERCA) and by Ca^{2+} extrusion into extracellular space via forward-mode Na^{+}/Ca^{2+} exchanger (NCX) and plasmalemmal Ca^{2+} ATPase (PMCA). We estimated the relative contribution of these three mechanisms from the rate constants (*k*) of single exponential curves fitted to decaying parts of the $I_{Ca,L}$- and caffeine-evoked CaTs, as described by Choi and Eisner.[11]

The decay of $I_{Ca,L}$-induced CaT (**Online-Figure 3A and B**) was fitted by an exponential curve:

$$[Ca^{2+}]_i(t) = ([Ca^{2+}]_{Amplitude} \cdot e^{-k_{syst} \cdot t}) + [Ca^{2+}]_{diast} \quad (1)$$

where $[Ca^{2+}]_i(t)$, $[Ca^{2+}]_{Amplitude}$ *and* $[Ca^{2+}]_{diast}$ represent the Ca^{2+} concentration at the timepoint *t,* the CaT amplitude and the diastolic Ca^{2+} level, respectively. The rate constant of decay k_{syst} is the reciprocal value of the time-constant of decay (τ, $k_{syst} = \frac{1}{\tau}$) and reflects the rate of combined Ca^{2+} transport by SERCA, NCX and PMCA. Therefore k_{syst} may be also expressed as the sum of the three separate rate constants:

$$k_{syst} = k_{SERCA} + k_{NCX} + k_{PMCA} \quad (2)$$

Application of caffeine leads to rapid depletion of SR Ca^{2+}, which is reflected by the caffeine-induced CaT (cCaT, **Online-Figure 3A,B**):

$$[Ca^{2+}]_i(t) = ([Ca^{2+}]_{cCaT-Ampl} \cdot e^{-k_{caff} \cdot t}) + [Ca^{2+}]_{diast} \quad (3)$$

with "$[Ca^{2+}]_{CaT-Ampl}$" representing the amplitude of cCaT and "k_{caff}" the rate constant of

decay of cCaT. Since caffeine prevents SR Ca^{2+} reaccumulation via SERCA (Ca^{2+} transport by SERCA under caffeine = 0, Ref.), the decay of cCaT depends largely on Ca^{2+} extrusion via NCX and PMCA:

$$k_{caff} = k_{NCX} + k_{PMCA} \tag{4}$$

Using this approach the contribution of SERCA to the decay of $I_{Ca,L}$-induced CaT can be estimated by subtracting the rate constant of cCaT (4) from the rate constant of $I_{Ca,L}$-induced CaT (Formula 2, **Online-Figure 3C**):

$$k_{SERCA} = k_{syst} - k_{caff} \tag{5}$$

For further evaluation of NCX and PMCA transport rates, contribution of NCX was additionally blocked by perfusion with Na^{+} and Ca^{2+} free bath solution. Under these conditions the rate constant of cCaTs "k_{0Na0Ca}" reflects the rate of Ca^{2+} transport by PMCA only (**Online-Figure 3A,B**):

$$k_{0Na0Ca} = k_{PMCA} \tag{6}$$

The rate constant of NCX can now be estimated by subtracting k_{0Na0Ca} (6) from k_{caff} (**Online-Figure 3C**):

$$k_{NCX} = k_{caff} - k_{0Na0Ca} \tag{7}$$

Relative contributions (**Online-Figure 3D**) were calculated by dividing the rate constant of the respective Ca^{2+} removal mechanism (k_{SERCA}, k_{NCX}, k_{PMCA}) by the rate constant of the $I_{Ca,L}$-induced CaT (k_{syst}).

Tables

Online-Table 1: Characteristics of patients used for functional studies

	Ctl	cAF
Patients, n	41	31
Gender, m/f	33/8	22/9
Age, y	69.6±1.5	69.7±1.4
Body mass index, kg/m^2	27.8±0.6	27.4±0.8
CAD, n	28	9**
MVD/AVD, n	7	13*
CAD+MVD/AVD, n	6	9
Hypertension, n	39	29
Diabetes, n	11	10
Hyperlipidemia, n	26	15
LVEF, %	49.3±2.6	48.3±2.5
Digitalis, n	1	3
ACE inhibitors, n	28	24
AT1 blockers, n	5	2
β-Blockers, n	33	25
Dihydropyridines, n	9	7
Diuretics, n	10	14
Nitrates, n	8	4
Lipid-lowering drugs, n	25	13

CAD, coronary artery disease; MVD/AVD, mitral/aortic valve disease; LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme; AT, angiotensin receptor.

*P<0.05 and **P<0.01 vs. SR from Fisher's exact test for categorical variables.

