Online Supplement

Detailed Methods

Isolation of canine left ventricular myocytes
All animal handling and laboratory procedures conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996), and to our Institutional Animal Care and Use Committee approved protocols (license no. 10/2011/DEMÁB).
Cell isolation was done with segment perfusion technique by enzymatic digestion of the extracellular matrix as described previously [1]. Chemicals and reagents were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) if not specified otherwise. Intramuscular application of 10 mg/kg ketamine hydrochloride (Calypsol, Richter Gedeon, Budapest, Hungary) and 1 mg/kg xylazine hydrochloride (Sedaxylan, Eurovet Animal Health BV, Bladel, The Netherlands) was used to achieve complete narcosis in adult healthy beagle dogs of either sex. Hearts were quickly removed in left lateral thoracotomy and washed in cold Tyrode solution containing (in mmol/L): NaCl 144, KCl 5.6, $CaCl_2$ 2.5, $MgCl_2$ 1.2, 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) 5, glucose 10 (pH = 7.4; adjusted with NaOH). Left anterior descending coronary artery was cannulated and perfused with a nominally Ca^{2+}-free JMM solution (Minimum Essential Medium Eagle, Joklik Modification, product no. M0518) gassed with a mixture of 95 % O_2 and 5 % CO_2 and supplemented with 2.5 g/L taurine, 200 mg/L NaH_2PO_4, 1.4 g/L $NaHCO_3$, 175 mg/L pyruvic acid, 13.5 mg/L allopurinol and 750 mg/L D-ribose, pH 6.8 at 37 ºC. Then atria were cut off and a wedge-shaped section of the left ventricular wall supplied by the left anterior descending coronary artery was perfused. Following further 5 min of perfusion to completely remove blood from the tissue, 0.9 g/L collagenase (type II, 245 U/mg; Worthington Biochemical Co., Lakewood, NJ, USA), 2 g/L bovine serum albumin (Fraction V.), and 50 µmol/L $CaCl_2$ were added to the JMM solution. During the 30-40 minutes long enzymatic digestion the solutions were kept at 37 ºC and gassed with a mixture of 95 % O_2 and 5 % CO_2.

Collection of myocytes from various regions of the left ventricle
Following the enzymatic digestion, a dermatome (C.R. Bard. Covington, GA, USA) was used to peel off slices thinner than 0.5 mm from both epicardial and endocardial surfaces of the left ventricle containing subepicardial (EPI) and subendocardial (ENDO) cells, respectively. These slices were handled separately, cut into small pieces and cells were released by gentle agitation. The remaining middle portion of the free left ventricular wall containing mainly midmyocardial (MID) cells was cut into pieces with a scalpel and myocytes were released by gentle agitation.
In separate experiments, cells were collected from the apical and basal parts of the midmyocardial layer of the left ventricular wall.
Cells of various parts of left ventricular wall were sedimented and filtered four times to remove big chunks. During this procedure, the Ca^{2+} concentration of the JMM solution was gradually restored to the final 1.8 mmol/L. Finally, cells were placed into MEM solution (Minimum Essential Medium Eagle, product no. M0643) supplemented with the followings: 2.5 g/L taurine, 200 mg/L NaH_2PO_4, 2.2 g/L $NaHCO_3$, 175 mg/L pyruvic acid, 13.5 mg/L allopurinol, 750 mg/L D-ribose (pH = 7.3, equilibrated with a mixture of 95 % O_2 and 5 % CO_2) and stored at 15 ºC until further use within 36 hours after isolation. The percentage of living cells (having clear cytoplasm, sharp edges and clear striations) was usually 30-60 % and only these cells were used for experiments.

Electrophysiology
Cells were placed in a plexiglass chamber with a volume of 1 mL and continuously superfused with Tyrode solution (see above for composition) supplied by a gravity driven system at a speed of 2 mL/min. During experiments the bath temperature was set to 37 ºC by a temperature controller (Cell MicroControls, Norfolk, VA, USA). Cells were visualized by inverted microscopes placed in a Faraday cage on an anti-vibration table (Newport, Rochester, NY, USA). Electrical signals were measured with intracellular amplifiers (MultiClamp 700A or 700B, Molecular Devices, Sunnyvale, CA, USA) after analogue-digital conversion (Digidata 1440A or 1332, Molecular Devices) and recorded with pClamp 10 software (Molecular Devices).

