The new antiarrhythmic drug vernakalant: ex vivo study of human atrial tissue from sinus rhythm and chronic atrial fibrillation

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Received 10 July 2012; revised 11 January 2013; accepted 15 January 2013; online publish-ahead-of-print 22 January 2013

Time for primary review: 20 days

Aims Vernakalant is a newly developed antiarrhythmic drug against atrial fibrillation (AF). However, its electrophysiological actions on human myocardium are unknown.

Methods and results Action potentials (APs) and ion currents were recorded in right atrial trabeculae and cardiomyocytes from patients in sinus rhythm (SR) and chronic AF. Vernakalant prolonged early repolarization in SR and AF, but late only in AF. AP amplitude (APA) and dV/dtmax were reduced in a concentration- and frequency-dependent manner with IC50, 1.3 Hz. Effective refractory period was increased more than action potential duration (APD) in SR and AF. INa was blocked with IC50s of 95 and 84 mM for SR and AF, respectively (0.5 Hz). Vernakalant did not reduce outward potassium currents compared with time-matched controls. However, area under the current–time curve was reduced due to acceleration of current decline with IC50s of 19 and 12 mM for SR and AF, respectively. Vernakalant had less effect on APD than the IKr blocker E-4031, blocked IK,ACh, and had a small inhibitory effect on IK1 at 30 mM. L-Type Ca2+ currents (SR) were reduced with IC50 of 84 mM.

Conclusion Rate-dependent block of Na+ channels represents the main antiarrhythmic mechanism of vernakalant in the fibrillating atrium. Open channel block of early transient outward currents and IK,ACh could also contribute.

Keywords Atrial fibrillation • Vernakalant • Human atrial action potentials • Na+ current • K+ currents • Atrial selectivity

1. Introduction

The high prevalence of atrial fibrillation (AF) in the ageing western population has fuelled the search for new therapeutic interventions. Conventional antiarrhythmic drugs are burdened with low efficacy and serious cardiac and extracardiac side effects. For instance, Na+ channel blockers with slow dissociation kinetics possess a clear propensity to provoke arrhythmias, in particular in patients with ischaemic heart disease.3 Proarrhythmic effects are also known for K+ channel blockers which can excessively prolong ventricular action potential duration (APD) leading to early afterdepolarizations that give rise to torsade-de-pointes arrhythmias.2

Vernakalant (RSD1235) is a novel ‘atrial-selective’ antifibrillatory drug. It was developed as a multiple ion channel blocker and targets Na+ channels (Nav1.5), various K+ channels, and native K+ currents with IC50 values in the micromolar concentration range (Kv1.5, 13 μM; Kv4.2, 38 μM; Kv4.3, 30 μM; hERG, 21 μM; IK,ACh, 10 μM; IK1 > 1 mM).3 Atrial selectivity refers to suppression of abnormal excitatory processes in the atria without proarrhythmic effects in the ventricles. Block of Na+ channels is a generally accepted

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antiarrhythmic mechanism (class I action according to 9) that was recently recognized to exhibit atrial selectivity when drug unbinding from the channels is potential dependent and rapid. 5 Potential dependency implies that drug affinity is higher at less negative membrane potential so that more drug will be bound and dissociation from the Na+ channels will be slower than at more negative potential. Since resting membrane potential (RMP) in atrium is less negative than in ventricle, more drug will remain bound to and block Na+ channels in atria than in ventricle, thereby exhibiting the atrial selectivity of Na+ channel block. Atrial-selective drug effects are also conferred by IKur, blockade, since Kv1.5 channels are predominantly expressed in the human atria 6 and IKur is undetectable in the human ventricle. 7 Increased inward rectifying K+ current can stabilize rotors. 8 However, only acetylcholine-activated current IKACh has been suggested to represent AF-specific drug targets, 9 although both constitutively active IKACh and Kir2.1 channel upregulation are hallmarks of the remodelled atria. 10 Block of IK1 is expected to be proarrhythmic because of its importance in the ventricles. As a result of its ion channel profile, vernakalant was shown to be atrial selective in humans. 11

Vernakalant has performed favourably in preclinical models of AF (see 12 for review). Several recent clinical studies demonstrated that vernakalant effectively and safely converts AF into sinus rhythm (SR). 13–15 The AVRO trial, for instance, compared intravenous application of vernakalant vs. amiodarone for conversion of recent onset AF. Ninety minutes after initiation of therapy 51.7% of the patients had been successfully converted in the vernakalant group vs. 5.2% in the amiodarone group. 13 These results were instrumental for European approval of vernakalant for the indication of intravenous conversion of recent onset AF.

