

SUPPLEMENTAL MATERIAL

PDE8 governs cAMP/PKA-dependent reduction of L-type calcium current in human atrial fibrillation

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Markus Kamler, Renate B. Schnabel, Istvan Baczkó, Anne Garnier, Hermann
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SUPPLEMENTARY FIGURES

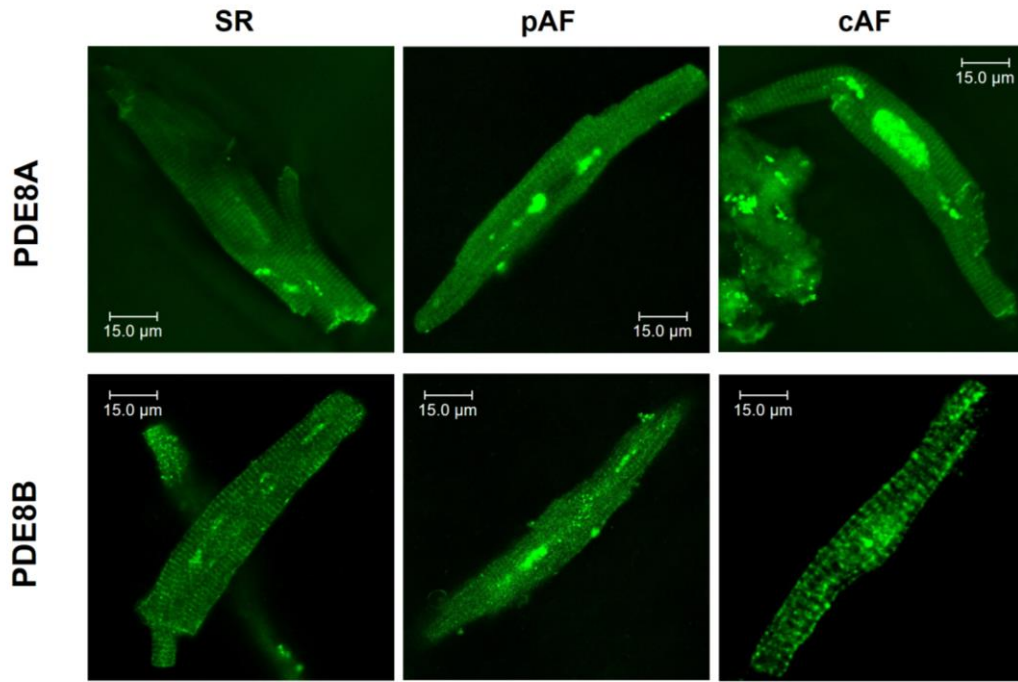


Figure S1. PDE8 localization in human atrial myocytes. Representative immunocytochemistry confocal images of PDE8A (top) showing its cytosolic distribution in isolated human atrial myocytes (HAMs) from sinus rhythm (SR), paroxysmal atrial fibrillation (pAF) and persistent (chronic) atrial fibrillation (cAF) patients (from left to right). Lower pictures show similar representative immunocytochemistry confocal images of PDE8B localization mainly at the plasma membrane, in HAMs from SR, pAF and cAF patients (from left to right). Gain was increased in order to obtain a better signal in images where the matched intensity was not sufficient to compare PDE8 isoform localizations.

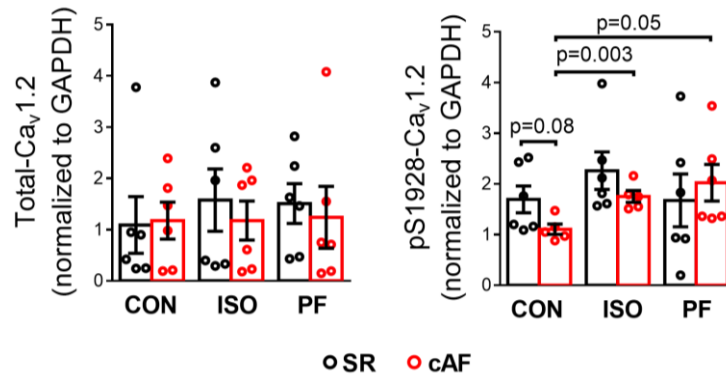


Figure S2. LTCC protein expression. Western-blot quantification of the protein expression of total-Ca_v1.2 (left, mean±SEM) and PKA phosphorylated pSer1928-Ca_v1.2 (right, mean±SEM) in atrial tissue homogenates from 6 SR and 6 cAF patients, at baseline and after 5 min stimulation with the selective PDE8 inhibitor PF-04957325 (30 nM) or the β -adrenoceptor agonist isoprenaline (ISO, 100 nM). GAPDH was used as loading control. *p<0.05 based on ANOVA with a Kruskal–Wallis test.

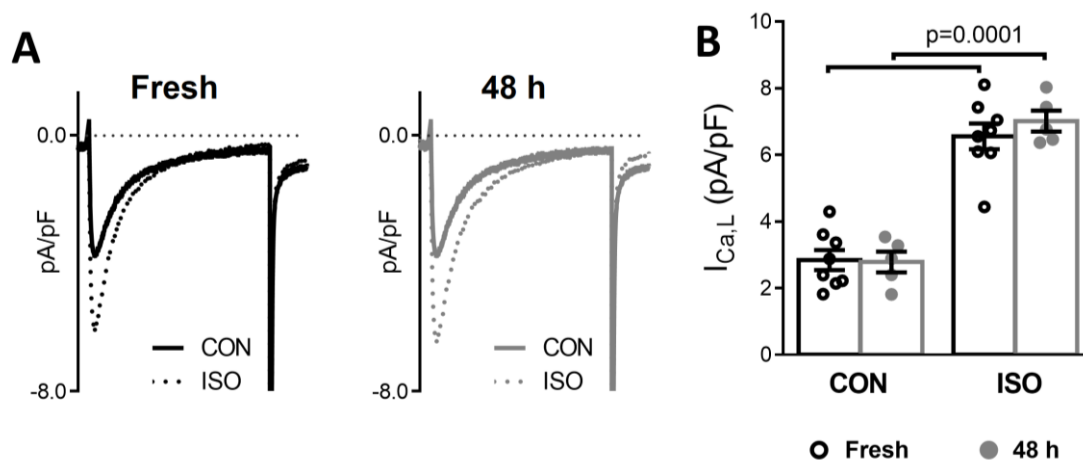


Figure S3. Functional characterization of human atrial myocytes in culture. From left to right: Representative L-type calcium current ($I_{Ca,L}$) patch-clamp recordings in freshly isolated (2 hours of culture) and after 48 hours of culture human atrial cardiomyocytes (HAMs). Quantification of $I_{Ca,L}$ density in cardiomyocytes from patients in sinus rhythm before (2 h) and after 48 hours of culture (48 h), at baseline (CON) and upon β -adrenoceptor stimulation with 100 nM isoprenaline (ISO). Individual and mean values of $I_{Ca,L}$ density in HAMs. # $p<0.05$ compared to CON based on ANOVA.

