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Rapid communication

Blockade of atrial-specific K^+-currents increases atrial but not ventricular contractility by enhancing reverse mode Na^+/Ca^{2+}-exchange

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Abstract

Background: AVE0118 (2′-{[2-(4-Methoxy-phenyl)-acetylamino]-methyl}-biphenyl-2-carboxylic acid (2-pyridin-3-yl-ethyl)-amide) blocks atrial ultrarapid delayed rectifier currents (I_{Kur}) and prolongs the atrial action potential (AP) plateau without affecting ventricular repolarisation. In patients with atrial contractile dysfunction due to atrial tachyarrhythmias, this response might increase atrial contractility without risk of ventricular proarrhythmia. This study was designed to evaluate the inotropic mechanisms of AVE0118.

Methods and results: In isometrically contracting atrial trabeculae, AVE0118 increased contractile force by 55.4% in sinus rhythm patients ($n=9$) and by 107.4% in patients with atrial fibrillation ($n=8$). In freshly isolated canine atrial myocytes studied under perforated patch current clamp (37 °C), AVE0118 increased myocyte fractional shortening from 3.8±0.6 to 9.6±0.8% and prolonged action potential duration at 30% repolarisation from 9±2 to 102±11 ms. Clamping cells to an AP waveform recorded during exposure to AVE0118 produced the same inotropic response as the drug itself. In action potential clamp, peak Ca^{2+} inward current (I_{CaL}) current declined from 5.5±1.3 pA/pF during control to 4.1±0.7 pA/pF when an AP recorded in the presence of AVE0118 was used as command waveform. However, I_{CaL} was more sustained with AVE0118 and the time integral did not change (135±37 vs. 173±30 pA/pF∗ms, p=ns). Importantly, blockade of reverse mode Na^+/Ca^{2+}-exchanger activity with 5 μM KBR7943 or using a Na^+-free pipette solution abolished the positive inotropic effect of the AP recorded in the presence of AVE0118. In ventricular myocytes AVE0118 did not elicit a positive inotropic response.

Conclusions: Block of I_{Kur} by AVE0118 enhances atrial contractility both in patients with sinus rhythm and atrial fibrillation. The positive inotropic effect is atrial-specific and due to the changes of the action potential configuration which enhances Ca^{2+} entry via reverse mode Na^+/Ca^{2+} exchange.

Keywords: Arrhythmia; Atrial function; E–c coupling; K-channel; Contractile function

1. Introduction

Morbidity and mortality of patients with atrial fibrillation (AF) are largely attributable to thromboembolic complications. Atrial thrombus formation and stroke occur not only during AF, but also after restoration of sinus rhythm [1]. Numerous studies document a drop of atrial blood flow velocity and an enhancement of spontaneous echo contrast

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early after cardioversion of AF [2,3]. Spontaneous echo contrast is a strong predictor of thromboembolic events [4], suggesting that low blood flow velocity favors the development of new atrial thrombi soon after cardioversion [2].

Use of positive inotropic drugs to improve atrial contractile function soon after cardioversion might reduce the risk of stroke during the post cardioversion period [5,6]. However, the limited efficacy and low safety of most of these compounds has limited the development of positive inotropic drugs to treat AF-induced atrial contractile dysfunction [7–9]. The ultrarapid delayed rectifier current (I_{Kur}) is an important determinant of repolarisation or refractoriness in the atria of dogs [10], pigs [11,12], goats [13], and humans [14]. In human atrial myocardium, block of I_{Kur} results in a prolongation and elevation of the plateau phase of the atrial action potential thereby eliciting a positive inotropic effect [15].

The newly developed biphenyl derivative AVE0118 blocks the transient outward current (I_{to}) and I_{Kur} [11,12]. Since I_{Kur} is only present in the atria, but not in the ventricle [16], block of this current selectively increases the atrial action potential plateau, which might increase atrial contractility without risk of significant ventricular proarrhythmia.

In the present study we tested the hypothesis that AVE0118 can restore atrial contractility in patients with AF. Furthermore, we investigated the cellular mechanisms of the positive inotropic effect of AVE0118.

2. Methods

2.1. Patients

Right atrial appendages were obtained from 17 patients undergoing open heart surgery. 8 patients were in chronic AF (≥3 months), the others were in sinus rhythm. Clinical characteristics of the patients are shown in Table 1. AF patients tended to have a lower ejection fraction and a higher NYHA class, without significant differences in these parameters. The only difference in medications was that digitalis was more frequently given to AF patients for ventricular rate control. The study was approved by the University of Maastricht ethical committee, and conforms with the principles outlined in the Declaration of Helsinki.

2.2. Contraction experiments in isolated human atrial trabeculae

Muscle strip preparations and isometric twitch tension measurements at the peak of the twitch tension-length relation (L_{max}) were performed as described previously [17,18]: immediately after surgical resection, atrial tissue was placed into 4 °C cold bathing solution (mM: $CaCl_2$ 2.0, NaCl 119.8, $MgCl_2$ 1.04, KCl 5.36, $NaHCO_3$ 22.6, NaH_2PO_4 0.42, glucose 5.05, ascorbic acid 0.28, Na_2EDTA 0.05; pH 7.4, gassed with 5% CO_2/95% O_2) and rapidly transferred to the laboratory. Thin myocardial strips were isolated in parallel to the muscle fiber direction under stereomicroscopic control.

Table 1
Patients— hemodynamic and clinical data

		Sinus rhythm (n=9)	Atrial fibrillation (n=8)	p-value
Age (y)		65±4	70±2	0.30
Mitral valve disease (n)		3	3	0.86
Aortic valve disease (n)		1	1	0.93
Coronary artery disease (n)		7	6	0.89
Gender (m/f)		(6/3)	(5/3)	0.86
NYHA class (n)				
I		1	0	–
II		3	1	–
III		4	5	–
IV		1	2	–
Mean		2.6± 0.3	3.1± 0.2	0.20
Ejection fraction (%)		51±6	47±2	0.56
Medication	Diuretics (n)	3	4	0.49
	Beta-blockers (n)	6	6	0.71
	Digitalis (n)	0	3	0.04*
	ACE-Inhibitors (n)	4	4	0.82
	Ca^{2+}-Antagonists (n)	4	1	0.15

*: $p<0.05$.