Although effects of vernakalant on cardiac APs and various ion currents have been reported in several species and expression systems, 3,11–17 comparable results are not available in isolated human atrial myocardium. Moreover, chronic AF is associated with electrical remodelling that induces robust AP alterations as evidenced by transformation of the typical spike-and-dome configuration of the SR AP into the triangular shape characteristic of permanent chronic AF. 18–20 Here we have studied the effects of vernakalant in human atrial cardiomyocytes and trabeculae both from patients in SR and chronic AF. In addition to comparing the effects of vernakalant and accepted IKur blockers on the shape of the atrial APs, we have also directly measured vernakalant’s action on INa, INaL, IKur, and IKACh in isolated human atrial cardiomyocytes. The aim of our study was to elucidate the putative mechanism of antiarrhythmic action by this novel drug in human tissue. Some of the data have been published previously in preliminary form. 21

### 2. Materials and methods

#### 2.1 Human tissue samples and patients’ characteristics

All work with human samples conforms to the Declaration of Helsinki. The study was approved by the ethics committee of Dresden University of Technology (No. EK790799). Each patient gave written, informed consent. Right atrial appendages were obtained from patients with SR and with long lasting AF (>6 months). The two groups differed significantly in age, in incidence of coronary artery disease (CAD), in CAD plus valvular disease, as well as in treatment with digitalis and lipid lowering drugs. For patients characteristics see Table 1.

#### 2.2 Electrophysiological recordings

APs were recorded with standard intracellular microelectrodes in atrial trabeculae. 19 Bath solution contained (in mM): NaCl 127, KCl 4.5, MgCl2 1.5, CaCl2 1.8, glucose 10, NaHCO3 22, NaH2PO4 0.42, equilibrated with O2–CO2 [95:5] at 36.5 ± 0.5°C, pH 7.4. Preparations were regularly stimulated for at least 1 h before data acquisition with a custom-made computer program (University of Szeged, Hungary) that also generated electrical stimuli. The following AP parameters were analysed: APA, RMP, AP duration at 20, 50, and 90% of repolarization (APD20, APD50, APD90, respectively), plateau potential defined as the mean potential (Vm) in the time window between 20 and 30% of repolarization (PLT20), and maximum upstroke velocity (dV/dtmax). The effective refractory period (ERP) was determined by applying an extra stimulus (2 × threshold voltage) at increasingly smaller intervals every 10th regular period until failure of initiating an extra AP. APs were analysed offline by using the LabChart software (ADInstruments, Spechbach, Germany).

Human myocytes were isolated enzymatically from atrial appendages and the conventional voltage-clamp technique in the ruptured patch configuration was used to measure ion currents (INa, INaL, IKACh, IK1, and IKs) as previously described. 22 The compositions of superfusion and pipette solutions used for measurements of ion currents are given in Supplementary material online, Table S3. The liquid-junction potential was calculated with JPCalc.

After forming a giga-ohm seal and breaking into the cell, capacitance was measured with small hyperpolarizing clamp steps from −40 to −42 mV. About 70% of series resistance and up to 100 pF of membrane capacitance were compensated electronically. Mean values are given in the legends to the figures.

<table>
<thead>
<tr>
<th>Table 1 Patients characteristics</th>
<th>SR</th>
<th>cAF</th>
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<tr>
<td>Patients, n</td>
<td>25</td>
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<tr>
<td>Gender, m/f</td>
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<tr>
<td>Lipid-lowering drugs, n</td>
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</table>

SR, sinus rhythm; cAF, chronic atrial fibrillation; CAD, coronary artery disease; MVD, mitral valve disease requiring valve replacement; AVD, aortic valve disease requiring valve replacement; LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme; AT, angiotensin receptor.