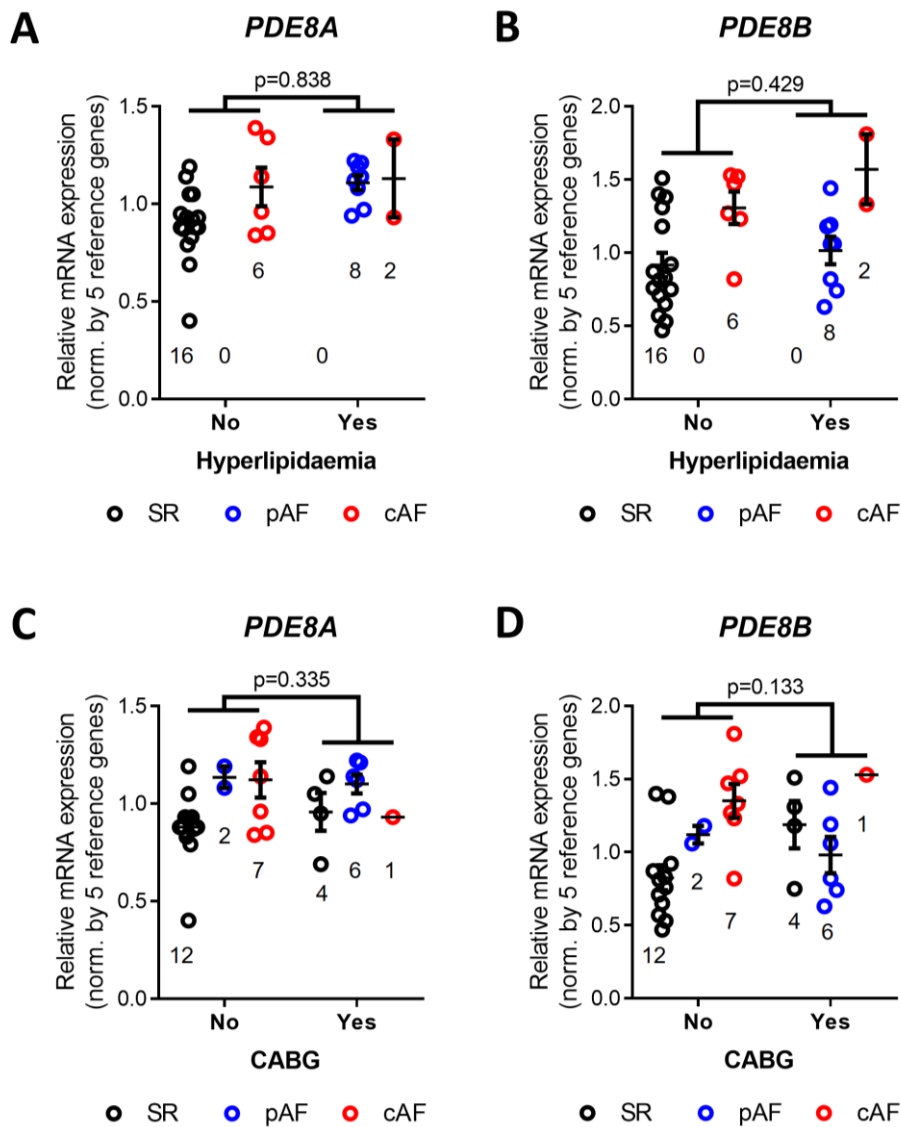


Figure S4. Relationship between hyperlipidaemia or cardiopulmonary bypass surgery and *PDE8A* or *PDE8B* mRNA expression. A-D, Two-way ANOVA analysis of atrial fibrillation rhythm (paroxysmal or persistent-chronic, pAF or cAF) status and hyperlipidaemia or bypass surgery (no vs. yes) for *PDE8A* (panels A and C) and *PDE8B* (panels B and D) mRNA expression levels. P values reflect significance level of the factor “hyperlipidaemia” or “bypass surgery” in two-way ANOVA. Numbers below symbols indicate number of patients. [Figure related to Supplementary Table 2.](#) Note that individual subgroups may be small, limiting the statistical power and robustness of these subanalyses.