Isometric force of contraction of all muscle strips was determined at 37 °C. Muscle strips were electrically driven at 1 Hz with 5 ms rectangular pulses applied at a voltage 25% above threshold. After equilibration for 30 to 45 min, muscles were stretched by increasing the resting tension from 1 mN stepwise by 0.5 mN until the muscle length providing maximal active force generation was reached (L_{max}). Muscle diameters of the stretched preparations were determined using ocular micrometers and were 0.62±0.21 mm (n=14) in sinus rhythm patients and 0.66±0.26 mm (n=11) in AF patients (p=n.s.). All preparations with a decline of active force generation by more than 10% within 20 min of stimulation at L_{max} were excluded from the study. The positive inotropic effect of AVE0118 was studied by cumulatively increasing the concentration of AVE0118 in the bathing solution. Steady-state conditions were reached within 10 min.

2.3. Isolation of canine atrial and ventricular myocytes

In 15 mongrel dogs anesthetized with pentobarbital sodium (30 mg/kg) and fentanyl citrate (15 μg/kg), a left lateral thoracotomy was performed. Hearts were quickly excised and placed in cold (4 °C) cardioplegic solution (pH 7.4). A small right atrial preparation (2×2 cm) including the right anterior tricuspid ring was dissected and perfused with cardioplegic solution. To ensure adequate atrial perfusion, all visible atrial coronary branches were tied off at the edges of the preparation. The perfusion was switched to nominally Ca^{2+}-free Tyrode containing (mM): NaCl 118, $NaHCO_3$ 25, KCl 4.8, $MgCl_2$ 1.2, KH_2PO_4 0.7, dextrose 4, mannitol 2, taurine 10, pyruvic acid 5, insulin 0.001, and penicillin–streptomycin (4 μ/ml), 37 °C, gassed with carbogen, pH 7.4. After 20 min collagenase (0.65 mg/ml, type II, Worthington Biochemical, Lakewood NJ) and BSA (0.65 mg/ml) were

added. The Ca^{2+}-concentration was increased to 0.5 mM in 4 steps of 125 μM after 20, 25, 30 and 35 min. After 40 min of collagenase perfusion the tissue was taken off the perfusion and gently agitated for further 10 min in the same solution. Myocytes were passed through a nylon mesh (200 μm), concentrated by low speed centrifugation and kept oxygenated at room temperature until used, within 8 h of isolation. Yields were in the range of 50 to 80% for viable, calcium tolerant myocytes. Only well striated, rod-shaped myocytes were used for the experiments.

Cells were allowed to adhere to laminin-coated culture dishes mounted in a thermal stage controller (Bioptech ΔT system, Butler, PA), maintained at 37 °C. Solutions were changed with a six port gravity flow system. Solutions were pre-warmed to 37 °C using a preheater (HPRE2, Cell MicroControls, Norfolk, VA). The study was approved by the local ethical committee and conforms with 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).

2.4. Ca^{2+}-transients

Canine atrial myocytes were loaded for 20 min with 1 μM fura-2/AM (Molecular Probes) at room temperature and studied on a Nikon Eclipse TE2000-S inverted microscope (Badhoevedorp, The Netherlands), imaged through a 40× fluorescence objective. Electrically paced (1 Hz field stimulation, 37 °C) myocytes were superfused with a normal Tyrode's solution containing (in mM) NaCl 135, KCl 5.4, $MgCl_2$ 1.0, NaH_2PO_4 0.3, glucose 10, $CaCl_2$ 2.0 and HEPES 20.0, at pH 7.4. Fluorescence measurements were performed using a dual-excitation single-emission fluorescence photomultiplier system (IonOptix, Milton, MA, USA). Excitation wavelengths of 340 nm and 380 nm were provided by a 75 W Xenon lamp and emission fluorescence was reflected through a 510-nm barrier filter to a photomultiplier tube (250 Hz sampling rate). Fluorescence signals were digitized on-line using an IBM-compatible computer and IonOptix fluorescence image acquisition software. Electrically stimulated fluorescence (F340/F380) transients were analyzed off-line using IonOptix fluorescence image analysis software.

2.5. Cellular electrophysiology and cell shortening

Patch pipettes were prepared from Corning 8161 glass capillaries (Warner Instrument Corp.), and the shanks were covered with Sylgard. Pipette tips were fire polished to a resistance of 2–3 MΩ when filled with pipette solution.

Action potentials were measured using the perforated patch technique in current clamp mode. Immediately prior to use, pipettes were front filled with a solution containing (mM): K-methane-ethylsulfonate 100, KCl 30, NaCl 10, Tris-HEPES 10, $MgCl_2$ 2, K_2EGTA 0.05, pH 7.2 by capillary action. The same solution containing 120 μg/ml nystatin was used for backfilling. The bathing solution contained (mM): NaCl 135, Na-HEPES 5, Na-acetate 3, KCl 3, $MgCl_2$ 1, $CaCl_2$ 2, glucose 5, pH 7.4. Interface potentials were nulled immediately before seal formation. As nystatin perforated the patch, access resistance declined to 10–30 MΩ within 5 to 10 min. Cells with access resistances above 30 MΩ were excluded from the study. Action potentials were elicited at 1 Hz, with a sampling rate of 20 kHz. Averaged ($n=12$) steady-state action potentials were used for analysis, and representative traces were stored for later use as command waveforms in action potential clamp experiments. From the averaged traces, we analyzed action potential durations (APD_{90} and APD_{30}) and the 'plateau potential' (average potential within a window 10 to 50 ms after the upstroke of the action potential). Cell shortening was measured both during current clamp and voltage clamp by video edge detection (VED104, Crescent Electronics, Salt Lake City, UT). Shortening was normalized to the distance between edge and tip of the pipette to obtain fractional shortening. In the voltage clamp experiments, trains of stylized typical 4-phase action potentials (holding potential −60 mV) were used as command potentials. We studied the effects of changing: 1) plateau duration, 2) plateau potential, 3) plateau prolongation without change of APD_{90}, and 4) the time required for final repolarisation (prolongation of APD_{90}

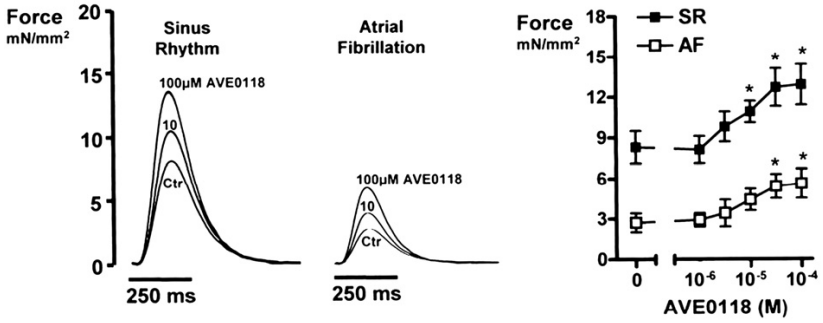

Fig. 1. Effect of AVE0118 on contractile force of atrial trabeculae from patients in sinus rhythm and patients in AF. Left panel: Representative force recordings. Right panel: Average data of 14 preparations from 9 patients in sinus rhythm and 11 preparations from 8 patients in AF. External field stimulation at 1 Hz, 37 °C. *: $p<0.05$ versus baseline.

without change in APD_{30}). When action potentials were applied to the myocytes (action potential clamp), the holding potential was set at −78 mV.