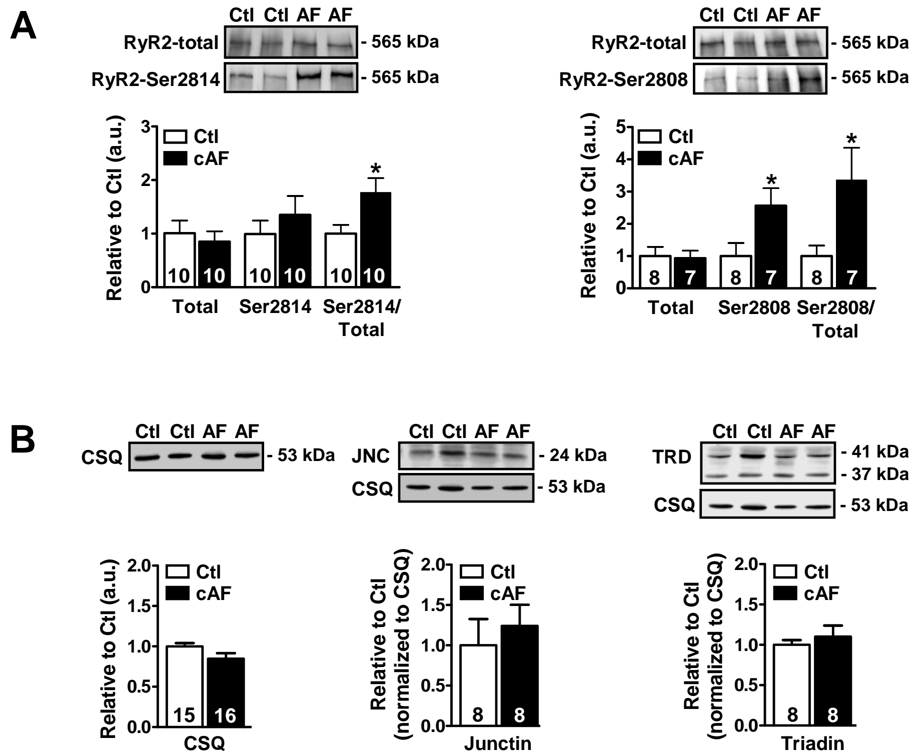
Online-Table 2: Characteristics of patients used for biochemistry

	Ctl	cAF
Patients, n	35	41
Gender, m/f	24/11	24/17
Age, y	66.5±1.7	68.9±1.3
Body mass index, kg/m^2	29.5±1.0	28.5±0.7
CAD, n	18	7**
MVD/AVD, n	11	17
CAD+MVD/AVD, n	6	17*
Hypertension, n	27	36
Diabetes, n	12	14
Hyperlipidemia, n	26	29
LVEF, %	52.3±1.9	47.3±2.5
Digitalis, n	3	12*
ACE inhibitors, n	22	25
AT1 blockers, n	3	5
β-Blockers, n	21	34*
Dihydropyridines, n	5	9
Diuretics, n	13	26*
Nitrates, n	3	8
Lipid-lowering drugs, n	23	19

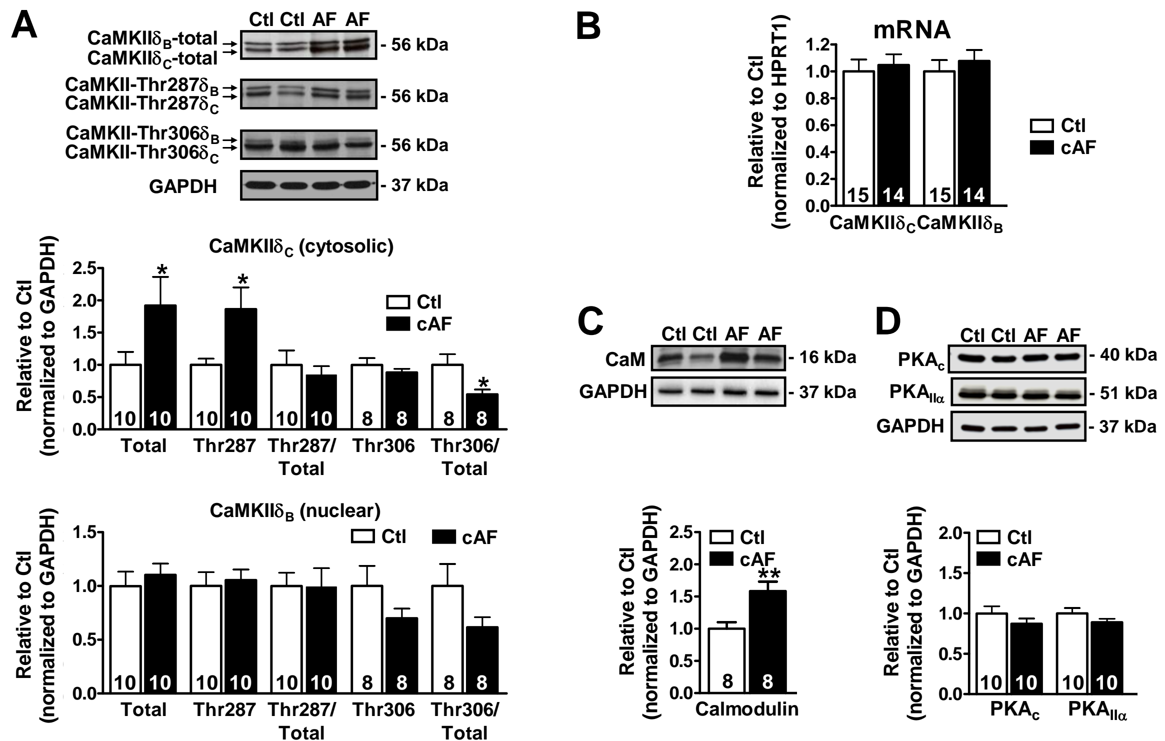
CAD, coronary artery disease; MVD/AVD, mitral/aortic valve disease; LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme; AT, angiotensin receptor.