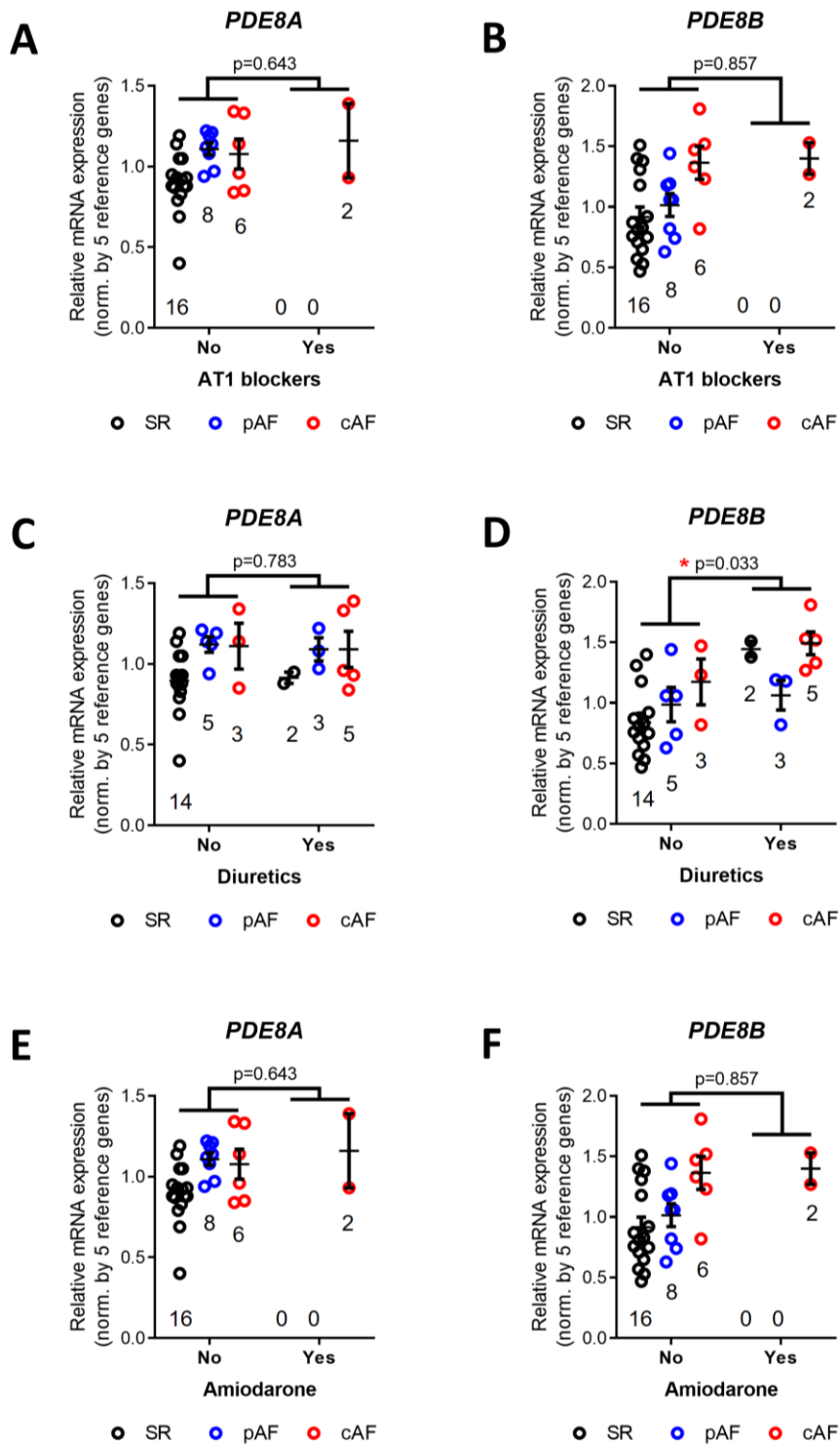


Figure S5. Relationship between angiotensin (AT) 1 blockers or diuretics or amiodarone use and *PDE8A* or *PDE8B* mRNA expression. A-F, Two-way ANOVA analysis of atrial fibrillation rhythm (paroxysmal or persistent-chronic, pAF or cAF) status and AT1 blockers or diuretics or amiodarone use (no vs. yes) for *PDE8A* (panels

A, **C** and **E**) and *PDE8B* (panels **B**, **D** and **F**) mRNA expression levels. P values reflect significance level of the factor “AT1 blockers” or “diuretics” or “amiodarone” in two-way ANOVA. Numbers below symbols indicate number of patients. **Figure related to Supplementary Table 2.** Note that individual subgroups may be small, limiting the statistical power and robustness of these subanalyses.

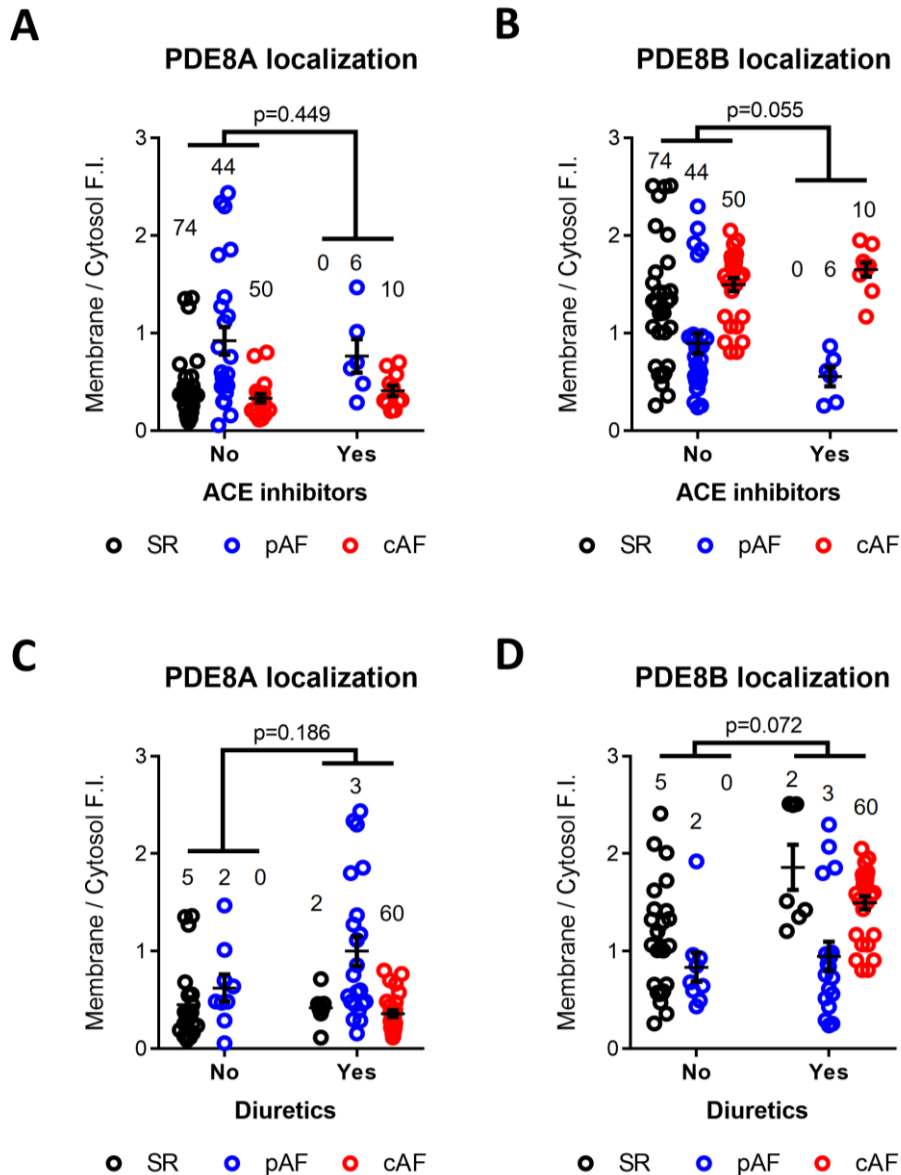


Figure S6. Relationship between angiotensin-converting enzyme (ACE) inhibitors or diuretics use and PDE8A or PDE8B localization. A-D, Two-way ANOVA analysis of atrial fibrillation rhythm (paroxysmal or persistent-chronic, pAF or cAF) status and ACE inhibitors or diuretics use (no vs. yes) for PDE8A (panels **A** and **C**) and PDE8B (panels **B** and **D**) localization. P values reflect significance level of the factor “ACE inhibitors” or “diuretics” in two-way ANOVA. Numbers above symbols indicate number of cells. **Figure related to Supplementary Table 5.** Note that individual subgroups may be small, limiting the statistical power and robustness of these subanalyses.

cAMP increase upon PDE8 inhibition

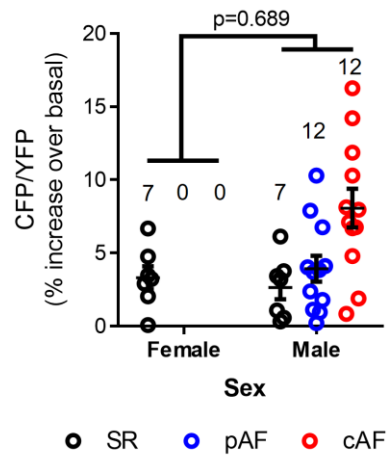


Figure S7. Relationship between sex and the increase in cAMP levels upon PDE8 inhibition. Two-way ANOVA analysis of atrial fibrillation rhythm (paroxysmal or persistent-chronic, pAF or cAF) status and sex (Female vs. Male) for the increase in cAMP levels upon PDE8 inhibition. P values reflect significance level of the factor “sex” in two-way ANOVA. Numbers above symbols indicate number of cells. **Figure related to Supplementary Table 6.** Note that individual subgroups may be small, limiting the statistical power and robustness of these subanalyses.