I_{to} and I_{Kur} (previously characterized as sustained outward current sensitive to submillimolar concentrations of 4-aminopyridine [14]) were measured during voltage steps ranging from −90 to +70 mV from a holding potential of −60 mV. Pipette and bathing solution were the same as above except for replacement of $CaCl_2$ with 0.2 mM $CdCl_2$ to suppress the L-type Ca^{2+} current (I_{CaL}).

I_{CaL} was measured in conventional whole cell patch clamp mode using a pipette solution containing (mM): CsCl 125, tetraethylammonium chloride (TEA-Cl) 20, Cs_2EGTA 10, MgATP 5, creatine phosphate 3.6, HEPES 10, pH 7.2. The bathing solution contained (mM): TEA-Cl 157, $CaCl_2$ 1, $MgCl_2$ 0.5, and HEPES 10 (pH 7.4 with CsOH). The seal was disrupted by gentle suction. Cells with access resistances above 12 MΩ were excluded from the experiments. For current measurement cells were depolarized from a holding potential of −60 mV to test potential ranging from −40 to +30 mV. During action potential clamp experiments, the holding potential was set at −78 mV.

2.6. Statistical analysis

All data are expressed as means±SEM. EC_{50}-values are given as means with 95% confidence intervals. Statistical significance of differences was evaluated with student's *t*-test or with repeated measures ANOVA. Significance of differences in medication or gender was calculated by Chi-square test. A *p*-value<0.05 was considered statistically significant.

3. Results

3.1. Positive inotropic effect of AVE0118 in human atrial myocardium

As reported in our previous studies [17,18], baseline force of contraction of right atrial trabeculae was reduced in the AF group by ~70% compared to patients in sinus rhythm (Sinus rhythm: 8.3±1.2 mN/mm^2 (n=14), AF: 2.7±0.7 mN/mm^2 (n=11), p<0.01). As shown in Fig. 1, AVE0118 increased trabecular force of contraction in both sinus rhythm (+4.6± 1.2 mN/mm^2, +55.4% over baseline) as well as in AF patients (+2.9±0.8 mN/mm^2, +107.4% over baseline). Contractile force in AF patients at 100 μM AVE0118 (5.6± 1.1 mN/mm^2) was slightly but not significantly lower than that in sinus rhythm patients in the absence of AVE0118 (8.3±1.2 mN/mm^2, p=0.12). Positive inotropic potency did not differ between the two groups (EC_{50} in sinus rhythm: 7.1 (5.5–11.3)μM, AF: 8.0 (6.1–14.5)μM, p=ns). In the three AF patients receiving digitalis, both baseline force of contraction (2.9±1.1 mN/mm^2) and the positive inotropic effect of AVE0018 (+2.6±1.2 mN/mm^2) were similar relative to patients not receiving the drug. In 4 muscle preparations of two patients in sinus rhythm force of contraction was similar

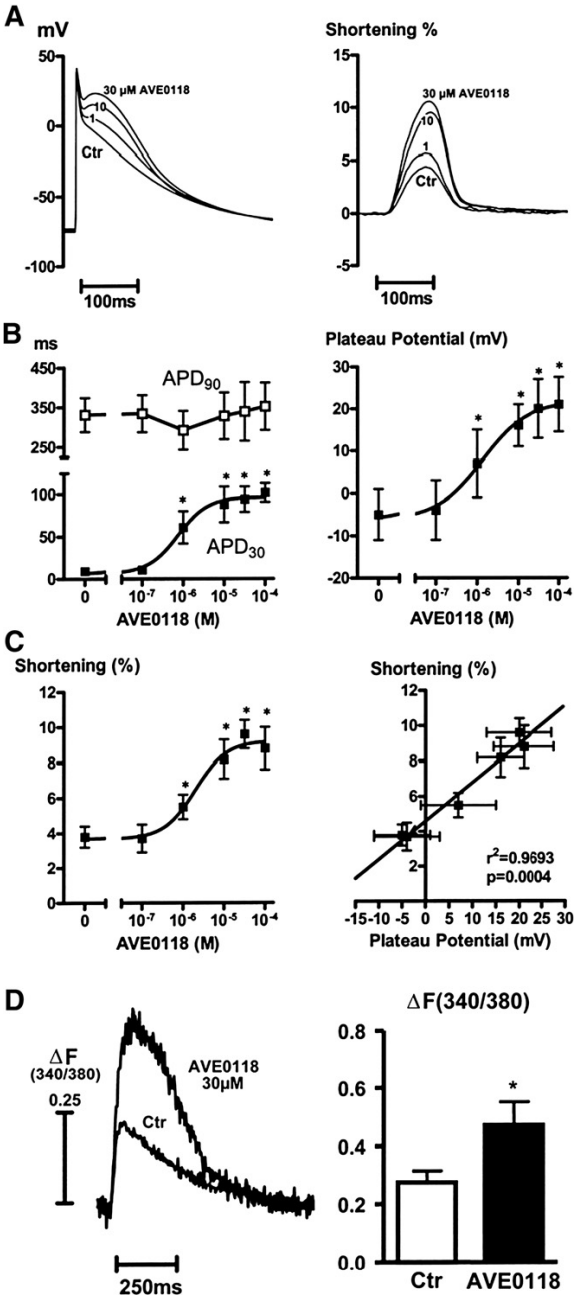

Fig. 2. Effect of AVE0118 on action potential, cell shortening, and Ca^{2+} transients of canine right atrial myocytes. A: Representative action potentials and shortening tracings. B: Effect of AVE0118 on action potential duration and plateau potentials. C: Effect on myocyte shortening and correlation between myocyte shortening and plateau potential. Cells were stimulated in current clamp mode, 37 °C, 1 Hz. N=8 cells, 5 dogs, *: p<0.05 versus baseline. D: The effect of AVE0118 on Ca^{2+}-transients of right atrial myocytes was measured during field stimulation at 1 Hz, 37 °C. N=19 cells, 4 dogs. *: p<0.05 versus control.