*$P<0.05$ and **$P<0.01$ vs. SR from Fisher's exact test for categorical variables.

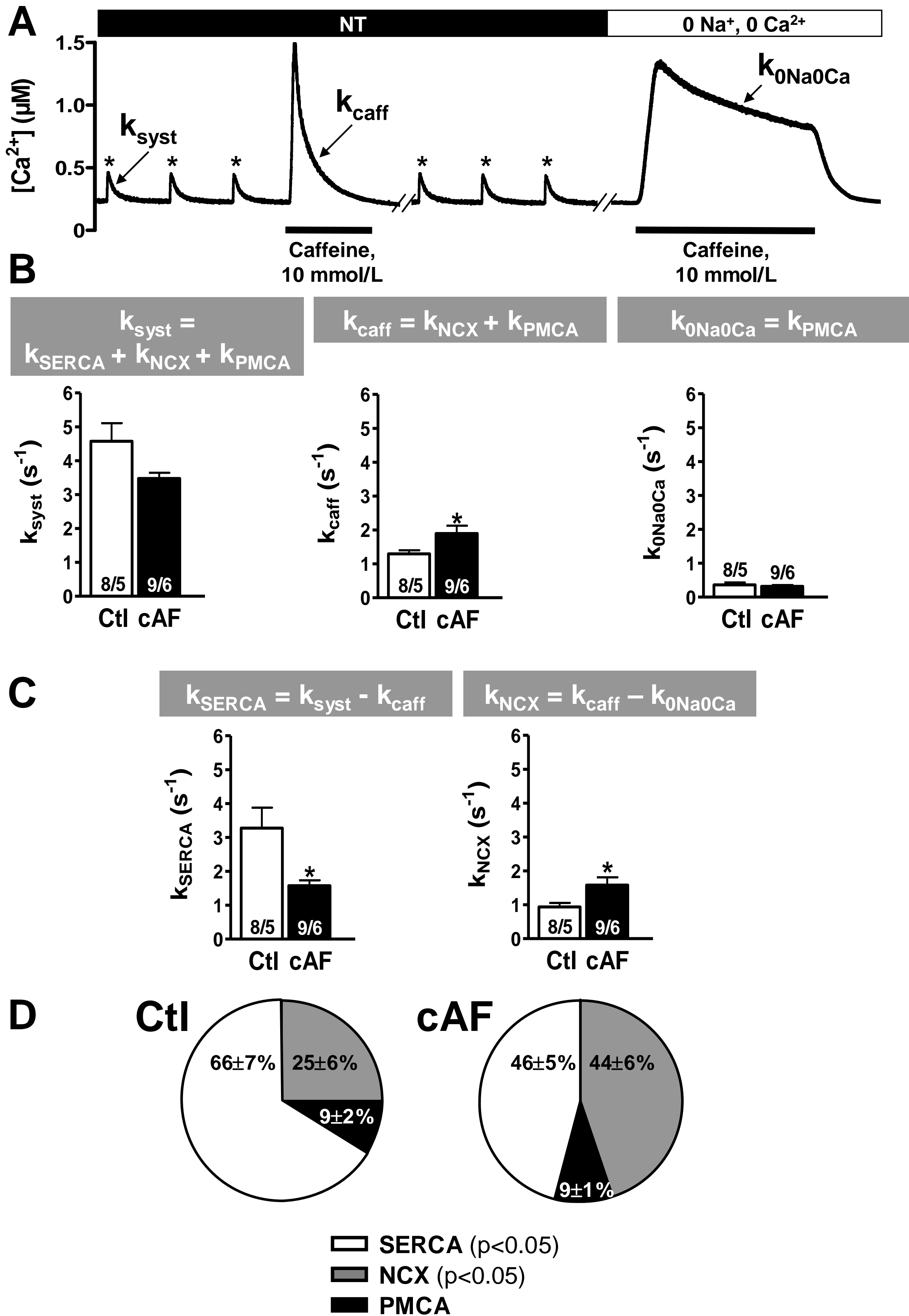
Online-Figures and Figure Legends



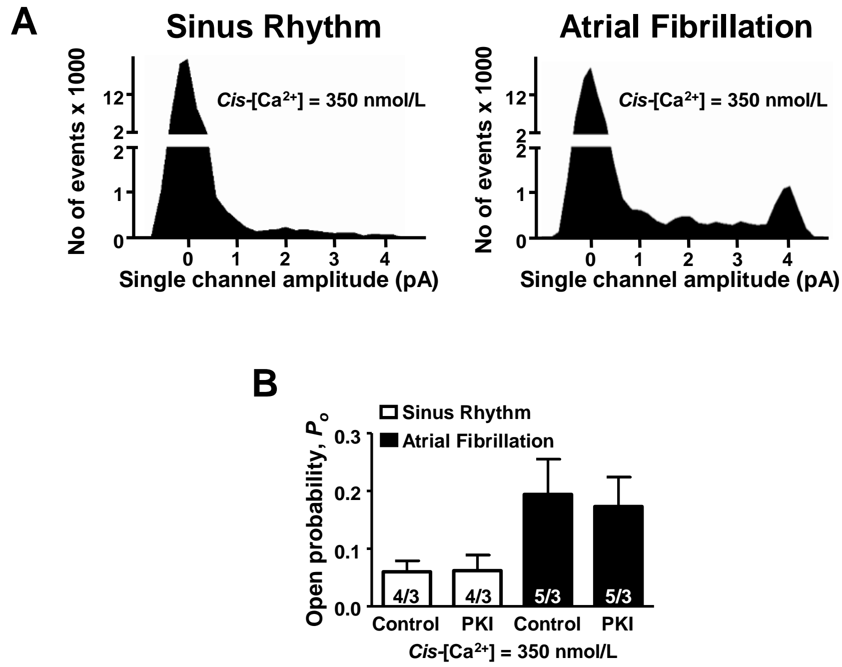
Online-Figure 1. A, Protein levels of total, Ser2814 and Ser2808 phosphorylated ryanodine receptor channels (RyR2) and **B,** Protein levels of calsequestrin, junctin, and triadin in atria of cAF vs. Ctl patients. *P<0.05 vs. corresponding Ctl.