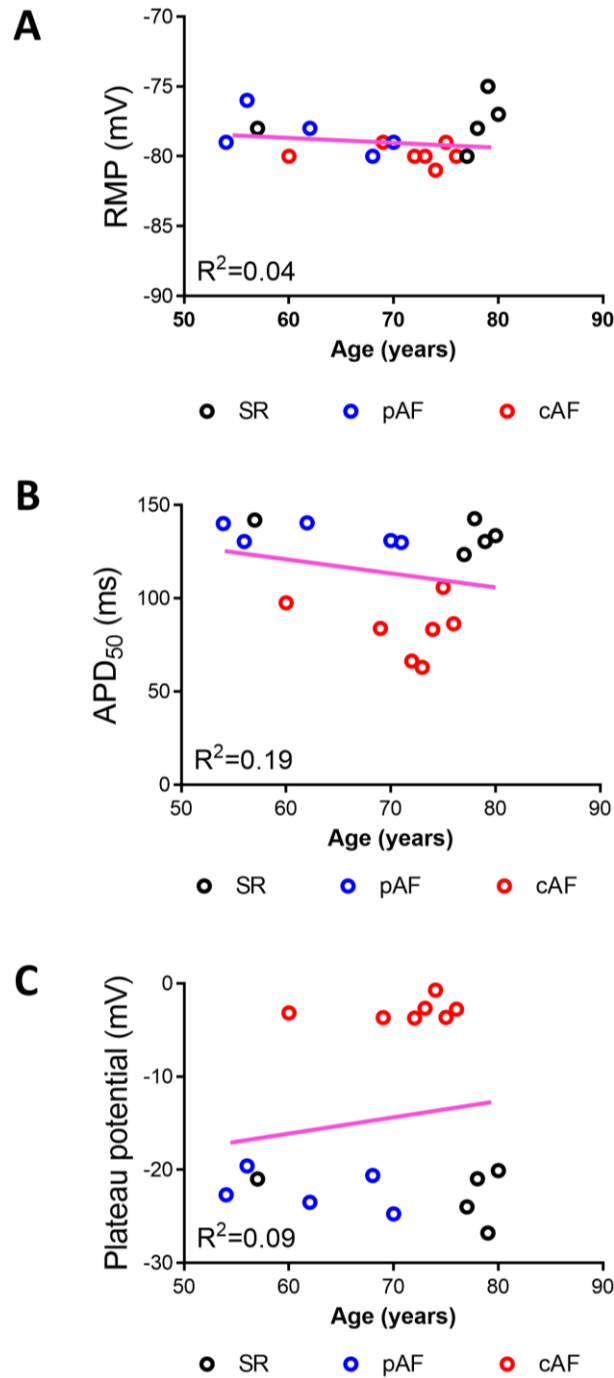


Figure S8. Age and rhythm dependency of the resting membrane potential (RMP) or the action potential duration at 50% of repolarization (APD₅₀) or the plateau potential parameters in human atrial trabeculae. Correlation between parameter value in sinus rhythm or paroxysmal or persistent-chronic (SR or pAF or cAF) and age, with corresponding correlation coefficients (R^2). **A**, RMP. **B**, APD₅₀. **C**, Plateau potential.

Figure related to Supplementary Table 8.

SUPPLEMENTAL MATERIAL

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SUPPLEMENTARY METHODS

RNA isolation, cDNA synthesis, real-time qPCR and quantification. Frozen tissue samples from 48 patients (16 SR, 8 pAF, 8 cAF, 8Ctl and 8 HF patients) were weighed and sited in pre-cooled tubes containing TRIzol® reagent (Invitrogen, Life Technologies, France) and rapidly subjected to automated grinding in a Bertin Precellys 24 (Bertin Technologies, France). Total RNA extraction was carried out using standard procedure according to the manufacturer's instructions. RNA concentration and purity were evaluated by optical density (Biophotometer, Eppendorf, BioServ, France). RNA integrity was analysed on a Bioanalyzer 2100 with the RNA6000 Nano Labchip Kit (Agilent Technologies, Santa Clara, CA, USA). First strand cDNA synthesis was performed from 1 µg of total RNA with random primers and MultiScribe™ Reverse Transcriptase according to the provided protocol (Applied Biosystems, Life Technologies, France). RNA integrity number (RIN) values were ranged between 5.6 and 8.5, with a mean of 6.9 ± 0.2 for SR, 6.4 ± 0.3 for pAF, 6.6 ± 0.2 for cAF, 7.0 ± 1.1 for Ctl and 7.4 ± 0.9 for HF. There were no differences between groups in RNA content. Real-time PCR assays were performed as previously described¹⁸ to study the expression of 2 target genes (*PDE8A*, *PDE8B*) related to specifically chosen²⁸ reference genes (*POLR2A*, *YWHAZ*, *GAPDH*, *IPO8*, *PPIA*). Experiments were performed in triplicates for each sample. An average Ct value was calculated for each group of patients. Relative gene expression ratio was determined using the $\Delta\Delta C_t$ method and normalized by the geometric mean of the set of stable reference genes. Normalization was performed using multiple reference genes instead of one in order to measure expression levels of targeted genes accurately. *POLR2A*, *YWHAZ*, *GAPDH*, *IPO8*, *PPIA* were chosen and validated as reference genes for human heart samples a previously described²⁶. The normalization factor was calculated using the geometric mean of these five reference genes instead of

the arithmetic mean in order to control better possible outliers and abundance differences between the different genes.

Western Blot analysis. 54 snap-frozen atrial samples were used to perform western blot. From those, 12 samples were first 5 min incubated with control buffer (CON), 30 nM PF-04957325 (PF), or 100 nM β -adrenergic agonist isoprenaline (ISO), and then snap-frozen. The samples were then homogenized in lysis buffer (Tris 0.03M, EDTA 0.005 M, NaF 0.03 M, 3% SDS, 10% Glycerol, Protease- and Phosphatase-Inhibitor Cocktail, Roche) using a homogenizer (MICCRA D-1). After three to five homogenization steps for 10 seconds each, always followed by cooling of the samples in liquid nitrogen, samples were incubated on ice for 1 hour. After centrifugation (900 g, 15 min, RT) supernatants were stored at -80°C until usage. Protein quantification was performed using BCA Protein Assay (Pierce BCA Protein Assay Kit, Thermo Scientific, #23227), samples were denatured at 95°C 5 min and 50 μ g of total protein were loaded on 8% SDS gels for SDS polyacrylamide gel electrophoresis. Proteins were transferred to nitrocellulose membrane (Amersham, #106.000.02) using a tank blot system. For immunoblot analysis, the following antibodies were used: PDE8A (Santa Cruz Biotechnology sc-30059, rabbit polyclonal antibody, 1:500 in 3% BSA, sample cooking 70°C 10 min), PDE8B (Santa Cruz Biotechnology sc-17234, goat polyclonal antibody, 1:200 in 3% BSA, 70°C 10 min), Cav1.2 α 1C (Alomone ACC-022, rabbit polyclonal antibody, 1:160 in 5% milk, 70°C 10 min), phosphospecific antibody anti-CH3P against Cav1.2-phosphoSer1928 (kindly provided by William A. Catterall, rabbit polyclonal antibody, 1:1000 in 5% milk, 70°C 10 min) and GAPDH (HyTest #5G4.6C5, mouse monoclonal antibody, 1:20,000 in 5% milk). For quantification, band densitometry analysis was done using ImageJ software.