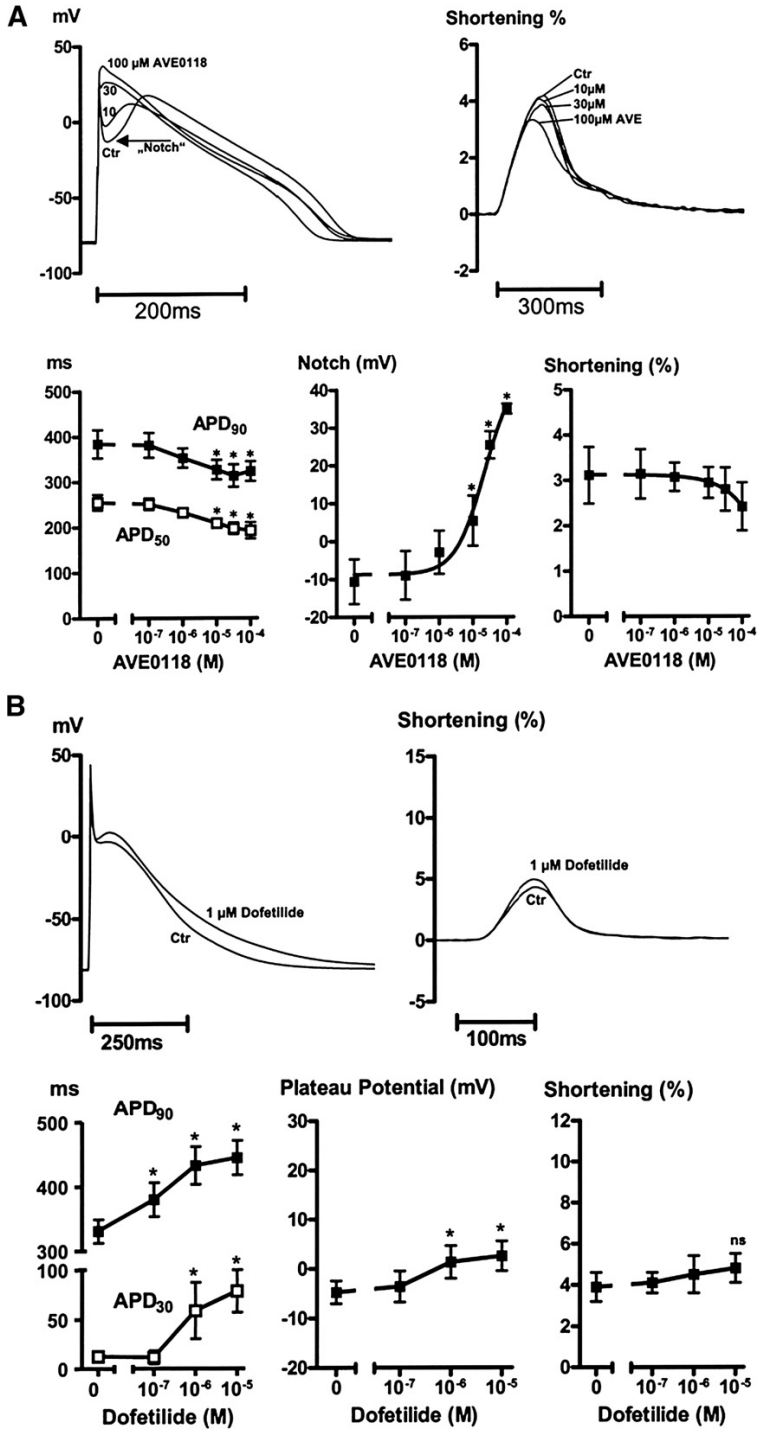

Fig. 3. A: Effect of AVE0118 on action potentials and cell shortening of right ventricular canine myocytes. Upper panels: Representative action potential and shortening tracings. Lower panels: Average effect of AVE0118 in 8 cells from 4 dogs. B: Effect of dofetilide on action potential and cell shortening of canine right atrial myocytes. Upper panels: Representative action potentials and shortening tracings. Lower panels: Effect of dofetilide on action potential duration and plateau potential and myocyte shortening. $N=7$ cells, 4 dogs. All cells were stimulated in current clamp mode, 37 °C, 1 Hz. *: $p<0.05$ versus baseline.

before (8.5±1.9 mN/mm^2) and after addition of vehicle (DMSO 0.1vol%, 7.9±2.1 mN/mm^2, n.s.).

3.2. Effects of AVE0118 and dofetilide on cell shortening, action potentials and Ca^{2+}-transients

Fig. 2A–C shows the effects of AVE0118 on the action potential and shortening of canine atrial myocytes stimulated under current clamp conditions (1 Hz, 37 °C). AVE0118 significantly prolonged APD_{30} from 9±2 ms to 102±11 ms. In contrast, APD_{90} was unchanged in the presence of the compound. Prolongation of APD_{30} increased the plateau potential (average potential 10 to 50 ms after action potential upstroke) from −5.1±6.1 mV to 20.9±6.5 mV. The vehicle used to solubilize AVE0118 (0.1 vol% DMSO) did not affect atrial action potential morphology (not shown). However, AVE0118 enhanced cell shortening 2.5-fold (from 3.8±0.6% to 9.6±0.8% at 30 μM). The half maximal positive inotropic effect was reached at 2.0 (1.2–3.5)μM. Cell shortening amplitude was strongly correlated with the plateau potential. AVE0118 (30 μM) significantly increased the amplitude of Ca^{2+}-transients in canine atrial myocytes stimulated with external field stimulation (1 Hz, 37 °C) from 0.27±0.04 to 0.47±0.08 (+71%, $p<0.05$, Fig. 2D).