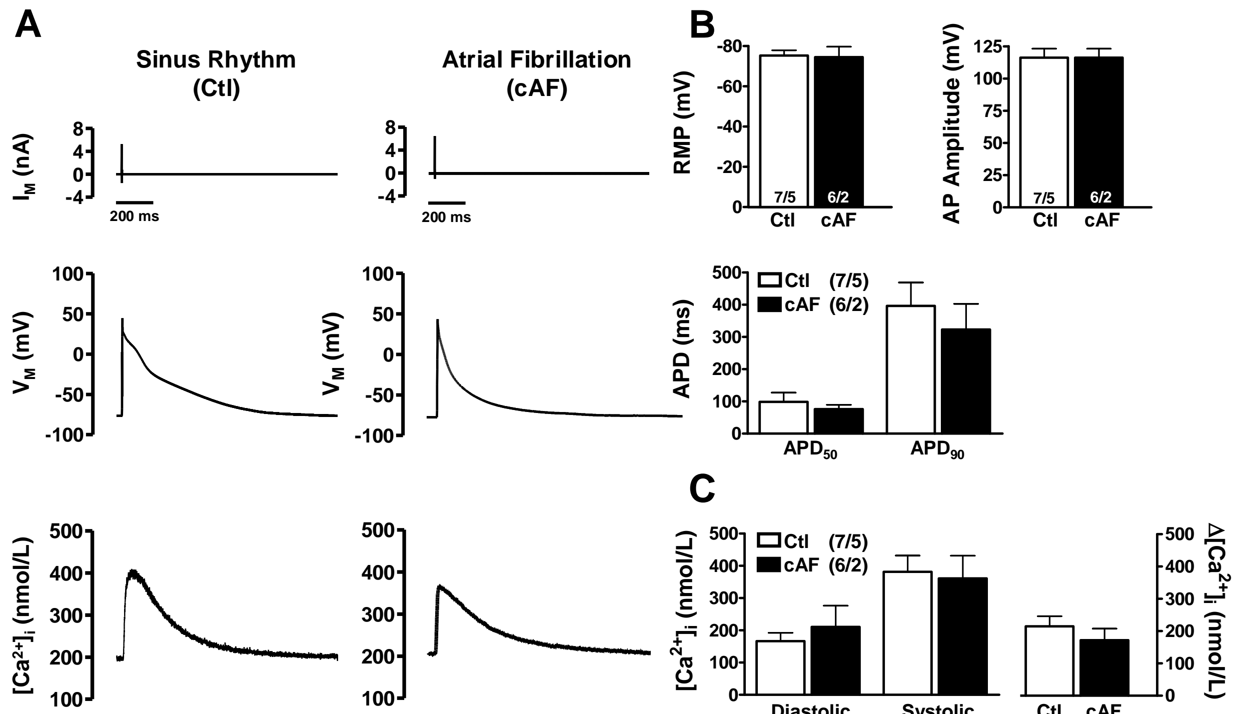
Online-Figure 2. Protein levels and autophosphorylation status of Ca^{2+}/calmodulin-dependent protein-kinase II (CaMKII) at stimulatory (Thr287) and inhibitory (Thr306/Thr307) phosphorylation sites, and of calmodulin, and catalytic and regulatory protein-kinase A isoforms (PKA_c and $PKA_{II\alpha}$, respectively) in atria of cAF vs. Ctl patients. *P<0.05 and **P<0.01 vs. corresponding Ctl.