Co-Immunoprecipitation. 15 snap-frozen samples were homogenized in immunoprecipitation buffer (10 mM HEPES, 5 mM EDTA, 500 mM Sucrose, 0.1% SDS,

0.5% Triton, 20 mM NaF, 1 mM Na₃VO₄, Protease- and Phosphatase-Inhibitor Cocktail). 500 µg of homogenate were incubated with 2 µg Cav1.2 antibody (Alomone; ACC-003) and 150 µl Protein G Sepharose beads (GE Healthcare) overnight. Subsequently, samples were washed 4x using PBS and centrifuged after each wash step (800 g, 2 min, 4°C). Samples were denatured at 95°C 5 min in 2x loading buffer. After centrifugation (800 g, 2 min, 4°C), 30 µl homogenate was loaded on 8-12% SDS gels. Detection of co-immunoprecipitated proteins were performed using specific antibodies against PDE8A (Santa Cruz Biotechnology sc-30059, rabbit antibody, 1:500), PDE8B (Santa Cruz Biotechnology sc-17234, goat antibody, 1:200), PKAIIα reg (Santa Cruz Biotechnology sc-908, rabbit antibody, 1:200) and PKAc (BD Biosciences 610980, mouse antibody, 1:200). For quantification, band densitometry analysis was done using ImageJ software.

Isolation and culture of HAMs. After surgical excision, tissue samples were placed into Custodiol® solution (Dr. Franz Köhler Chemie GmbH, Bensheim, Germany) for the transport to the laboratory. Cell isolations from 47 patients were carried out as previously described⁷. Tissue was cut into small cubes and digested in Enzyme solution I [Stop Ca²⁺ free solution containing 0.5 mg ml⁻¹ collagenase (Worthington type 1, 240 U mg⁻¹; Lakewood, New Jersey, USA), 0.5 mg ml⁻¹ proteinase (Sigma type XXIV, 11 U mg⁻¹; St. Louis, Missouri, USA) and 2% bovine serum albumin (BSA; Sigma, St. Louis, Missouri, USA)] at 200 rpm and 37°C 30 min. Next, tissue cubes were transferred to Stop Ca²⁺ free solution [in mM: 88 Sucrose, 88 NaCl, 5.4 KCl, 4 NaHCO₃, 0.3 NaH₂PO₄, 1.1 MgCl₂, 10 HEPES, 20 Taurine, 10 Glucose, 5 Na⁺ pyruvate. 7.4 pH at RT; 5% BSA, and 2 µM blebbistatin (Sigma, St. Louis, Missouri, USA)] and moved up and down in a Pasteur pipette. The remaining tissue was digested for five more rounds maximum with Enzyme solution II (Ca²⁺-free solution containing 0.4 mg ml⁻¹ collagenase and 2% BSA) at 200 rpm and 37°C 15 min. HAMs were harvested by centrifugation (500 rpm, 5 min,

RT). For patch-clamp experiments, HAMs were resuspended in Stop Ca^{2+} free solution and Ca^{2+} was re-introduced by a gentle increase to 1 mmol/L. For Förster-resonance energy transfer (FRET) experiments, HAMs were resuspended in plating medium M1 [MEM (M4780; Sigma, St Louis, Missouri, USA) containing 2 mmol/L Ca^{2+} , 2.5% fetal bovine serum (FBS, Invitrogen, Cergy-Pontoise, France), 1% penicillin-streptomycin and 2 μM blebbistatin]. HAMs were then plated on Laminin-coated dishes (Cellvis Glasbottom dish, 29 mm with 10 mm bottom well). After 2-hours at 37°C and 5% CO_2 , medium was changed to medium M2 (FBS-free M1) containing adenovirus and HAMs were kept in culture at 37°C and 5% CO_2 for 48-hours. The multiplicity of infection (MOI) was 200 PFU/cell. Only striated, rod-shaped HAMs were used.

Perforated patch-clamp in freshly-isolated and cultured HAMs. Whole-cell perforated patch-clamp configuration was used to record $I_{\text{Ca,L}}$ in 16 HAMs from 10 patients as previously described^{4,11}. Pipettes had a resistance between 1.5–3 $\text{M}\Omega$ and were filled with internal solution containing (in mM): aspartic acid 109, CsCl 47, MgCl_2 1, Mg_2ATP 3, Na_2 -phosphocreatine 5, Li_2GTP 0.42, HEPES 10, adjusted to pH 7.2 with CsOH. Amphotericin (250 $\mu\text{g}/\text{mL}$) was added to the pipette solution before each experiment. Extracellular solution contained (in mM): CaCl_2 2, MgCl_2 1.8, NaCl 127.1, NaHCO_3 4, NaH_2PO_4 0.33, D-glucose 10, pyruvic acid 5, HEPES 10, MgCl_2 1.8, adjusted to pH 7.4 with NaOH. Amphotericin-B (250-mg/mL) was added to the tip of the pipette solution. For $I_{\text{Ca,L}}$ measurement, cells were depolarized from -80 to -50 and to 0 mV during 200 ms at 0.5 Hz, and the current-voltage (I-V) dependence was determined by depolarizing the myocytes to potentials between -40 and +50 mV (in increments of 10 mV). A 50 ms pre-pulse of -50 mV was used to inactivate voltage-dependent Na^+ -currents. K^+ -currents were blocked by replacing all K^+ -ions with external and internal Cs^+ . Myocytes were voltage-clamped at room temperature using an EPC-10 patch-clamp

amplifier (HEKA Elektronik, Germany). The maximal amplitude of whole-cell $I_{Ca,L}$ was measured at 0 mV as the difference between the peak inward current and the current at the end of the depolarization step. $I_{Ca,L}$ amplitudes were corrected for membrane capacitance. Membrane capacitance was calculated as the time integral of current responses to 1-mV hyperpolarizing step changes in membrane potential. Experiments were performed under stable access resistance only when it had decreased to a value only 5-fold higher than intrinsic pipette resistance. No series resistance compensation was employed.

FRET-based live-cell imaging of sarcolemmal cAMP in HAMs. FRET measurements were performed on 38 living HAMs from 18 patients, transduced with adenovirus encoding pm-Epac1-camps to measure cAMP at the membrane²⁹. Cells were maintained in a K⁺-Ringer solution containing (in mM): 144 NaCl, 5.4 KCl, 1 MgCl₂, 1 CaCl₂, 10 Hepes, adjusted to 7.4 pH with NaOH at RT and images were taken every 5 seconds. The FRET system used consist of an LED light source (pE-100, CoolLED), a beam splitter (DV2, Photometrix) and a camera (CMOS camera optiMOS, QImaging), together with a standard inverted microscope (Nikon eclipse Ti-4) and a 60x/1.5 oil immersion objective. To excite the donor fluorophore (CFP) a single-wavelength light-emitting diode (LED, 440 nm) was used. The LED was controlled by an Arduino digital-to-analogue input-output board (Arduino). A Dual View beam splitter (Cube 05-EM, 505 dcxr, D480/30m, D535/40) was used to split the emission light into donor (CFP) and acceptor (YFP) channels. Single channels intensities were recorded with a CMOS camera (QImaging). The software Micro Manager 1.4.5 was used to perform time-laps image acquisition. Microsoft Excel was used for data analysis. The corrected FRET ratio was calculated as follows: FRET ratio cAMP measurements= $YFP - (B * CFP) / CFP$. Bleed through (B) corrected FRET traces were normalized to baseline and FRET response was quantified.