In ventricular myocytes, AVE0118 slightly shortened APD_{50} and APD_{90} and increased the "notch"-potential

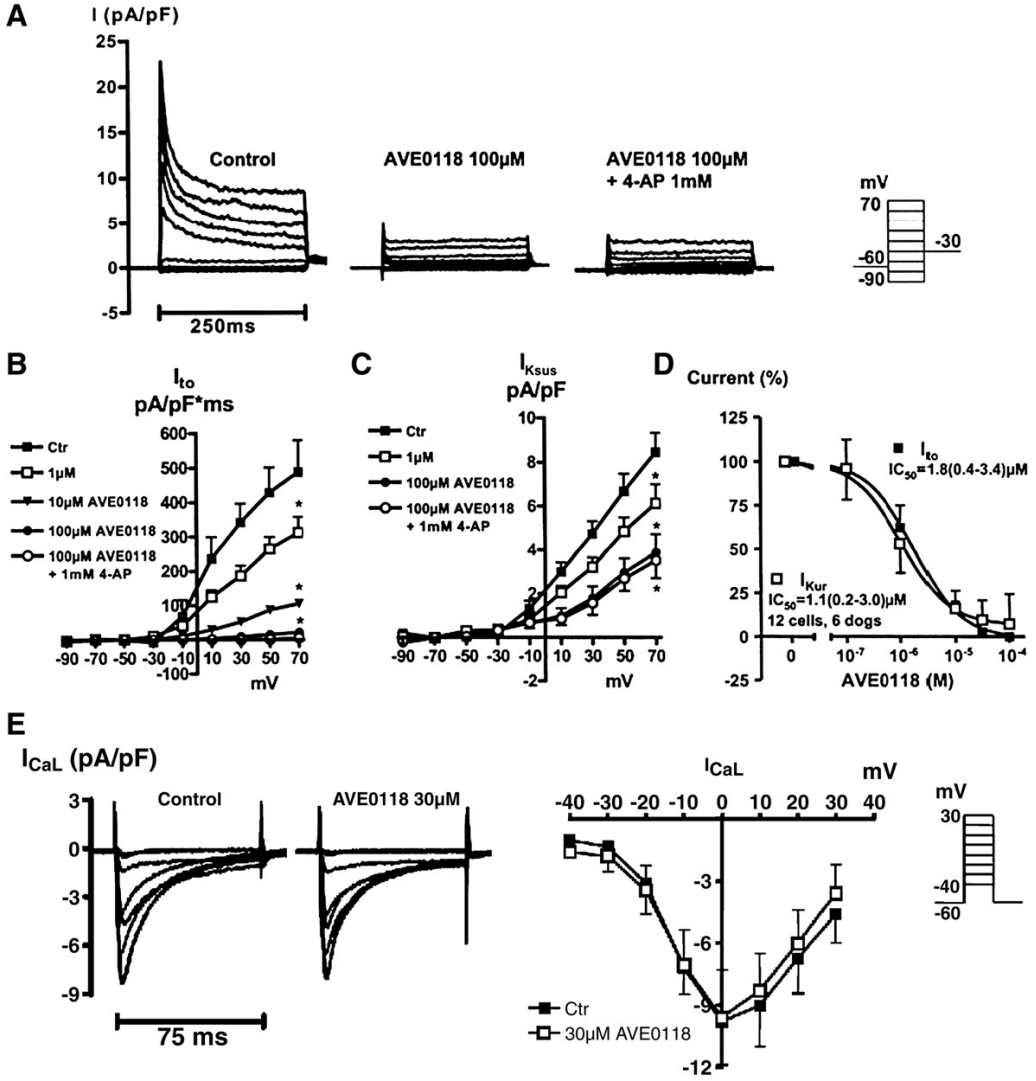

Fig. 4. Effect of AVE0118 on transient (I_{to}) and sustained outward current (I_{Ksus}), and the Ca^{2+} inward current (I_{CaL}) in canine atrial myocytes. 4-AP=4-aminopyridine. A: Representative current tracings and voltage protocol of K^+-currents. B and C: Average current–voltage relationships of I_{to} and I_{sus}. D: Relative K^+-current inhibition (12 cells isolated from 6 dogs) E: Representative current tracings, current–voltage relationship, and voltage protocol of I_{CaL} (9 cells from 3 dogs). *: $p<0.05$ versus control.

(Fig. 3A), with no positive inotropic effect. A slight decline in cell shortening at high drug concentrations was not statistically significant.

Unlike AVE0118, the I_{Kr}-blocker dofetilide clearly prolonged final repolarisation and only slightly increased the plateau potential in atrial myocytes resulting in a prolongation of both APD_{30} and APD_{90} (Fig. 3B). Dofetilide did not significantly enhance cell shortening.

3.3. AVE0118 blocks I_{to} and I_{Kur}, but not I_{CaL}

In canine atrial myocytes, depolarizing voltage steps from a holding potential of −60 mV produced a rapidly activating outward current which subsequently inactivated to a steady-state current (Fig. 4). The activating and inactivating pattern of this current strongly resembles the currents in human atrial myocytes, also composed of I_{to} and sustained currents (I_{Ksus}) (Fig. 4A) [19]. The latter contains a 4-aminopyridine-sensitive component which is usually referred to as I_{Kur} [12,14]. AVE0118 dose-dependently blocked both I_{to} and I_{Ksus} (Fig. 4B–D). At 100 μM, AVE0118 blocked ~98% of I_{to} and ~55% of I_{Ksus}. 1 mM 4-aminopyridine given on top of AVE0118 blocked the residual I_{to} but did not change I_{Ksus} significantly, indicating that 100 μM AVE0118 blocked virtually all I_{Kur}. IC_{50}-values for AVE0118 inhibition of I_{Kur} (1.1(0.2–3.9 μM) and I_{to} (time integral) (1.8(0.4–3.4)μM) resembled those reported by others [12]. 30 μM AVE0118 did not affect I_{CaL} peak current density, kinetics or current–voltage relationship (Fig. 4E).