A
NT
0 Na+, 0 Ca2+
[Ca2+] (µM)
1.5
1.0
0.5
0
ksyst
kcaff
k0Na0Ca
Caffeine,
10 mmol/L
Caffeine,
10 mmol/L
B
ksyst =
kSERCA + kNCX + kPMCA
kcaff = kNCX + kPMCA
k0Na0Ca = kPMCA
ksyst (s-1)
kcaff (s-1)
k0Na0Ca (s-1)
8/5
9/6
Ctl cAF
C
kSERCA = ksyst - kcaff
kNCX = kcaff – k0Na0Ca
kSERCA (s-1)
kNCX (s-1)
D
Ctl
cAF
66±7%
25±6%
9±2%
46±5%
44±6%
9±1%
SERCA (p<0.05)
NCX (p<0.05)
PMCA

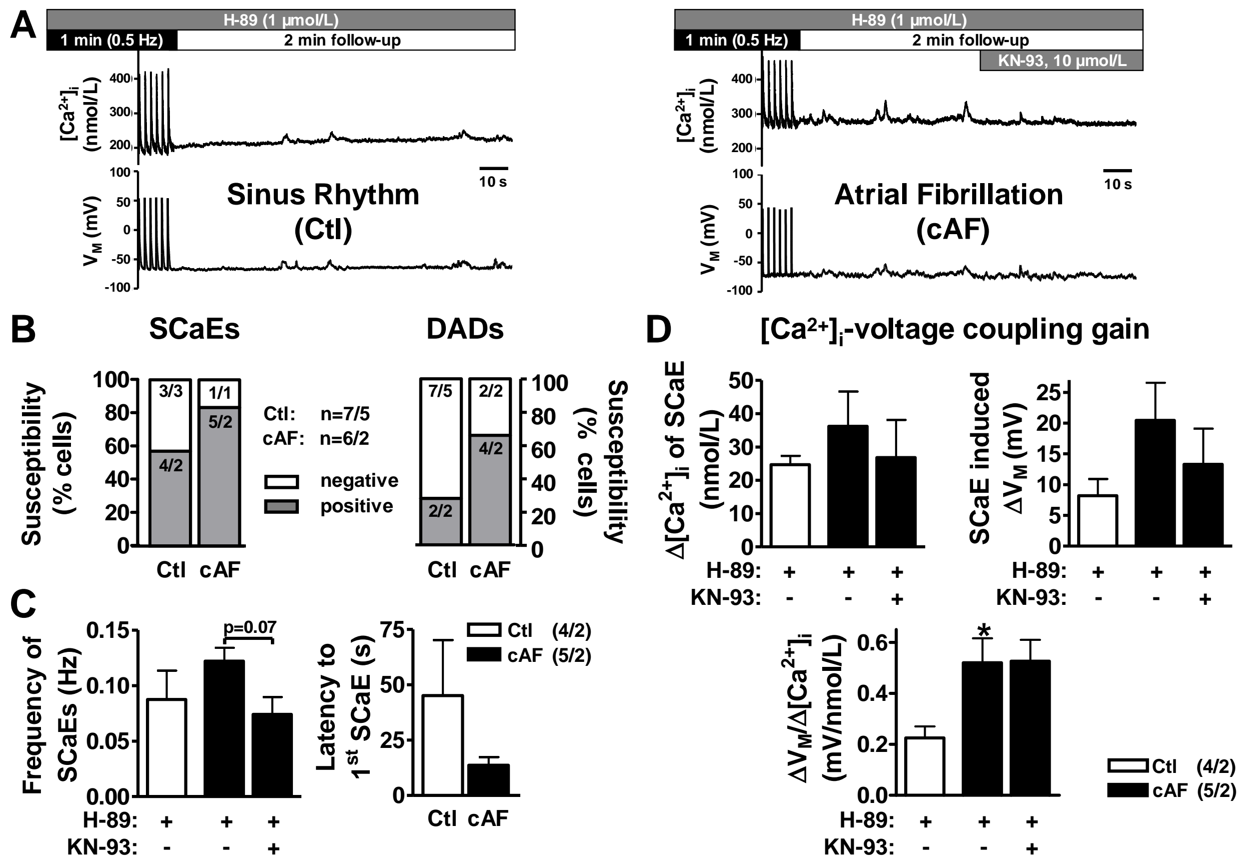
Online-Figure 3: Estimation of Ca^{2+} transport rates of SERCA, NCX and PMCA. **A,** Representative recording to illustrate the experimental protocol used to estimate Ca^{2+} transport by SERCA, NCX and PMCA. Caffeine (10 mmol/L) application following steady-state stimulation (*) with $I_{Ca,L}$ activation at 0.5 Hz (1 minute) resulted in SR Ca^{2+} depletion reflected by caffeine induced Ca^{2+} transient (cCaT). After washout the same myocyte was stimulated again at 0.5 Hz with $I_{Ca,L}$ activation to regain SR Ca^{2+} load. Finally, caffeine (10 mmol/L) was applied again while perfusion with Na^+- and Ca^{2+}- free bath solution to quantify SERCA and NCX independent Ca^{2+} removal mechanisms, which are suggested to be largely mediated by PMCA. **B,** Mean±SEM of rate constants of monoexponential curves fitted to the decay of electrically stimulated CaT (k_{syst}, left panel), caffeine evoked CaT (k_{caff}, middle panel) and caffeine evoked CaT in absence of Na^+ and Ca^{2+} in the bath solution (k_{0Na0Ca}, right panel). **C,** Mean±SEM of calculated rate constants of SERCA (k_{SERCA}) and NCX (k_{NCX}). **D,** Relative contributions of SERCA, NCX and PMCA to diastolic Ca^{2+} removal. *$P<0.05$ vs. corresponding values in Ctl myocytes. Numbers indicate myocytes/patients. For further details see Supplemental methods section.