Recording of action potentials
Action potentials were measured with 3 mol/L KCl containing borosilicate microelectrodes having a tip resistance of 20-50 MΩ with a sampling rate of 50 kHz. 0.3-5 s cycle length steady-state pacing was achieved using supra-threshold current pulses (1-2 ms long, 120-130 % of threshold) produced by an electronic stimulator (DS-R3; Főnixcomp Ltd, Hungary). Upon the off-line analysis of APs the following parameters were determined in ten consecutive APs then averaged: APD_{90} value (duration of the AP from the peak to 90 % of repolarization), V_{max} (maximal rate of depolarization, maximal dV/dt) and resting membrane potential (RMP). Phase-1 was

measured either as the membrane potential difference between the AP peak and the smallest membrane potential value preceding the dome of the AP in spike and dome configuration or as the membrane potential difference between the AP peak and any clearly detectable change in repolarization rate. Where even this was not recognizable with absolute certainty the value of phase-1 was considered to be zero. In some AP experiments 1 mmol/L 4-aminopyridine (4-AP) was used on EPI cells where it was diluted to its final concentration in Tyrode solution from a stock of 0.25 mol/L and concentrated HCl was used to adjust its pH to 7.4.

Voltage-clamp studies

Membrane currents were recorded using the patch-clamp technique [2] in whole-cell configuration. Borosilicate glass micropipettes having tip resistances of 2-3 MΩ after filling with pipette solution containing (in mmol/L) K-aspartate 110, KCl 45, K_2-ATP 3, $MgCl_2$ 1, HEPES 5 were used to preserve the normal Ca^{2+} homeostasis. The pH of the pipette solution was adjusted to 7.2 using KOH and its measured osmolality was 287-290 mmol/kg. After establishing high (1-10 GΩ) resistance seal by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by further suction and/or by applying 1.5 V electrical pulses for 1 ms. The series resistance was typically 4-6 MΩ. Experiments were discarded when the series resistance was high (>8 MΩ) or substantially increased (>20 %) during the measurement.

$I_{Cl(Ca)}$ was defined as 9-AC-sensitive current ($I_{9\text{-}AC}$) obtained by subtracting the current signals recorded after 8 min perfusion of 0.5 mmol/L 9-AC from those measured in control condition (in Tyrode solution), in the absence of 9-AC [1, 3]. In conventional voltage-clamp measurements, $I_{Cl(Ca)}$ was determined within the voltage range of −100 to +100 mV using 200 ms-long voltage pulses with 20 mV increments arising from the holding potential of −40 mV and applied at a rate of 0.2 Hz. Sampling rate was 10 kHz. Current amplitudes were calculated as a difference between peak and pedestal values.

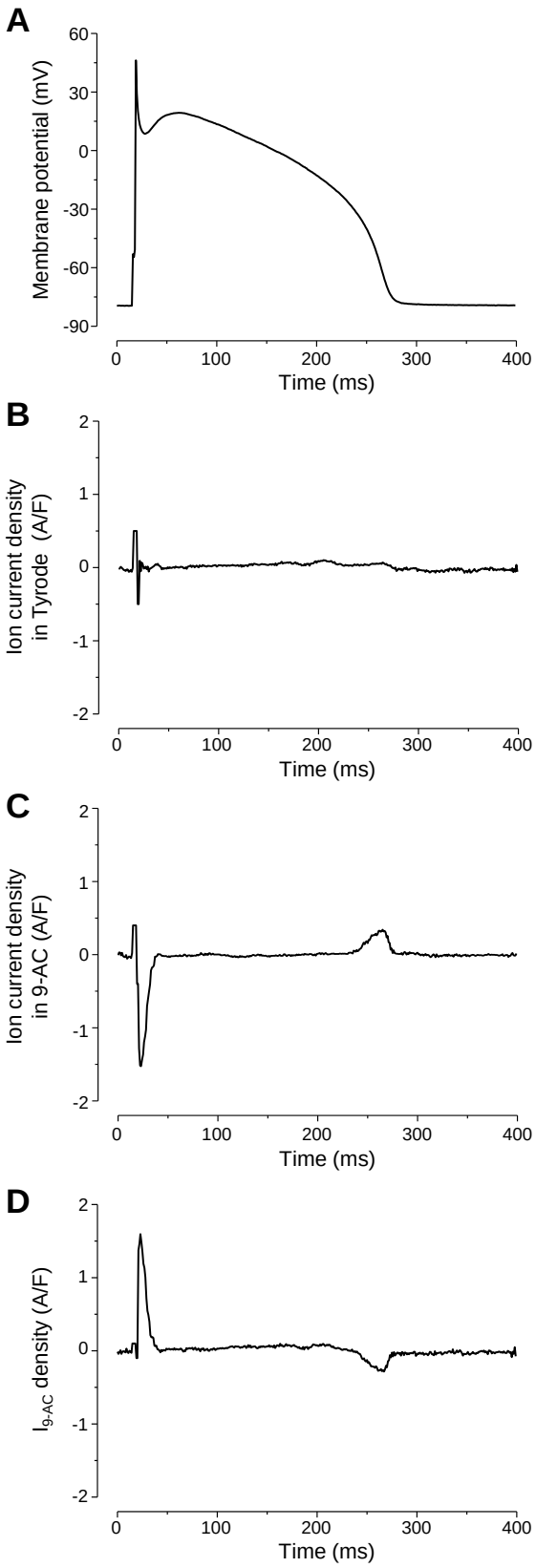
In experiments where L-type Ca^{2+} current ($I_{Ca,L}$) and transient outward K^+ current (I_{to1}) were studied the following pipette solution was used (in mmol/L): K-aspartate 100, KCl 45, K_2-ATP 3, $MgCl_2$ 1, HEPES 5, BAPTA 10, pH = 7.2 (adjusted using KOH). In case I_{to1} measurements the external solution was Tyrode solution supplemented with nisoldipine and E4031, both 1 μmol/L to block $I_{Ca,L}$ and the rapid component of delayed rectifier K^+ current (I_{Kr}), respectively. Upon measuring $I_{Ca,L}$, the external Tyrode solution contained E4031 and HMR1556, both 1 μmol/L to block I_{Kr} and the slow component of delayed rectifier K^+ current, respectively, as well as 3 mmol/L 4-AP to block I_{to1}. $I_{Ca,L}$ was evoked by 400 ms long depolarizations to +5 mV followed by a 20 ms long prepulse from the holding potential of −80 mV applied in every 5 s. I_{to1} was evoked from the holding potential of −80 mV with 200 ms long depolarizations to +60 mV followed by a 5 ms long prepulse to −40 mV in every 5 s. Sampling rate was 2 and 10 kHz for $I_{Ca,L}$ and I_{to1}, respectively.