3.4. Cell shortening during voltage clamp

To study the effect of action potential morphology on atrial myocyte shortening, shortening was first evaluated during trains of stylized 4-phase action potentials. We evaluated the effect of changing plateau duration (Fig. 5A), plateau potential (Fig. 5B), prolongation of the plateau without change of APD_{90} (Fig. 5C), and the time required for final repolarisation (prolongation of APD_{90} without change in APD_{30}, Fig. 5D) on cellular shortening of 8 atrial myocytes isolated from 5 dogs. Plateau prolongation strongly enhanced fractional shortening from 1.9±0.8% at 50 ms to 16.6±2.7% at 600 ms ($p<0.05$). Increasing plateau potential amplitude increased shortening from 0.22±0.05% at −50 mV to 17.8±2.6% at +60 mV ($p<0.05$). Prolongation of the plateau duration by 150 ms without changing APD_{90} nearly doubled shortening of the atrial myocytes ($p<0.05$). In contrast, prolongation of APD_{90} by 200 ms did not significantly enhance shortening. To study the effect of the change in action potential configuration induced by I_{to}/I_{Kur}-block on cell shortening, action potential clamp experiments were performed using the action potentials recorded in the absence and presence of AVE0118 (30 μM) as command waveforms, and atrial myocyte shortening was studied under voltage clamp conditions. Experiments were

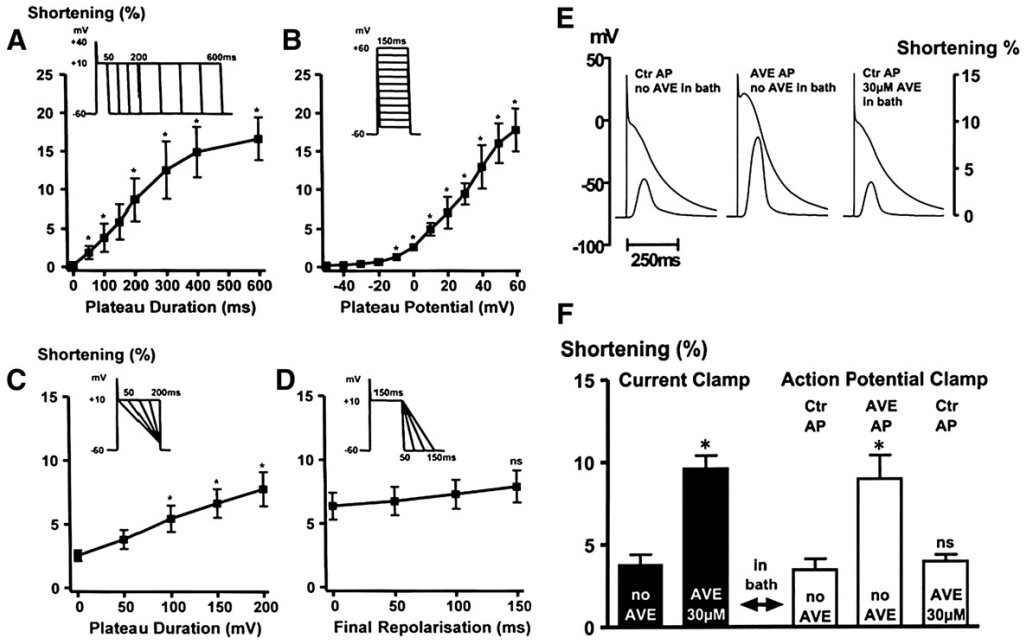

Fig. 5. A–D: Effect of AP configuration on myocyte shortening. Insets show shape of the stylized APs used as command potentials. Stimulation at in voltage clamp 1 Hz, 37 °C. $N=8$ cells from 5 dogs. *: $p<0.05$ versus control. E and F: Inotropic effect related to the change of the action potential configuration induced by AVE0118. "Ctr AP" = action potential recorded under control conditions, "AVE AP" = action potential recorded in the presence of 30 μM AVE0118. E: Action potential command waveforms and shortening tracings of atrial myocytes during action potential clamp experiments. F: Myocyte shortening during current clamp (same trabeculae as in Fig. 2) and action potential clamp experiments. Stimulation at 1 Hz, 37 °C. $N=8$ cells from 5 dogs. *: $p<0.05$ versus control.

performed in the absence and presence of AVE0118 (30 μM) in the bathing solution (Fig. 5E and F). With no AVE0118 in the bathing solution, switching from an action potential obtained under control conditions to an action potential recorded in the presence of 30 μM AVE0118 enhanced shortening from 3.6±0.6% to 8.7±1.5%, $p<0.05$. This positive inotropic effect was not significantly different from the effect provoked by the drug itself under current clamp conditions. Importantly, voltage clamp with an action potential waveform recorded during control conditions completely abolished the positive inotropic effect of 30 μM AVE0118 added to the bathing solution.

3.5. Effect of AVE0118 on Ca^{2+} entry in atrial myocytes

To test the effect of AVE0118 on Ca^{2+} entry via the L-type Ca^{2+} channel, I_{CaL} measurements were performed using the action potential clamp technique. Fig. 6A shows I_{CaL} current traces and summary data during application of a control action potential and action potentials recorded in the presence of different concentrations of AVE0118. Peak inward current declined in a dose-dependent manner to 75% of baseline at 30 μM AVE0118. Because the plateau potential declined more slowly in the presence of AVE0118, and passed the window of maximal channel activation (~0–5 mV) later during the action