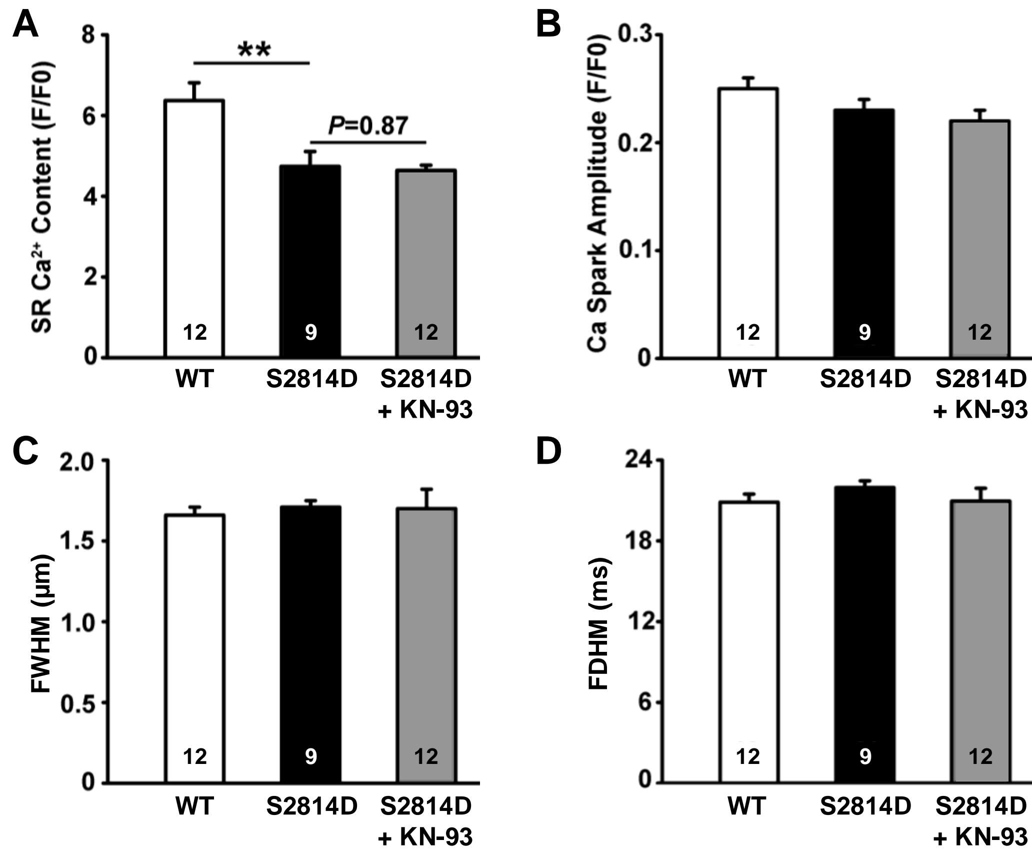
Online-Figure 4. A, Representative current amplitude histograms of RyR2 single-channel recordings from a Ctl and a cAF patient. **B,** Mean±SEM for open probability (P_o) of RyR2 before and during perfusion with the PKA-inhibitory peptide PKI (10 μmol/L). Numbers indicate channels/patients.