Action potential voltage-clamp (APVC) experiments were conducted according to the method described previously [1, 4]. Action potentials were recorded in whole-cell configuration of current-clamp mode from the myocytes superfused with Tyrode solution with the sampling rate of 50 kHz. The pipette solution was identical to that used for conventional voltage-clamp (see above). Cells were continuously paced through the patch-pipette with supra-threshold depolarizing pulses of 1-2 ms duration delivered at steady stimulation frequency of 1 Hz so as a 1-2 ms gap between the stimulus artifact and the upstroke of the AP could occur. Subsequent APs were recorded and immediately analyzed from each cell. One of these APs, recorded always in Tyrode solution, having duration (and overall shape) closest to the average, was delivered to the same cell at the identical frequency as command voltage after switching the amplifier to voltage-clamp mode. Current signals were sampled at 50 kHz. The current trace obtained under these conditions is a horizontal line positioned at the zero level except for the very short segment corresponding to the action potential upstroke (Supplementary Fig. 1B). In some experiments, previously recorded typical ENDO and EPI APs were used as a voltage command. To study $I_{Cl(Ca)}$, its pharmacological inhibitor (0.5 mmol/L 9-AC) was used to reveal it from the net membrane current (Supplementary Fig. 1C). The profile of $I_{Cl(Ca)}$ was determined by subtracting the post-drug curve recorded after the action of 9-AC reached steady-state (usually within 8 minutes) from the pre-drug one:

$I_{9\text{-}AC}$ = I (measured prior to the application of 9-AC) – I (measured in the presence of 9-AC) (Supplementary Fig. 1D).

Ion currents were normalized to cell capacitance, determined in each cell using hyperpolarizations from +10 to −10 mV for 15 ms. Cell capacitance was 125±8 pF in the average of the 83 myocytes studied.

9-AC and the non-specific β-adrenergic agonist, isoproterenol (ISO) were dissolved in DMSO and distilled water, respectively and diluted to their final concentrations in Tyrode solution. E4031, nisoldipine and HMR1556 were diluted from DMSO stock solutions. Equivalent volume of DMSO did not affect any of the physiological parameters studied. The osmolality of all extracellular solutions was carefully adjusted to 295 ± 3 mmol/kg with a vapor pressure osmometer (Vapro 5520, Wescor Inc., Logan, UT, USA).



Supplementary Fig. 1. 9-AC sensitive current ($I_{9\text{-}AC}$) measured with APVC.
Representative AP (A) recorded in Tyrode solution on a midmyocardial cell. That same AP was used as a stimulus waveform on the same cell in subsequent part of the experiment. The AP evoked current after normalization to cell capacitance in Tyrode solution (B) and in the presence of 0.5 mmol/L 9-AC (C). Representative $I_{Cl(Ca)}$ current density profile (D) obtained by subtracting the curve on panel C from that shown on panel B.