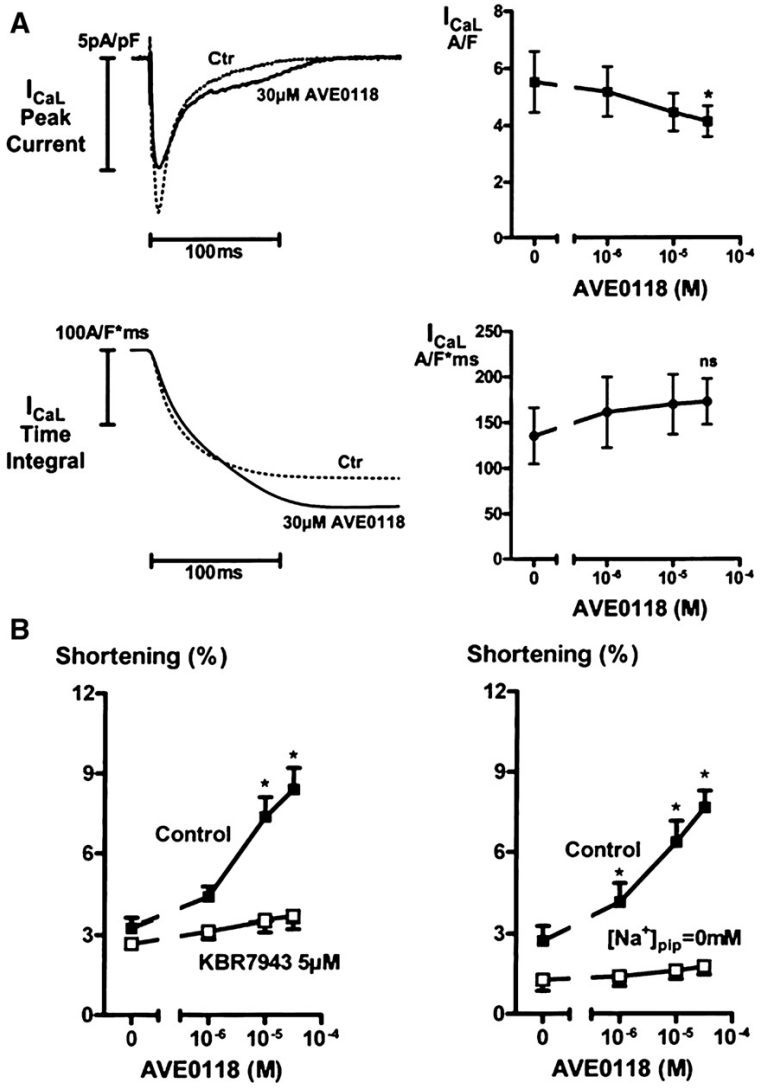

Fig. 6. A: Peak current (upper panels) and time integral (lower panels) of I_{CaL} during action potential clamp experiments. Action potentials recorded during control and various concentrations of AVE0118 were used as command waveforms. Stimulation at 1 Hz, 37 °C. $N=9$ cells, 5 dogs. *: $p<0.05$ versus baseline. B: Inotropic effect of clamping atrial myocytes to action potentials recorded in the presence of various concentrations of AVE0118. During control, "AVE0118-like" action potentials significantly increased myocyte shortening. Both, KBR7943 (9 cells from 5 dogs) and using a Na^+-free pipette solution (8 cells from 6 dogs) completely abolished this positive inotropic effect. Stimulation in action potential clamp at 1 Hz, 37 °C. $N=10$ cells, 4 dogs. *: $p<0.05$ versus control.

potential, a second component of inward current occurred during the later phase of the action potential. The second component reflects the balance between increased driving force of I_{CaL}, recruitment of additional channels, and progressive channel inactivation. The smaller peak current in the presence of AVE0118, along with less pronounced inactivation, makes Ca^{2+} influx via I_{CaL} more stable and less concentrated in the early phase of the action potential. Analysis of the current time integral (lower panels) reveals that the total amount of Ca^{2+} entering the cells is only slightly and insignificantly increased when the cells were clamped to action potentials recorded in the presence of AVE0118.

As the Ca^{2+}-transient was increased and influx via I_{CaL} was not significantly enhanced, we evaluated the effect of AVE0118 on Ca^{2+} entry via reverse mode of the Na^+/Ca^{2+}-exchanger. KBR7943, a blocker of the reverse mode Na^+/Ca^{2+}-exchange [20], was used to evaluate the contribution of the exchanger to the positive inotropic effect of AVE0118. Fig. 6B shows that action potential clamp using the waveform of action potentials recorded in the presence of AVE0118 elicited a positive inotropic effect similar to that of the drug itself in current clamp experiments. When myocytes were clamped to a control action potential, addition of 5 μM KBR7943 to the bathing solution did not significantly affect cellular shortening. However, the positive inotropic effect of clamping the cells to action potentials recorded in the presence of AVE0118 was completely abolished by this treatment. Similarly, omission of Na^+ from the pipette solution (Fig. 6B) also blunted the positive inotropic effect of AVE0118.

4. Discussion

Atrial contractility is decreased in AF, largely due to electrical remodeling that is associated with a downregulation of L-type calcium currents [17,21–23]. The present study demonstrates that combined block of I_{to} and I_{Kur} enhances force of contraction of isolated human atrial trabeculae, both in patients in sinus rhythm or AF. In canine right atrial myocytes, we found that the positive inotropic effect of I_{to}/I_{Kur}-block was due to changes in the shape of the action potential, resulting in a secondary enhancement of reverse mode Na^+/Ca^{2+}-exchanger activity. This mechanism represents a new positive inotropic principle which is atrial-specific and which might be useful for the treatment of atrial contractile dysfunction induced by atrial tachyarrhythmias.

4.1. I_{to}/I_{Kur}-block enhances contractile force of atrial trabeculae isolated from AF patients

As reported previously, contractile force of isolated atrial trabeculae from AF patients is ~70% lower than that of patients in sinus rhythm [17,18]. This phenomenon likely reflects the clinical observation that after cardioversion of AF, atrial contractility is significantly impaired. AVE0118 strongly increased contractile force in AF patients, nearly reaching the contractile force generated by trabeculae from patients in sinus rhythm at baseline. We recently demonstrated that AVE0118 completely restored atrial contractility in chronically instrumented goats although atrial contraction was virtually absent after cardioversion of AF [24]. These observations indicate that AVE0118 might have the potential to significantly improve or even to restore atrial contractile function after termination of the arrhythmia in patients.

4.2. Modulation of atrial contractility by the shape of the atrial action potential

During an action potential, Ca^{2+} enters myocytes via L-type Ca^{2+} channels and reverse mode Na^+/Ca^{2+}-exchanger activity. Both pathways are strongly voltage dependent, but Ca^{2+} entry via I_{CaL} depends both on the potential and on the kinetics of the current. As a consequence, myocyte shortening is dependent on the shape of the action potential.

In our voltage clamp experiments, cell shortening was strongly dependent on both plateau duration and plateau potential of the test waveform. While a close relationship between action potential duration and the resulting contractile force was documented decades ago [25], the dependence of atrial cell shortening on the plateau potential is less well appreciated in the literature. In ventricular voltage clamped myocytes, cell shortening is paralleled by the bell-shaped current–voltage relationship of I_{CaL}, with low contraction amplitude at positive potentials of >40 mV [26,27]. In contrast, in the canine atrial myocytes studied here cell shortening continued to increase at positive potentials of up to 60 mV. A similar voltage-shortening relationship was recently reported in trout atrial myocytes. In this study, the increase of cell shortening at positive potentials was not blocked by nifedipine and was dependent on a sustained Ni^{2+}-sensitive outward current [28]. Also, in rabbit atrial myocytes Ca^{2+} entry via reverse Na^+/Ca^{2+}-exchange was able to trigger release of Ca^{2+} from the sarcoplasmic reticulum at positive potentials [29]. These observations suggest that at positive membrane potentials, atrial myocyte shortening significantly depends on reverse mode Na^+/Ca^{2+}-exchange as an alternate route for Ca^{2+} entry.