*p < 0.05 values from non-paired Student’s t-test for continuous variables and from the χ² test for categorical variables.
2.3 Chemicals and drugs
Vernakalant was provided by Merck Research Laboratories. Tertiapin-Q was a gift from Xention (Cambridge, UK). All other compounds were purchased from Sigma (Steinheim, Germany).

2.4 Data analysis and statistics
For determining IC_{50} values, sigmoidal functions were fitted to the mean data points of concentration response curves using GraphPad Prism (GraphPad Software Inc., San Diego, USA).

Differences between continuous data were compared by paired (AP parameters) or unpaired Student’s t-test (with Welch’s correction if indicated) or one-way ANOVA. P < 0.05 was considered statistically significant.

3. Results
3.1 Effects of vernakalant on human right atrial action potentials
Based on the previously reported IC_{50} values for ion channels expressed in human embryonic kidney cells and on the therapeutic plasma concentration range measured in patients, the effects of vernakalant on human atrial APs were studied at 10 and 30 μM (Figure 1). At a frequency of 1 Hz, AP parameters of pre-drug control significantly differed in preparations from patients in SR and AF yielding the typical spike and dome and triangular shape in SR and AF, respectively. In SR preparations, RMP was not significantly less negative compared with AF preparations, APA was smaller, plateau potential was more negative, APD_{20} was shorter, and APD_{90} was longer than in AF (see Supplementary material online, Tables S1 and S2). Moreover, SR and AF preparations exhibited distinctly different responses to vernakalant. In SR trabeculae, the most prominent effects of vernakalant (30 μM) were a significant slowing of early repolarization causing a widening of the spike, with some depression of the plateau phase, but little effect on final repolarization (APD_{90}), a rate-dependent reduction of the maximum upstroke velocity dV/dt_{max} and APA. In AF, the prolonging effects of vernakalant on early and late repolarization phase (APD_{20} and APD_{90}, respectively) were variable in size, but nevertheless statistically significant for vernakalant (10 μM) at 0.5, 1, and 3 Hz (see Supplementary material online, Table S2). The ERP was significantly lengthened, and there was a trend for a larger increase in ERP than in APD_{90}, although this did not reach the level of statistical significance. In SR trabeculae paced at 3 Hz, 30 μM vernakalant prolonged APD_{90} by 29.6 ± 19.4 ms, whereas mean ERP increased by 60.4 ± 13.8 ms. In AF trabeculae, APD_{90} and ERP were prolonged by 25.1 ± 11.7 and 45.0 ± 9.6 ms, respectively (for a complete list of all AP parameters for pre-drug control, 10 and 30 μM vernakalant at stimulation rates of 0.5, 1, and 3 Hz in comparison with time-matched controls (TMCs), see Supplementary material online, Table S1 for SR and Supplementary material online, Table S2 for AF).

The frequency dependence of vernakalant’s effect on dV/dt_{max} was studied with the following experiment. After equilibration, but under pre-drug control conditions, frequency was increased in small steps for about 1 min (Figure 1B) until the preparations failed to respond to excitation (i.e. at 5 and 6 Hz in the SR and AF preparations, respectively). Upon return to 1 Hz, excitability recovered completely within 5 min. The muscles were then exposed to the first concentration of vernakalant (10 μM) for 20 min before the next series of increasing stimulation rates was started until excitation failed again. Then stimulation rate was returned back to 1 Hz, followed by addition of 30 μM vernakalant and renewed increase in stimulation rate. With 30 μM of vernakalant, excitation failed at even lower rates than with 10 μM. Moreover, suppression of dV/dt_{max} with a given drug concentration was larger at higher stimulation rates, suggesting strong frequency dependence of drug effect. Figure 1C compares dV/dt_{max} depression by 10 and 30 μM vernakalant expressed in percent of the pre-drug control values at the same frequency. Both in SR and AF preparations, vernakalant depressed maximum upstroke velocity to a larger extent at higher stimulation rates. This graph also allows a rough estimate of the frequency dependence of the IC_{50} value for vernakalant: at 3 Hz, 50% of block was achieved with a concentration between 10 and 30 μM, whereas at 4 Hz and higher frequencies, less than 10 μM was sufficient for half-maximum block.