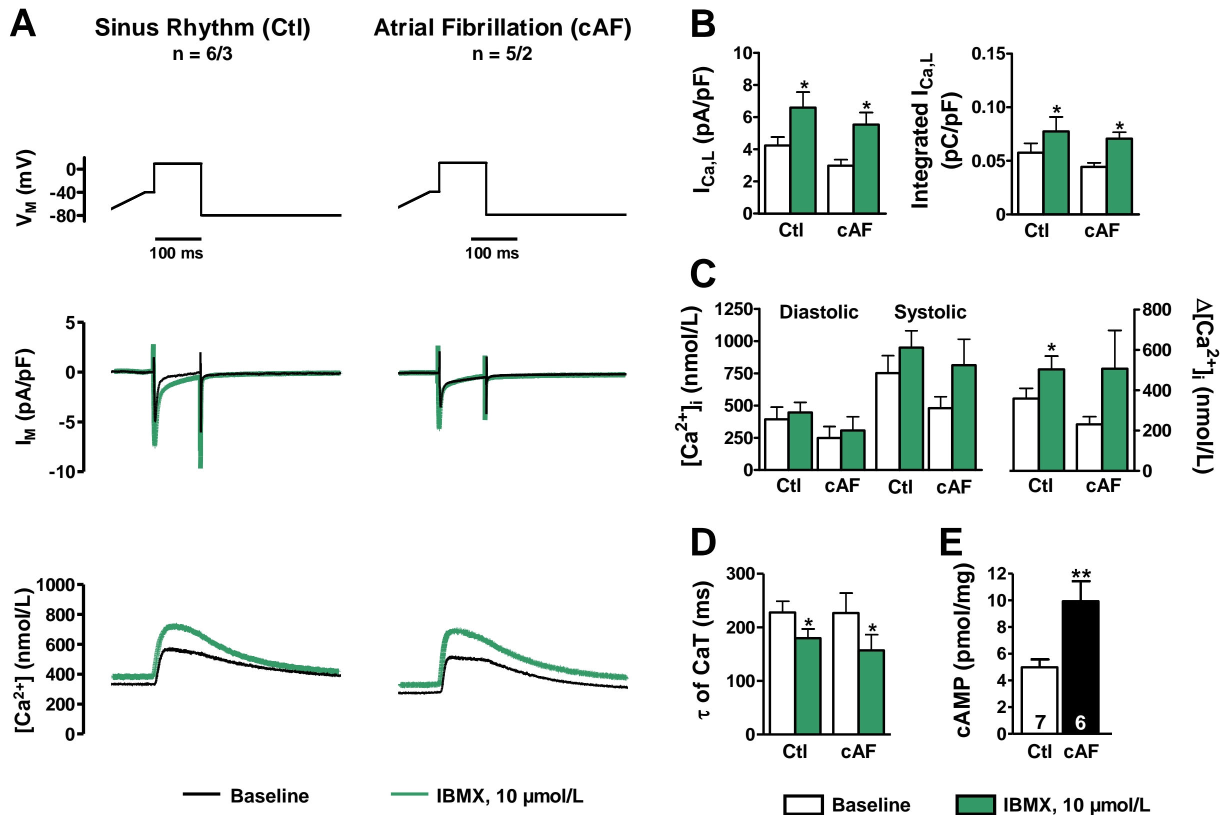
Online-Figure 5. Simultaneous recordings of membrane voltage (V_m) and $[Ca^{2+}]_i$ in atrial myocytes from Ctl and cAF patients in perforated-patch configuration after incubation with the PKA-inhibitor H-89 (1 µmol/L; 30 mins). **A,** Current-clamp protocol (0.5 Hz, top) together with simultaneous recordings of triggered AP (middle) and CaT (Fluo-3, bottom) in a Ctl (left) and in a cAF (right) myocyte. **B,** Mean±SEM resting membrane potential, AP amplitude, APD_{50} and APD_{90}, respectively. **C,** Mean±SEM diastolic and systolic $[Ca^{2+}]_i$ levels (left) and resulting CaT-amplitude (right). Numbers indicate myocytes/patients.