Analysis of variability of AP repolarization

A series of 50 consecutive action potentials evoked by 1 Hz steady-state pacing was recorded as described earlier and analyzed offline to estimate short-term variability of repolarization (SV) using the following formula:

$$SV = \frac{\sum_{i=1}^{n}(|APD_{i+1} - APD_i|)}{n\sqrt{2}}$$

where SV is short-term variability, APD_i and APD_{i+1} indicate the APD_{90} values of the i^{th} and $i+1^{th}$ APs, respectively, and n denotes the number of consecutive beats analyzed [5, 6]. Poincaré diagrams made out of 50 consecutive APD_{90} values were used to visualize drug-induced changes in SV. To analyze further the variability of repolarization, the difference between consecutive APD_{90} values were grouped in ms ranges and the overall probability of their appearance was calculated in each cell. Then the average of these data was plotted (see Fig. 6E, F in manuscript) to illustrate the changes in beat-to-beat variability of APD.

Measurement of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$)

Myocytes were loaded in Tyrode solution with the membrane-permeant form of 5 µmol/L Fluo-3 in the presence of Pluronic F-127 for 25 min at room temperature. A period of minimum 30 min was allowed for de-esterification of the dye at room temperature then cells were stored at 15 ºC until the experiment. Loaded cells were field-stimulated through a pair of platinum wires. Rectangular pulses, having durations of 1–3 ms and amplitudes of twice the threshold, were generated by an electronic stimulator (DS-R3; Főnixcomp Ltd, Hungary) and delivered at a steady-state frequency of 1 Hz. Cells were visualized using a 40x oil immersion objective (CFI S-Fluor 40x oil, Nikon). The excitation wavelength of 500 nm was obtained with an excitation monochromator and an on-line connected microcomputer (DeltaScan, Photon Technology International, New Brunswick, NJ, USA) from the light of a xenon arc lamp (Ushio Deutschland GmbH, Steinhöring, Germany). Fluorescence emission was monitored at 526 nm using a R1527P photomultiplier tube (Hamamatsu Photonics Deutschland GmbH., Herrsching am Ammersee, Germany) at an acquisition rate of 500 Hz. FeliX32 Software & BryteBox Interface (Photon Technology International, New Brunswick, NJ, USA) was used during recording where nonspecific background fluorescence was deducted from signals. Ten consecutive $[Ca^{2+}]_i$ transients were analyzed off-line where fluorescence was normalized to diastolic fluorescence obtained right before each transient then averaged. Changes in intracellular free Ca^{2+} levels were approximated by this normalized averaged fluorescence signal and presented as F/F_0 on Figures.

Protein sample preparation and Western blot analysis

Enzymatically isolated cardiomyocytes obtained from various transmural regions (EPI, ENDO, MID) of the left ventricle and from the apical and basal region of the left ventricular midmyocardial layer were used for molecular biology studies. Cells were harvested with centrifugation and lysed by ultrasound treatment in lysis buffer (20 mmol/L TRIS-HCl, 5 mmol/L EGTA, pH=7.4 supplemented with 1 % protease inhibitor cocktail). Sodium dodecyl sulfate and mercapto-ethanol were added in an appropriate amount and the samples were heated to 100°C for 10 minutes. Samples were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7.5 % gels loaded with 40 µg protein per lane). Following electrophoresis proteins were transferred into nitrocellulose membrane (Bio-Rad), blocked with 5 % dry milk in phosphate-buffered saline (PBS; pH 7.4) and probed with primary antibodies: anti-TMEM16A (Santa Cruz Biotechnology, Dallas, TX, USA, sc-135235; rabbit, 1:200), anti-Bestrophin-3 (Santa Cruz Biotechnology, Dallas, TX, USA, sc-70147, goat, 1:200). Irrespective of the primary antibodies, the secondary antibodies were a horseradish peroxidase-conjugated goat anti-rabbit IgG and donkey anti-goat IgG (1:1000, Bio-Rad, Bio-Rad Laboratories, Hercules, CA, USA), respectively. Immunoreactive bands were visualized using an enhanced chemiluminescence Western blotting Pico or Femto detection kit (Thermo Scientific, Rockford, IL, USA) in a Fujifilm Labs-3000 dark box (Fujifilm, Tokyo, Japan). To quantify expression levels, background-corrected densitometry was performed using ImageJ software (NIH, Bethesda, MD, USA). The optical densities of the TMEM16A- and Bestrophin-3-specific bands were normalized to those of the β-actin-specific ones of the samples (1:200; Santa Cruz).
Specificity of the antibodies used to detect TMEM16A and Bestrophin-3 was tested earlier (see supplementary material of [1]: www.jmmc-online.com/cms/attachment/2057751712/2061725178/mmc1.doc)