The shape of the cardiac AP is determined by current flow through various different ion channels. Therefore, we also performed voltage clamp experiments to establish the profile of vernakalant’s action on ion channels in native human right atrial cardiomyocytes.

3.2 Effects of vernakalant on I_{Na}
Sodium currents were studied with reduced extracellular Na+ concentration (5 mM) in order to avoid loss of voltage control due to the huge initial current surge at physiological Na+ concentrations. Peak Na+ current amplitudes normalized to cell capacitance were significantly lower (~20%) in cardiomyocytes from patients in AF than from SR patients (Figure 2). Vernakalant produced a concentration-dependent block of peak I_{Na} amplitude. Fitting sigmoidal functions to the data points and assuming complete block of I_{Na} by high concentrations of vernakalant (Figure 2B) yielded IC_{50} values of 95 μM in SR and 84 μM in AF cells at a stimulation rate of 0.5 Hz. The current–voltage (IV) curves (Figure 2C and D) illustrate the potential dependence of block. The reversal potential for I_{Na} of +15 mV was very close to the calculated liquid-junction potential of +11.4 mV. We were not able to investigate vernakalant’s effect on late Na+ current (I_{Na,late}) because we could not detect any I_{Na,late} in SR or AF cells.

3.3 Effects of vernakalant on I_{to}/I_{Kur}
In SR cells, K+ currents activated with 500 ms long clamp steps from a holding potential of –60 mV to a test potential of +50 mV, consisted of a large peak transient component (I_{peak}) and a steady-state component (I_{late}). I_{peak} was markedly smaller in AF than in SR, consistent with down regulation of Kv4.3 channels (conducting transient outward current I_{to}) in AF (Figure 3A). Increasing concentrations of vernakalant (1–100 μM) slightly reduced I_{peak} and I_{late} in both groups of cells; however, similar changes also occurred in TMC experiments (‘run-down’) (Figure 3B and C). Because of the discrepancy between this finding and the vernakalant-induced prolongation in APD during the early phase of repolarization (‘widening of spike’), we analysed the drug’s effect during the initial 50 ms of a clamp step to +50 mV by calculating the area under the current–time curve (AUC) (Figure 4A and B). The AUC remained constant in TMCs. In SR and AF cells, vernakalant accelerated the apparent rate of inactivation of the initial rapidly declining current component, producing a significant, concentration-dependent reduction in outward charge transfer during the early phase of the clamp step both in SR and AF (Figure 4C and D). The IC_{50} values obtained by curve fitting to
cumulative concentration–response curves for AUC were 19 and 12 μM for SR and AF, respectively.

### 3.4 Effects of vernakalant on \(I_{Kr}\)

In human enzymatically dissociated cardiomyocytes, we have never been able to detect any \(I_{Kr}\), although the selective \(I_{Kr}\) blocker E-403125 prolongs APD in human papillary muscle. Therefore, direct effects of vernakalant on \(I_{Kr}\) in native human atrial myocytes could not be tested. However, in atrial multicellular trabeculae, E-4031 significantly prolonged APD (Figure 5) with \(EC_{50}\) values of 181 and 201 nM in SR and AF preparations, respectively (\(n=7\) in each group) (Figure 5C and D). Vernakalant, however, was ineffective in SR and slightly increased APD at higher frequencies (see Supplementary material online, Figure S1D and E). It should be noted, however, that also in the absence of drug, \(I_{Ca,L}\) declined with increasing frequency, probably due to incomplete recovery from inactivation during shorter intervals. With 100 μM vernakalant, suppression of \(I_{Ca,L}\) was limited to about 50%.