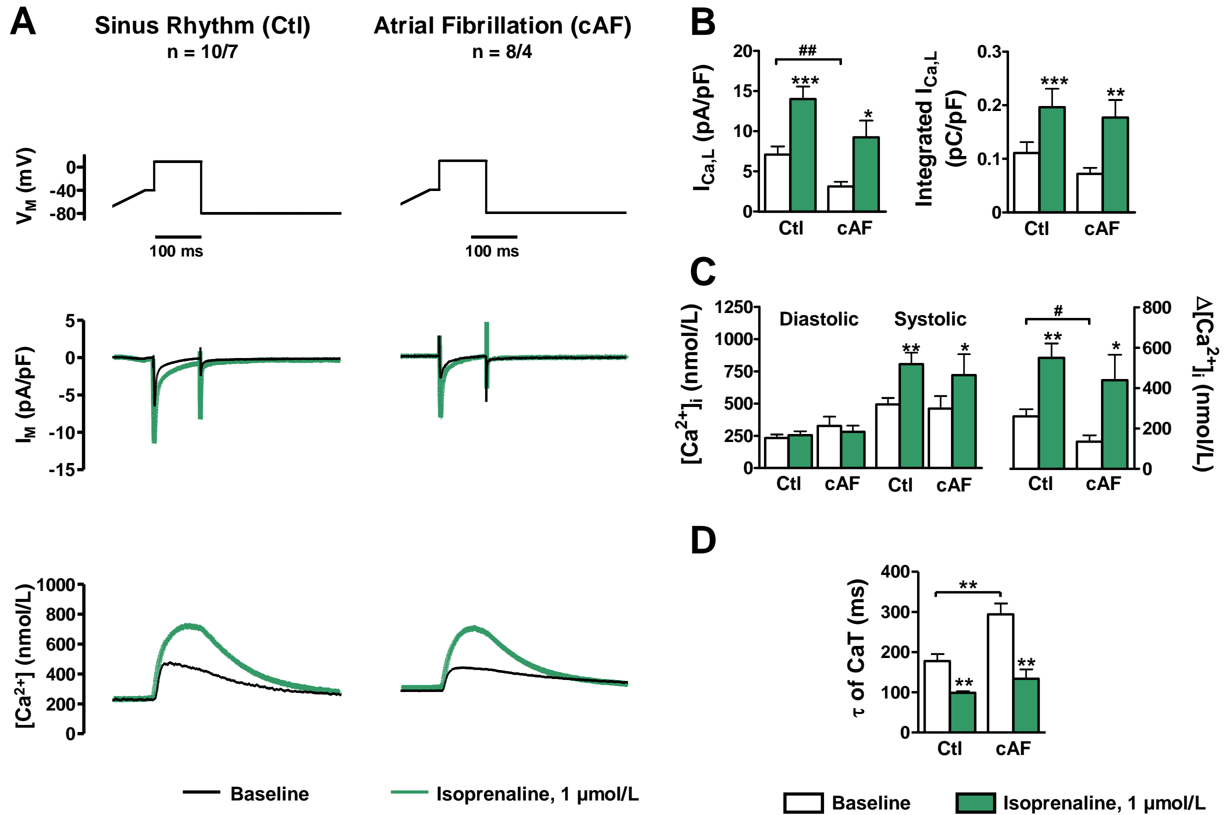
Online-Figure 6. Incidence of SCaEs and corresponding DADs in atrial myocytes from Ctl and cAF patients in perforated-patch configuration after incubation with the selective PKA-inhibitor H-89 (1 µmol/L, 30 mins). **A,** Representative recordings of $[Ca^{2+}]_i$ (Fluo-3) and corresponding membrane-voltage (V_m) oscillations (DADs/triggered APs) in a Ctl and a cAF-myocyte, respectively, following steady-state stimulation for 1-minute at 0.5 Hz. After 1-minute follow-up period the selective CaMKII-inhibitor KN-93 (10 µmol/L) was applied to cAF myocytes. **B,** Enhanced susceptibility to spontaneous Ca^{2+}-release events (SCaEs) and SCaE-induced DADs in cAF vs. Ctl. **C,** Mean±SEM for frequency (left) and latency (right) of SCaEs. **D,** Mean±SEM amplitude of SCaEs (top left), magnitude of corresponding V_m-change (top right), and the calculated $[Ca^{2+}]_i$-membrane voltage coupling gain (bottom). *P<0.05 vs. corresponding mean in Ctl. Numbers indicate myocytes/patients.



Online-Figure 7. Characteristics of Ca^{2+} sparks in atrial myocytes from WT and S2814D knock-in mice. Mean±SEM of SR Ca^{2+} content (F/F0) measured by rapid application of caffeine (10 mmol/L), spark amplitude (F/F0), full-width half-maximum (FWHM) and full-duration half-maximum (FDHM) of Ca^{2+} sparks in WT myocytes, S2814D myocytes and S2814D myocytes treated with KN-93 (10 µmol/L). Numbers indicate numbers of myocytes.



Online-Figure 8. Effect of 3-isobutyl-1-methylxanthine (IBMX, 10 µmol/L) on $I_{Ca,L}$-triggered Ca^{2+} transients (CaT) in sinus rhythm (Ctl) and atrial fibrillation (AF). **A,** Voltage-clamp protocol (0.5 Hz, top) together with simultaneous recordings of $I_{Ca,L}$ (middle) and triggered CaT (bottom) in atrial myocytes from Ctl (left) and cAF (right) patients before (baseline) and after application of IBMX (1µmol/L). **A-D**, Corresponding mean±SEM for Peak-$I_{Ca,L}$ (B, left) and integrated $I_{Ca,L}$ (B, right), diastolic and systolic $[Ca^{2+}]_i$ levels (C, left), CaT amplitude (C, right) and the time-constant (τ) of decay of $I_{Ca,L}$-triggered CaT (D). **E,** Mean±SEM of cAMP levels in right atrial biopsies from sinus rhythm and cAF patients. *P<0.05 and **P<0.01 vs. corresponding mean baseline values and Ctl myocytes, respectively. Numbers indicate myocytes/patients.



Online-Figure 9. Effect of isoprenaline (1 µmol/L) on $I_{Ca,L}$-triggered Ca^{2+} transients (CaT) in sinus rhythm (Ctl) and atrial fibrillation (AF). **A,** Voltage-clamp protocol (0.5 Hz, top) together with simultaneous recording of $I_{Ca,L}$ (middle) and triggered CaT (bottom) in atrial myocytes from Ctl (left) and cAF (right) patients before (baseline) and after application of isoprenaline. **A-D**, Corresponding mean±SEM for Peak-$I_{Ca,L}$ (B, left) and integrated $I_{Ca,L}$ (B, right), diastolic and systolic $[Ca^{2+}]_i$ levels (C, left), CaT amplitude (C, right) and the time-constant (τ) of decay of $I_{Ca,L}$-triggered CaT (D). *P<0.05, **P<0.01, ***P<0.001, [#]P<0.05 and [##]P<0.01 vs. corresponding mean baseline values and Ctl myocytes, respectively. Numbers indicate myocytes/patients.

References

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