Immunocytochemistry and confocal imaging. HAMs were fixed with paraformaldehyde (PFA; 2%) for 15 min. After 15 min incubation at RT, PFA was removed and neutralized with Glycine 0.1 M for 10 min. Myocytes were then 3x washed 5 min with phosphate-buffered saline (PBS) and permeabilized with Triton X-100 (0.5%) for 15 min. HAMs were rinsed again three times with PBS and blocked with 1% BSA in PBS for 40 min. Myocytes were then labeled with a primary rabbit polyclonal PDE8A antibody (Santa Cruz Biotechnology sc-30059, 1:200) or a goat polyclonal PDE8B antibody (Santa Cruz Biotechnology sc-17234, 1:200), diluted in PBS containing 10% goat serum and 0.25% Triton X-100. After an overnight incubation at 4°C, HAMs were washed three times using 1% BSA and then incubated for 2 h with AlexaFluor® 488 conjugated anti-mouse IgG to reveal the PDE8 staining. Images of 74 myocytes from 7 SR patients, 50 myocytes from 5 pAF patients, and 60 myocytes from 7 cAF patients were acquired using a Carl Zeiss (Oberkochen, Germany) LSM 510 confocal laser scanning microscope. Optical section series were obtained with a Plan Apochromat 63x objective (NA 1.4, oil immersion). The fluorescence was observed with a BP 505-550 nm emission filter under 488-nm laser illumination, respectively.

Sharp-electrode AP-recordings. APs were recorded with standard intracellular microelectrodes in atrial trabeculae from 5 SR, 5 pAF and 7 cAF patients³⁰. Trabeculae were then mounted on the organ bath and perfused with 50-mL of recirculating (flow rate 7 mL/min), oxygenized Tyrode's solution containing (in mM): NaCl 127, KCl 4.5, MgCl₂ 1.5, CaCl₂ 1.8, glucose 10, NaHCO₃ 22, NaH₂PO₄ 0.42, equilibrated with 5 % CO₂ in 95 % O₂ at 36 °C, pH 7.4. Microelectrodes filled with 2.5 M KCl. Pipette resistances were between 20 and 80 MΩ. Preparations were stimulated at 1-Hz for at least 1-h before data acquisition.

Chemicals. 3-isobutyl-1-methylxanthine (IBMX) was from AppliChem (Darmstadt, Germany). PF-04957325 (PF, MCE, Sweden), Isoprenaline (ISO), laminin, and all other chemicals were from Sigma-Aldrich (Deisenhofen, Germany).

Table S1: Clinical characteristics of all the patients included in the study

	SR (n = 67)	pAF (n = 38)	cAF (n = 60)	p-value
Age, years	70.2 ± 1.1	69.3 ± 1.5	71.2 ± 1.2	0.416
Male gender, n (%)	49 (73%)	23 (61%)	50 (83%)	0.504
Body mass index, kg/m ²	27.5 ± 1.2	27.3 ± 0.7	26.5 ± 0.8	0.746
Arterial hypertension, n (%)	35 (52%)	14 (37%)	42 (70%)	0.083
Diabetes, n (%)	15 (22%)	16 (42%)	19 (32%)	0.125
Hyperlipidaemia, n (%)	48 (72%)	16 (42%)	43 (70%)	0.008
LVEF, %	58.7 ± 1.4	55.2 ± 1.7	55.1 ± 1.2	0.098
Indication for surgery				
CABG, n (%)	22 (33%)	13 (34%)	15 (25%)	0.493
AVD, n (%)	8 (12%)	4 (11%)	16 (27%)	0.077
MVD, n (%)	7 (11%)	11 (29%)	13 (22%)	0.053
CABG+ MVD/AVD, n (%)	29 (43%)	10 (26%)	15 (25%)	0.074
Others, n (%)	1 (2%)	0 (0%)	1 (1%)	0.739
Medication				
Digitalis, n (%)	1 (2%)	2 (5%)	10 (16%)	0.008
Beta-blockers, n (%)	34 (51%)	29 (76%)	46 (77%)	0.010
ACE inhibitors, n (%)	36 (54%)	22 (58%)	28 (47%)	0.418
AT1 blockers, n (%)	10 (15%)	1 (3%)	6 (10%)	0.149
Diuretics, n (%)	10 (15%)	15 (40%)	25 (42%)	0.006
Nitrates, n (%)	6 (9%)	0 (0%)	5 (8%)	0.172
Amiodarone, n (%)	0 (0%)	3 (8%)	7 (12%)	0.021
Statins, n (%)	19 (28%)	14 (37%)	19 (31%)	0.759

Data presented as numbers (%) or as mean ± SEM; Ctl: control-sinus rhythm, pAF: paroxysmal atrial fibrillation, cAF: persistent (chronic) atrial fibrillation, MVD/AVD, mitral/aortic valve disease, LVEF - left ventricular ejection fraction, CABG: coronary artery bypass graft surgery, ACE - angiotensin-converting enzyme; AT – angiotensin.

Table S2: Clinical characteristics of the patients employed for RT-qPCR experiments

	SR (n = 16)	pAF (n = 8)	cAF (n = 8)	p-value
Age, years	69.7 ± 2.1	76 ± 4.0	76.6 ± 1.8	0.053
Male gender, n (%)	11 (69%)	6 (75%)	7 (87%)	0.378
Body mass index, kg/m ²	27.6 ± 1	29.9 ± 0.7	26.4 ± 0.7	0.097
Arterial hypertension, n (%)	2 (12%)	4 (50%)	4 (50%)	0.079
Diabetes, n (%)	1 (6%)	0 (0%)	0 (0%)	0.607
Hyperlipidaemia, n (%)	0 (0%)	8 (100%)	2 (25%)	0.0001
LVEF, %	62.1 ± 1.3	58.1 ± 2.3	59.1 ± 2.7	0.246
Indication for surgery				
CABG, n (%)	4 (25%)	6 (75%)	1 (12%)	0.019
AVD, n (%)	1 (6%)	0 (0%)	2 (25%)	0.201
MVD, n (%)	0 (0%)	0 (0%)	1 (12%)	0.223
CABG+ MVD/AVD, n (%)	11 (69%)	2 (25%)	4 (50%)	0.135
Medication				
Digitalis, n (%)	0 (0%)	1 (12%)	0 (0%)	0.223
Beta-blockers, n (%)	7 (44%)	4 (50%)	6 (75%)	0.356
ACE inhibitors, n (%)	9 (56%)	3 (37%)	2 (25%)	0.331
AT1 blockers, n (%)	0 (0%)	0 (0%)	2 (25%)	0.045
Diuretics, n (%)	2 (12%)	3 (37%)	5 (62%)	0.045
Nitrates, n (%)	0 (0%)	0 (0%)	0 (0%)	0.172
Amiodarone, n (%)	0 (0%)	0 (0%)	2 (25%)	0.045
Statins, n (%)	4 (25%)	3 (37%)	2 (25%)	0.799

Data presented as numbers (%) or as mean ± SEM; Ctl: control-sinus rhythm, pAF: paroxysmal atrial fibrillation, cAF: persistent (chronic) atrial fibrillation, MVD/AVD, mitral/aortic valve disease, LVEF - left ventricular ejection fraction, CABG: coronary artery bypass graft surgery, ACE - angiotensin-converting enzyme; AT – angiotensin.