Isolation of rabbit left ventricular myocytes and recording drug-sensitive currents during AP voltage clamp

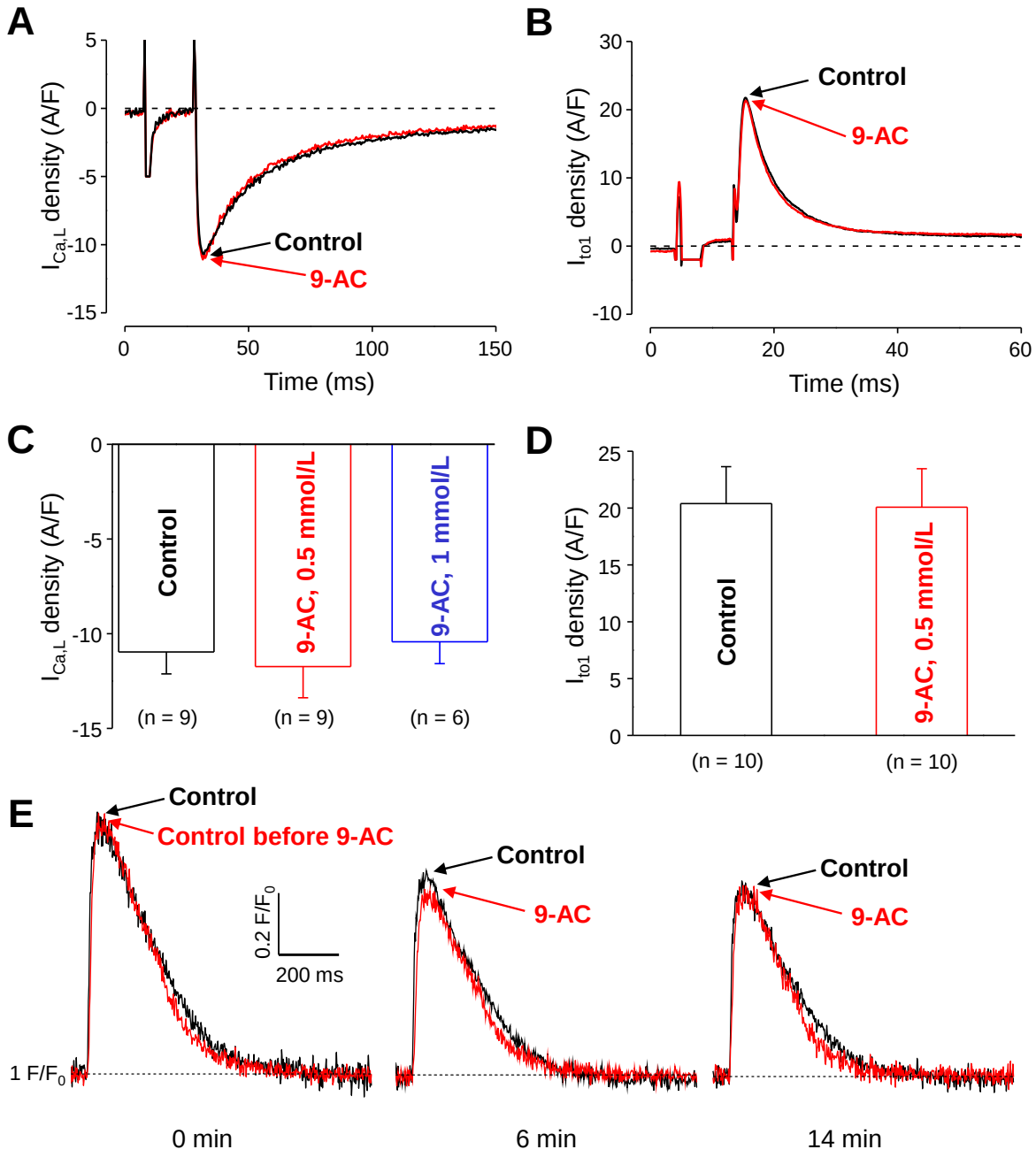
All animal handling and laboratory procedures conform to the approved protocols of the Institutional Animal Care and Use Committee at University of California, Davis confirming to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (8th edition, 2011). Chemicals and reagents were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) if not specified otherwise.
Ventricular myocytes were isolated from adult New Zealand White rabbits (male, 3–4 months old, 2.5–3 kg) by a standard enzymatic technique using 1 g/L collagenase type II (Worthington Biochemical Co., Lakewood, NJ, USA) and 0.05 g/L protease type XIV as previously described [4].