At plateau potentials less than −10 mV, a 150 ms duration test action potential waveform was unable to elicit any shortening response. This observation stresses the fact that excitation–contraction coupling is strongly dependent on the potential during the early plateau phase of the AP, whereas the final repolarization phase is less important. In keeping with this, prolongation of final repolarization did not change cell shortening, while plateau prolongation without any change in APD_{90} produced a strong positive inotropic effect. The observed differences between dofetilide and AVE0118 on cell shortening are thus closely related to their differential effects on action potential morphology. AVE0118 significantly increases plateau potential but does not change APD_{90}, leading to an increase in contractile force. In contrast, dofetilide has only a small effect on plateau potential, but significantly prolongs APD_{90}. As the amount of Ca^{2+} entering the cells

during final repolarisation is small, dofetilide does not produce a significant positive inotropic effect.

4.3. Mode of positive inotropic action of AVE0118

Recently, Wettwer et al. showed that the I_{to}/I_{Kur}-blocker 4-aminopyridine (4-AP) prolongs the plateau phase of the human atrial action potential in patients with sinus rhythm or AF [15]. Computer modeling suggested that this change of the shape of the action potential would result in an increase of I_{CaL}. Concordant with this hypothesis, we found that I_{to}/I_{Kur}-block with AVE0118 increased the amplitude of Ca^{2+}-transients in canine right atrial myocytes during field stimulation. Furthermore, our data provide direct evidence that the positive inotropic effect of AVE0118 is exclusively related to the change in the shape of the atrial action potential. Application of action potentials recorded in the presence of AVE0118 produced an increase in cell shortening similar to that of the drug itself. More importantly, action potential clamp with a control action potential waveform in the presence of drug abolished the effect of AVE0118 added to the bathing solution. This result suggests that mechanisms unrelated to the change in shape of the atrial action potential do not contribute significantly to the inotropic effect of AVE0118, and demonstrates that the positive inotropic effect of I_{to}/I_{Kur}-block in atrial myocytes can be studied by clamping the myocytes to action potentials recorded in the presence of this compound.

At first glance, the change of the action potential configuration induced by AVE0118 should seem to have consequences for the kinetics of I_{CaL}, which might explain the positive inotropic action of the compound. Thus, it was somewhat surprising that we found that peak I_{CaL} density declined when action potentials recorded in the presence of AVE0118 were used as command waveforms in action potential clamp. This is likely due to the fact that initial ("phase one") repolarisation is delayed in the presence of AVE0118, due to I_{to}-block. Since the potential during the initial repolarization is more positive than the peak of the current–voltage relationship (~0–5 mV in canine atrial myocytes), fewer Ca^{2+} channels are activated early during the action potential. However, as the potential passes the window of optimal activation, more channels become activated and the current is more sustained. As a result, the current time integral which represents the amount of Ca^{2+} entering the cells during the action potential tends to be even higher in the presence of AVE0118. In our experiments this effect was insignificant and already present at the lowest concentration of AVE0118 studied (1 μM). Most of the positive inotropic effect, however, occurred at higher concentrations of the drug. Therefore, changes of time course or time integral of I_{CaL} are unlikely to contribute significantly to the positive inotropic effect of AVE0118. Further, the I_{CaL} measurements were performed in the presence of a high intracellular EGTA concentration which depresses Ca^{2+}-dependent inactivation of I_{CaL}. Since AVE0118 increases the amplitude of the Ca^{2+}-transient, we can not rule out that our measurements slightly overestimate the effect of AVE0118 on the I_{CaL} time integral. This also argues against an important role of I_{CaL} in the positive inotropic effect of the compound.

An alternate explanation for the positive inotropic effect of I_{to}/I_{Kur}-block is that the more positive potential during the plateau of the atrial action potential shifts the balance between forward- and reverse mode Na^+/Ca^{2+}-exchanger activity to enhance the reverse mode. In reverse mode, Ca^{2+} entry contributes to refilling of the sarcoplasmic reticulum. If this hypothesis is correct, block of the Na^+/Ca^{2+}-exchanger reverse mode should significantly attenuate the positive inotropic effect of I_{to}/I_{Kur}-block. Indeed, we found that application of action potentials recorded in the presence of AVE0118 significantly increased cell shortening, and this effect was completely abolished in the presence of 5 μM KBR7943, a concentration known to block reverse mode Na^+/Ca^{2+}-exchange [30]. The same was found when Na^+ was omitted from the pipette solution, which also attenuates reverse mode Na^+/Ca^{2+}-exchange. Thus, the positive inotropic effect of AVE0118 appears to be due to a change in action potential configuration, secondarily enhancing reverse mode Na^+/Ca^{2+}-exchange. This mechanism helps explain why AVE0118 effectively enhances atrial contractility in patients with AF. In AF patients, I_{CaL} is downregulated by ~70% which will decrease the efficacy of positive inotropic interventions that primarily target I_{CaL}. We previously demonstrated that the L-type Ca^{2+} channel agonist BayK8644 failed to increase atrial contractility in AF patients [17]. In contrast, the Na^+/Ca^{2+}-exchanger is upregulated [18], and here we show that AVE0118, using the Na^+/Ca^{2+}-exchanger as an indirect target, can effectively increase atrial contractility in patients with AF.

Importantly, in canine ventricular myocytes AVE0118 neither prolonged repolarization nor enhanced contractility, emphasizing the atrial-specific nature of the effects of I_{to}/I_{Kur}-block. The slight shortening of the action potential in ventricular myocytes might be explained by earlier and enhanced activation of I_{Kr} due to block of I_{to} [31].

4.4. Limitations

In dogs, the atrial action potential is slightly shorter and the degree of shortening due to AF is more pronounced than in humans [32,33]. Therefore, in these two species the relative contribution of I_{to} and I_{Kur} to repolarization, and the effect of block of these currents on the shape of the action potential might differ, with potential consequences for the extent of the positive inotropic effect of the drug. Even though in the present study I_{to}/I_{Kur}-block produced a significant positive inotropic effect in trabeculae isolated from the atria of patients with chronic AF and despite the fact that AVE0118 was shown to restore atrial contraction after cardioversion in chronically instrumented goats [24], it still remains to be determined whether AVE0118 significantly improves atrial contractile function in patients after termination of AF. Also, although we did not observe obvious

signs of Ca^{2+}-overload in the presence of AVE0118 (Ca^{2+}-waves or delayed after depolarisations) we cannot rule out the possibility that excessive concentrations of AVE0118 might result in increased Ca^{2+}-load of atrial myocytes.

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