Table S3: Clinical characteristics of the patients employed to compare control and heart failure in RT-qPCR experiments

	Ctl (n = 8)	HF (n = 8)	p-value
Age, years	44 ± 4.7	43.6 ± 6.3	0.939
Male gender, n (%)	5 (62%)	5 (62%)	0.999
Body mass index, kg/m ²	n.a.	28.1 ± 1.5	-
Arterial hypertension, n (%)	n.a.	6 (75%)	-
Diabetes, n (%)	n.a.	2 (25%)	-
Hyperlipidaemia, n (%)	n.a.	3 (37%)	-
LVEF, %	n.a.	27.6 ± 4.3	-
Indication for surgery			
Transplant, n (%)	0 (0%)	8 (100%)	-
Medication			
Digitalis, n (%)	n.a.	0 (0%)	-
Beta-blockers, n (%)	n.a.	6 (75%)	-
ACE inhibitors, n (%)	n.a.	3 (37%)	-
AT1 blockers, n (%)	n.a.	1 (12%)	-
Diuretics, n (%)	n.a.	7 (87%)	-
Nitrates, n (%)	n.a.	0 (0%)	-
Amiodarone, n (%)	n.a.	0 (0%)	-
Statins, n (%)	n.a.	1 (12%)	-

Data presented as numbers (%) or as mean ± SEM; Ctl: control-sinus rhythm, HF: heart failure, MVD/AVD, mitral/aortic valve disease, LVEF - left ventricular ejection fraction, CABG: coronary artery bypass graft surgery, ACE - angiotensin-converting enzyme; AT – angiotensin; n.a. – not available.

Table S4: Clinical characteristics of the patients employed for western-blotting and co-immunoprecipitation experiments

	SR (n = 28)	pAF (n = 14)	cAF (n = 27)	p-value
Age, years	70.6 ± 1.5	69 ± 1.6	69.8 ± 1.6	0.832
Male gender, n (%)	24 (86%)	10 (71%)	18 (67%)	0.248
Body mass index, kg/m ²	27.1 ± 1.7	27.9 ± 1	26.8 ± 1.4	0.906
Arterial hypertension, n (%)	20 (71%)	7 (50%)	21 (75%)	0.184
Diabetes, n (%)	7 (28%)	9 (64%)	9 (33%)	0.066
Hyperlipidaemia, n (%)	9 (32%)	5 (36%)	6 (22%)	0.598
LVEF, %	57.1 ± 2.5	52.2 ± 3.8	51.5 ± 1.7	0.376
Indication for surgery				
CABG, n (%)	11 (39%)	4 (29%)	8 (30%)	0.689
AVD, n (%)	3 (10%)	1 (7%)	6 (22%)	0.333
MVD, n (%)	4 (14%)	4 (29%)	7 (26%)	0.459
CABG+ MVD/AVD, n (%)	9 (32%)	5 (36%)	6 (22%)	0.598
Others, n (%)	1 (3%)	0 (0%)	0 (0%)	0.331
Medication				
Digitalis, n (%)	1 (3%)	1 (7%)	4 (15%)	0.336
Beta-blockers, n (%)	16 (57%)	11 (78%)	21 (78%)	0.184
ACE inhibitors, n (%)	17 (61%)	9 (64%)	17 (63%)	0.972
AT1 blockers, n (%)	3 (10%)	1 (7%)	1 (4%)	0.609
Diuretics, n (%)	4 (14%)	5 (36%)	6 (22%)	0.288
Nitrates, n (%)	4 (14%)	0 (0%)	5 (18%)	0.245
Amiodarone, n (%)	0 (0%)	1 (7%)	2 (7%)	0.348
Statins, n (%)	5 (18%)	6 (43%)	5 (18%)	0.152

Data presented as numbers (%) or as mean ± SEM; Ctl: control-sinus rhythm, pAF: paroxysmal atrial fibrillation, cAF: persistent (chronic) atrial fibrillation, MVD/AVD, mitral/aortic valve disease, LVEF - left ventricular ejection fraction, CABG: coronary artery bypass graft surgery, ACE - angiotensin-converting enzyme; AT – angiotensin.

Table S5: Clinical characteristics of the patients employed for immunostaining experiments

	SR (n = 7)	pAF (n = 5)	cAF (n = 7)	p-value
Age, years	72.1 ± 6.2	72.4 ± 4.9	68.9 ± 6.9	0.574
Male gender, n (%)	4 (57%)	5 (100%)	7 (100%)	0.083
Body mass index, kg/m ²	27.7 ± 1.1	27.5 ± 0.9	27.3 ± 0.8	0.954
Arterial hypertension, n (%)	2 (29%)	1 (20%)	4 (57%)	0.499
Diabetes, n (%)	2 (29%)	3 (60%)	4 (57%)	0.602
Hyperlipidaemia, n (%)	1 (14%)	1 (20%)	3 (43%)	0.558
LVEF, %	59.7 ± 2.7	57.5 ± 2.5	62.6 ± 4.4	0.891
Indication for surgery				
CABG, n (%)	0 (0%)	1 (20%)	0 (0%)	0.263
AVD, n (%)	2 (29%)	0 (0%)	2 (29%)	0.494
MVD, n (%)	2 (29%)	2 (40%)	3 (43%)	0.999
CABG+ MVD/AVD, n (%)	3 (43%)	2 (40%)	2 (29%)	0.999
Medication				
Digitalis, n (%)	0 (0%)	0 (0%)	2 (29%)	0.304
Beta-blockers, n (%)	3 (43%)	5 (100%)	4 (57%)	0.206
ACE inhibitors, n (%)	0 (0%)	4 (80%)	4 (57%)	0.022
AT1 blockers, n (%)	2 (29%)	0 (0%)	1 (14%)	0.747
Diuretics, n (%)	2 (29%)	3 (60%)	7 (100%)	0.021
Nitrates, n (%)	0 (0%)	0 (0%)	0 (0%)	-
Amiodarone, n (%)	0 (0%)	0 (0%)	0 (0%)	-
Statins, n (%)	3 (43%)	2 (40%)	5 (71%)	0.602

Data presented as numbers (%) or as mean ± SEM; Ctl: control-sinus rhythm, pAF: paroxysmal atrial fibrillation, cAF: persistent (chronic) atrial fibrillation, MVD/AVD, mitral/aortic valve disease, LVEF - left ventricular ejection fraction, CABG: coronary artery bypass graft surgery, ACE - angiotensin-converting enzyme; AT – angiotensin.