Isolated ventricular myocytes were transferred to a temperature-controlled Plexiglas chamber (Cell Microsystems, Research Triangle Park, NC, USA) and continuously superfused with a bicarbonate-containing Tyrode (BTY) solution having the following composition (in mmol/L): NaCl 125, $NaHCO_3$ 25, KCl 4, $CaCl_2$ 1.2, $MgCl_2$ 1, HEPES 10, and Glucose 10; pH was set to 7.4 with NaOH. Electrodes were fabricated from borosilicate glass (World Precision Instruments, Sarasota, FL, USA) with tip resistances of 2–2.5 MΩ when filled with internal solution. The internal solution contained (in mmol/L): K-Aspartate 108, KCl 25, NaCl 5, Mg-ATP 3, HEPES 10, cAMP 0.002, phosphocreatine dipotassium salt 10, and EGTA 0.01; pH was set to 7.2 with KOH. It is important to note that this composition preserved the physiological Ca^{2+} cycling [7]. A prerecorded "typical" rabbit ventricular AP waveform was used as the voltage command (canonical AP-clamp) and delivered at 2 Hz steady-state frequency to measure drug-sensitive currents, where the profile of each drug-sensitive current was determined by subtracting the post-drug curve from the pre-drug one. $I_{Cl(Ca)}$ was dissected from the net membrane current using its pharmacological inhibitors 9-AC (0.5 mmol/L) or CaCCinh-A01 (30 µmol/L) in separate experiments. Ca^{2+}-activated K^+ current was measured as 100 nmol/L apamin-sensitive current under the same conditions. Current densities were calculated by normalizing ion currents to cell capacitance. Axopatch 200B amplifier (Axon Instruments Inc., Union City, CA, USA) was used for measurements and the signals were digitized at 50 kHz by a Digidata 1440A A/D converter (Molecular Devices, Sunnyvale, CA, USA). All experiments were conducted at 36±0.1°C.

Supplementary results

9-AC is a suitable tool to measure $I_{Cl(Ca)}$ in ventricular myocytes

Our study was aimed to characterize the contribution of $I_{Cl(Ca)}$ to spatial and temporal heterogeneity of left ventricular repolarization. This could only be achieved if it is absolutely certain that the applied blocker of $I_{Cl(Ca)}$, 9-AC does not influence any other current responsible for the cardiac ventricular AP. Previously we have shown that 0.5 mmol/L 9-AC is suitable to study the contribution of $I_{Cl(Ca)}$ to the AP contour [3]. That publication evaluated the possible direct actions of 9-AC on $I_{Ca,L}$, I_{Kr}, the slow component of delayed rectifier K^+ current (I_{Ks}), and the inward rectifier K^+ current (I_{K1}). None of those currents were influenced by 9-AC [3]. It must be noted however that those measurements were done using 10 mmol/L EGTA in the pipette solution. Later we showed that $I_{9\text{-}AC}$, which we considered to be $I_{Cl(Ca)}$, still persists in that condition as 10 mmol/L EGTA is not able to fully eliminate $I_{Cl(Ca)}$ due to its slow Ca^{2+} buffering ability [1]. It was also shown that Ca^{2+} entry via L-type Ca^{2+} channels is an absolute necessity for the activation of $I_{Cl(Ca)}$ as the $I_{9\text{-}AC}$ was absent in the presence of nisoldipine [1]. Therefore in our previous article, where the specificity of 9-AC was tested [3] the lack of action on I_{Kr} and I_{Ks} are very likely due to the lack of 9-AC action on those channels as nisoldipine was applied to block $I_{Ca,L}$ in those measurements. In case of I_{K1} measurements, although nisoldipine was not used but it is not possible that $I_{Ca,L}$ was activated by a hyperpolarizing voltage step evoked from -80 mV [3]. Therefore only $I_{Ca,L}$ remains to be tested if it is influenced by 9-AC as 10 mmol/L EGTA was also in the pipette solution in our more recent publication as well [1]. Another critical current which should not be influenced by 9-AC during the study of $I_{Cl(Ca)}$ is I_{to1} as it is also active in the early part of the AP [8] therefore would largely overlap with 9-AC-sensitive current measured in APVC. Supplementary Fig. 2 shows the result of those experiments where 10 mmol/L BAPTA was applied in the pipette solution to avoid any possible activation of $I_{Cl(Ca)}$ during measurements of $I_{Ca,L}$ where nisoldipine could not be used. We have shown previously that the application of 10 mmol/L BAPTA in the pipette solution is suitable to eliminate $I_{Cl(Ca)}$ as it strongly and rapidly buffers Ca^{2+} therefore prevents the activation of $I_{Cl(Ca)}$ [1]. In case of recording I_{to1}, nisoldipine was also applied in the perfusion which makes the activation of $I_{Cl(Ca)}$ impossible especially in the presence of 10 mmol/L BAPTA. In the presence of 0.5 mmol/L 9-AC the density of neither $I_{Ca,L}$ nor I_{to1} was different compared to control condition (Supplementary Fig. 2). Moreover, $I_{Ca,L}$ was also examined in the presence of 1 mmol/L 9-AC where again no significant change was observed (Supplementary Fig. 2C).