### 3.6 Effects of vernakalant on \(I_{K1}\) and \(I_{K,ACh}\)

Inward rectifier K⁺ current was measured during a 1000 ms ramp pulse from −100 to +40 mV (pulse frequency 0.2 Hz) and current amplitude was analysed in the inward branch at −100 mV. \(I_{K,ACh}\) was activated two times in succession by stimulating muscarinic receptors with carbachol (2 μM) for 2 min, followed by a 4 min wash-out period and renewed exposure (S1 and S2, respectively).
Vernakalant (30 μM) was added 2 min prior to S2. Diary plots of basal current and I\textsubscript{K,ACH} are shown in Figure 6A. During exposure to carbachol (CCh), current amplitude at −100 mV rapidly increased, and slowly decreased again due to desensitization. Moreover, S2 was always smaller, i.e. ≏0.8 of S1 due to incomplete recovery from desensitization. Vernakalant (30 μM) significantly depressed I\textsubscript{K,ACH} in SR cells (Figure 6B). Effects of vernakalant on AF cells could not be estimated because of non-uniform effects of carbachol in AF cells. Using the selective I\textsubscript{K,ACH} channel blocker tertiapin, we have shown previously that I\textsubscript{K,ACH} becomes constitutively active in chronic AF because basal current is significantly reduced with tertiapin only in AF but not in SR, and a similar trend is observed in the present experiments (Figure 6C and D). Vernakalant (30 μM), however, significantly reduced basal current in SR by 15.5 ± 3.8% (from 8.2 ± 1.0 to 7.2 ± 1.0 pA/pF). In AF, basal current was reduced by 7.6 ± 2.7% (from 16.9 ± 2.9 to 15.5 ± 2.6 pA/pF; Figure 6E and F); however, this difference did not reach the level of statistical significance. In contrast, tertiapin had no effect on basal current in SR, indicating lack of constitutively active I\textsubscript{K,ACH}, but significantly reduced basal current in AF—as expected from constitutively active I\textsubscript{K,ACH} in this arrhythmia. Thus vernakalant has a small blocking effect on I\textsubscript{K1} in SR.

4. Discussion

Vernakalant’s electrophysiological and pharmacological properties have mainly been demonstrated in expression systems or animal models of AF. The major findings of our present study in human atrial tissue are that vernakalant prolonged early repolarization without elevating the plateau phase of the AP like other I\textsubscript{to/IKur} blockers but reduced APA and maximum upstroke velocity \(\frac{dV}{dt}\max\) in a rate-dependent manner. This suggests that Na\textsuperscript{+} channel block dominates over I\textsubscript{Kur} block with respect to AP changes in isolated trabeculae.

4.1 Effects on action potentials

Typical I\textsubscript{to/IKur} blockers, such as 4-aminopyridine or AVE0118, elevate the plateau phase of human atrial APs and produce a significant positive inotropic effect. Vernakalant also elevates the plateau and prolongs APD in cardiomyocytes from rat ventricle—which unlike human ventricle—expresses functional Kv1.5 channels. Similarly, vernakalant elevates the plateau in dog left atrium, consistent with I\textsubscript{Kur} inhibition. In contrast, vernakalant did not elevate the AP plateau or enhance force of contraction in human atrial trabeculae (unpublished result). This is unlike other I\textsubscript{Kur} blocking
drugs that elevate AP plateau and produce a robust positive inotropic response. These results suggest that IKur block by vernakalant was not sufficient to elevate AP plateau in human atrial AP from SR patients. The reason for this finding is not entirely clear. Enzymatic destruction of targeted channels can be excluded because APs were recorded in intact trabeculae. Moreover, a recent report of the rate-dependent effects of vernakalant in dog left atrial preparations showed that sodium channel block was the major contributor. The significant effect of vernakalant on outward current during the initial tens of ms of a clamp step (AUC in Figure 4) explains the ‘widening of the spike’ of the AP in SR, whereas in AF this may even produce the increase in APD20 consistent with further inhibition of already downregulated early repolarizing current (i.e. IKur and Ito).