Table S6: Clinical characteristics of the patients employed for FRET experiments to measure cAMP in living myocytes

	SR (n = 6)	pAF (n = 6)	cAF (n = 6)	p-value
Age, years	65.7 ± 2.5	63.5 ± 2.6	71.3 ± 3.9	0.504
Male gender, n (%)	3 (50%)	6 (100%)	6 (100%)	0.074
Body mass index, kg/m ²	27.4 ± 0.9	27.3 ± 0.7	26.8 ± 0.7	0.843
Arterial hypertension, n (%)	5 (83%)	1 (16%)	5 (83%)	0.999
Diabetes, n (%)	0 (0%)	2 (33%)	2 (33%)	0.735
Hyperlipidaemia, n (%)	1 (16%)	1 (16%)	3 (50%)	0.473
LVEF, %	55.3 ± 3.2	55.3 ± 2.1	54.2 ± 2.9	0.554
Indication for surgery				
CABG, n (%)	4 (66%)	2 (33%)	2 (33%)	0.349
AVD, n (%)	1 (16%)	2 (33%)	2 (33%)	0.999
MVD, n (%)	0 (0%)	2 (33%)	0 (0%)	0.294
CABG+ MVD/AVD, n (%)	1 (16%)	1 (16%)	1 (16%)	0.999
Others, n (%)	0 (0%)	0 (0%)	1 (16%)	0.999
Medication				
Digitalis, n (%)	0 (0%)	0 (0%)	1 (16%)	0.999
Beta-blockers, n (%)	4 (66%)	5 (83%)	5 (83%)	0.999
ACE inhibitors, n (%)	5 (83%)	3 (50%)	3 (50%)	0.818
AT1 blockers, n (%)	0 (0%)	0 (0%)	1 (16%)	0.999
Diuretics, n (%)	2 (33%)	2 (33%)	2 (33%)	0.999
Nitrates, n (%)	0 (0%)	0 (0%)	0 (0%)	-
Amiodarone, n (%)	0 (0%)	1 (16%)	1 (16%)	0.999
Statins, n (%)	3 (50%)	1 (16%)	2 (33%)	0.818

Data presented as numbers (%) or as mean ± SEM; Ctl: control-sinus rhythm, pAF: paroxysmal atrial fibrillation, cAF: persistent (chronic) atrial fibrillation, MVD/AVD, mitral/aortic valve disease, LVEF - left ventricular ejection fraction, CABG: coronary artery bypass graft surgery, ACE - angiotensin-converting enzyme; AT – angiotensin.

Table S7: Clinical characteristics of the patients employed for patch-clamp experiments to measure $I_{Ca,L}$

	SR (n = 5)	cAF (n = 5)	p-value
Age, years	68.8 ± 3.7	71.8 ± 2.3	0.444
Male gender, n (%)	4 (80%)	5 (100%)	0.999
Body mass index, kg/m ²	26.8 ± 0.9	26.2 ± 0.7	0.821
Arterial hypertension, n (%)	5 (100%)	4 (80%)	0.999
Diabetes, n (%)	2 (40%)	0 (0%)	0.444
Hyperlipidaemia, n (%)	4 (80%)	1 (20%)	0.999
LVEF, %	60.2 ± 1.7	56.4 ± 2.7	0.429
Indication for surgery			
CABG, n (%)	3 (60%)	1 (20%)	0.524
AVD, n (%)	0 (0%)	2 (40%)	0.444
MVD, n (%)	0 (0%)	1 (20%)	0.999
CABG+ MVD/AVD, n (%)	2 (40%)	1 (20%)	0.999
Medication			
Digitalis, n (%)	0 (0%)	0 (0%)	
Beta-blockers, n (%)	2 (40%)	5 (100%)	0.444
ACE inhibitors, n (%)	2 (40%)	0 (0%)	0.444
AT1 blockers, n (%)	3 (60%)	0 (0%)	0.167
Diuretics, n (%)	0 (0%)	2 (40%)	0.141
Nitrates, n (%)	1 (20%)	0 (0%)	0.999
Amiodarone, n (%)	0 (0%)	2 (40%)	0.444
Statins, n (%)	3 (60%)	2 (40%)	0.999

Data presented as numbers (%) or as mean ± SEM; Ctl: control-sinus rhythm, pAF: paroxysmal atrial fibrillation, cAF: persistent (chronic) atrial fibrillation, MVD/AVD, mitral/aortic valve disease, LVEF - left ventricular ejection fraction, CABG: coronary artery bypass graft surgery, ACE - angiotensin-converting enzyme; AT – angiotensin.

Table S8: Clinical characteristics of the patients employed for sharp electrode experiments to measure actions potentials in atrial tissue

	SR (n = 5)	pAF (n = 5)	cAF (n = 7)	p-value
Age, years	74.2 ± 4.3	63.4 ± 3.7	72 ± 2.2	0.033
Male gender, n (%)	3 (60%)	3 (60%)	6 (85%)	0.519
Body mass index, kg/m ²	27.5 ± 1.2	27.3 ± 0.7	26.5 ± 0.8	0.955
Arterial hypertension, n (%)	1 (20%)	1 (20%)	4 (57%)	0.378
Diabetes, n (%)	3 (60%)	2 (40%)	4 (57%)	0.999
Hyperlipidaemia, n (%)	1 (20%)	1 (20%)	4 (57%)	0.378
LVEF, %	64.8 ± 2.5	55.3 ± 2.1	61.3 ± 3.7	0.778
Indication for surgery				
CABG, n (%)	0 (0%)	0 (0%)	3 (43%)	0.081
AVD, n (%)	1 (20%)	1 (20%)	2 (29%)	0.999
MVD, n (%)	1 (20%)	3 (60%)	1 (14%)	0.293
CABG+ MVD/AVD, n (%)	3 (60%)	1 (20%)	1 (14%)	0.293
Medication				
Digitalis, n (%)	0 (0%)	0 (0%)	3 (43%)	0.081
Beta-blockers, n (%)	2 (40%)	4 (80%)	6 (85%)	0.293
ACE inhibitors, n (%)	3 (60%)	3 (60%)	2 (29%)	0.568
AT1 blockers, n (%)	2 (40%)	0 (0%)	1 (14%)	0.434
Diuretics, n (%)	2 (40%)	2 (40%)	3 (43%)	0.999
Nitrates, n (%)	1 (20%)	0 (0%)	0 (0%)	0.588
Amiodarone, n (%)	0 (0%)	1 (20%)	0 (0%)	0.588
Statins, n (%)	1 (20%)	2 (40%)	3 (43%)	0.830

Data presented as numbers (%) or as mean ± SEM; Ctl: control-sinus rhythm, pAF: paroxysmal atrial fibrillation, cAF: persistent (chronic) atrial fibrillation, MVD/AVD, mitral/aortic valve disease, LVEF - left ventricular ejection fraction, CABG: coronary artery bypass graft surgery, ACE - angiotensin-converting enzyme; AT – angiotensin.