Supplementary Fig. 2. Lack of 9-AC action on $I_{Ca,L}$, I_{to1} and Ca-transients.
Representative $I_{Ca,L}$ (A) and I_{to1} (B) currents normalized to cell capacitance in control (black) and in the presence of 0.5 mmol/L 9-AC (red). Average results for $I_{Ca,L}$ where 1 mmol/L 9-AC (blue column) was also applied after 0.5 mmol/L 9-AC (red column) (C). Average results for I_{to1} (D). Representative Ca-transients recorded using Fluo-3-AM fluorescent dye in field-stimulated myocytes at a steady-state frequency of 1 Hz in the absence (Control, black) and in the presence of 0.5 mmol/L 9-AC (red curves) at time 0 min (left), 6 min (middle) and 14 min (right) (E). Columns and bars indicate mean ± SEM values, n shows the number of experiments.

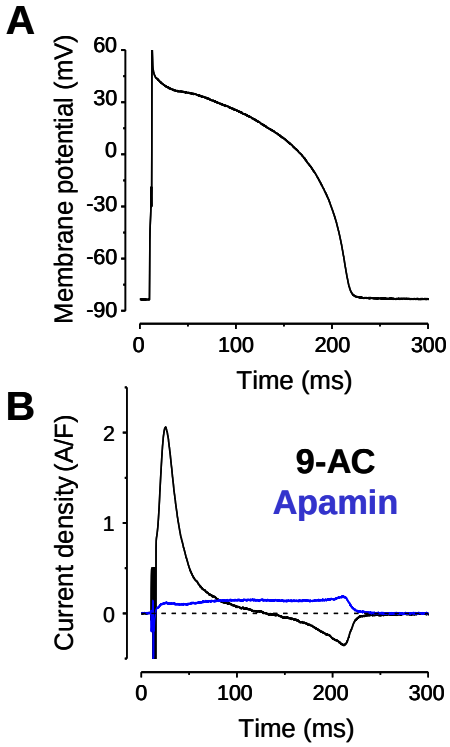
The possible action of 9-AC on $[Ca^{2+}]_i$ was also examined using Fluo-3-AM fluorescent dye in field-stimulated cells paced at a steady-state rate of 1 Hz. The action of 0.5 mmol/L 9-AC on peak systolic fluorescence was not significantly different from what was measured in the lack of 9-AC (vehicle containing time matched control measurements, Supplementary Fig. 2E and Supplementary Table 1). The decay time constant of Ca-transients was not changed during the examined time period (14 minutes recording) in the 9-AC treated cells just as in time matched controls (Supplementary Table 1).

Supplementary Table 1
Ca-transient parameters before (initial) and during application of 0.5 mmol/L 9-AC and time matched controls

	Control (n=8)	9-AC (n=8)			Control (n=8)	9-AC (n=8)
peak systolic fluorescence (F/F_0) (initial)	1.79±0.08	1.78±0.14		decay time constant (ms) (initial)	197±17	193±10
peak systolic fluorescence (F/F_0) (6 min)	1.65±0.07	1.53±0.13		decay time constant (ms) (6 min)	157±23	172±23
peak systolic fluorescence (F/F_0) (14 min)	1.59±0.05	1.55±0.15		decay time constant (ms) (14 min)	186±29	166±25

9-AC-sensitive current is completely different form apamin-sensitive current but identical to CaCCinh-A01-sensitive current

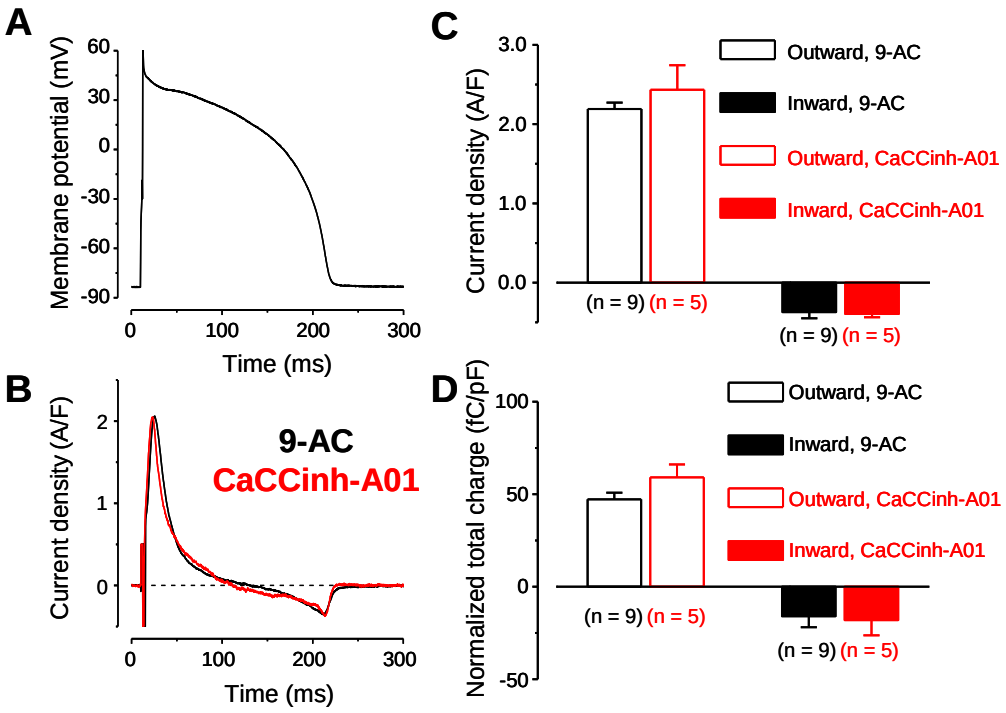
We also took precaution to examine whether the small conductance Ca^{2+}-activated K^+ current (I_{SK}) might potentially interfere with the 9-AC-sensitive current. However, I_{SK} has a relatively small magnitude in healthy ventricular myocytes of larger mammals, and its blockade exerts minimal effect on APD_{90} of those control cells [9, 10]. We recorded I_{SK} as 100 nmol/L apamin-sensitive current under APVC to evaluate the profile of small conductance Ca^{2+}-activated K^+ current (Supplementary Fig. 3). This current was outward during the whole duration of AP in healthy rabbit ventricular myocytes (Supplementary Fig. 3). In contrast, the 9-AC-sensitive current showed a significantly larger outward component in the early phase of the AP and a smaller inward component in later AP time course (Supplementary Fig. 3), similarly to that seen in canine cells (Fig. 3 and 5).



Supplementary Fig. 3. $I_{9\text{-}AC}$ and I_{apamin} measured with AP voltage clamp.
AP command waveform (A) and drug-sensitive currents (B) of 0.5 mmol/L 9-AC (black trace) or 100 nmol/L apamin (blue trace).

The 9-AC-sensitive current recorded in rabbit myocytes was identical to the CaCCinh-A01-sensitive current (Supplementary Fig. 4). CaCCinh-A01 is another inhibitor of $I_{Cl(Ca)}$ [11] that has become available recently and blocks approximately 90 % of the human TMEM16A current expressed in HEK 293 cells [12]. CaCCinh-A01 shows large difference in its chemical structure to that of 9-AC and is considered to have higher potency and selectivity for $I_{Cl(Ca)}$ over other chloride currents and transporters, including CFTR channel and the voltage-gated chloride channels [13]. The inhibitor-sensitive current profiles were similar regardless of the used blocker

(0.5 mmol/L 9-AC versus 30 µmol/L CaCCinh-A01) and composed of an early narrow outward current having a peak value of 2.19±0.08 and 2.43±0.31 A/F in 9-AC and CaCCinh-A01, respectively. The outward peak was followed by a smaller late inward current reaching its maximum (−0.05±0.01 and −0.06±0.01 A/F in 9-AC and CaCCinh-A01, respectively) towards the end of the AP (Supplementary Fig. 4C). These peak current densities did not differ between the two types of blocker-sensitive currents. Similarly, the normalized total charge values of both inward and outward currents were not significantly different depending on the type of blocker (Supplementary Fig. 4D). According to our knowledge, this is the first report of using CaCCinh-A01 to measure $I_{Cl(Ca)}$ in cardiac myocytes. The fact that these two drugs dissect out identical currents under APVC suggests that acute application of 9-AC has negligible effect on other chloride currents shaping AP in healthy ventricular myocytes and validates the approach of using 9-AC to investigate $I_{Cl(Ca)}$.



Supplementary Fig. 4. $I_{9\text{-AC}}$ and $I_{CaCCinh\text{-}A01}$ measured with AP voltage clamp.
AP command waveform (A) and drug-sensitive currents (B) of 0.5 mmol/L 9-AC (black trace, B) or 30 µmol/L CaCCinh-A01 (red trace). Peak current densities (C) and total charges (D) measured during outward (left column pair) and inward currents (right column pair). Columns and bars indicate mean ± SEM values, n shows the number of experiments.

Supplemental References

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