Vernakalant concentration-dependently attenuated APA and reduced dV/dt max. These findings support Na+ channel blocking effect of vernakalant, since dV/dt max reflects availability of Na+ channels. Vernakalant significantly prolonged ERP, i.e. at 3 Hz by ~60 ms in SR and by ~45 ms in AF with 30 μM. These increases compare well with previously reported in vivo measurements after intravenous drug application, where ERP was prolonged by ~31 ms at a cycle length of 600 ms. There was a trend for larger prolongation in ERP than in APD90, although not reaching the level of statistical significance. Nevertheless, this is in line with post-repolarization refractoriness due to Na+ channel block.

Moreover, dV/dt max was reduced by vernakalant in a strongly frequency-dependent manner with less than 10 μM required for half-maximum block at stimulation rates higher than 3 Hz. Since reduction of dV/dt max impairs impulse conduction, this highly frequency-dependent effect of vernakalant most likely explains the good clinical efficacy of the drug in acute conversion of recent onset AF into SR.

Nevertheless, we have also analysed the direct effects of vernakalant on human atrial I Na.

4.2 I Na and I Na,late

In AF, peak atrial I Na was moderately smaller than in SR, confirming published data. Vernakalant blocked I Na with similar potency in SR and AF cells, the IC50 values were 95 and 84 μM, respectively, at pulse frequency 0.5 Hz, and correspond well to results in rat ventricular cells taking into account the strong potential and frequency dependence of I Na block by vernakalant. The authors reported IC50 values of 43 and 9 μM for pacing rates of 1 and 20 Hz, respectively, at a holding potential of ~80 mV, and 107 μM for 1 Hz at ~120 mV. Vernakalant was reported to suppress I Na,late measured with Nav1.5 expressed in HEK cells; however, with a similar protocol we failed to detect I Na,late in human atrial SR and AF preparations.

4.3 I to/I Kur

In human atrial cardiomyocytes, the transient outward current I to and the ultra-rapidly activating K+ current IKur exhibited substantial temporal overlap at physiological temperature. As a crude distinction, peak and late current were considered to represent I to and IKur.
respectively. In comparison with TMC, vernakalant did not significantly reduce either peak or late outward current of human atrial cardiomyocytes at physiological temperature, but attenuated early charge transfer (AUC) by apparent acceleration of inactivation of the initial components of transient outward currents. Since Kv1.5 channels expressed in a mouse fibroblast cell line inactivate rapidly at physiological temperature, the apparent acceleration of current inactivation observed during the initial 50 ms of the clamp step in native cardiomyocytes could be due to block of Ito or IKur or both. The IC50 values for the effects on AUC (19 and 12 \( \mu \text{M} \)) were within the range reported for expressed Kv1.5 (13 \( \mu \text{M} \)) and Kv4.3 (30 \( \mu \text{M} \)) and hence do not allow to distinguish between Ito and IKur. Moreover, modelling of vernakalant binding to Kv1.5 channels suggests a high affinity to activated open channels. In any case, enhanced decline of early outward current can explain the ‘widening of spike’ and increased APD 20 as observed in AP recordings from SR and AF patients.

The small size of vernakalant-induced block of IKur in native atrial cardiomyocytes was surprising given the efficacious IKur block reported in expression systems. In native atrial cells, vernakalant neither affected Ipeak nor ILate. Only when early outward current was analysed by measuring the AUC during the initial 50 ms could we detect any significant block of outward current. Therefore, it is not surprising that vernakalant did not elevate AP plateau like other Ito/IKur blockers, as for instance AVE0118.

### 4.4 IKr/ICa,L

In our hands, we could not detect IKr in human atrial cardiomyocytes which may be related to the use of the ‘chunk’ rather than ‘perfusion’ isolation method or to destruction of hERG channels by serine proteases used for enzymatic dissociation. Nevertheless, in multicellular preparation, the IKr blocker E-4031 prolonged APD with similar EC50 values in SR and AF trabeculae suggesting the presence of functional hERG channels in intact atrial tissue strips. Interestingly, human tissue remodelled by chronic AF maintained its sensitivity to the class III E-4031, whereas in a goat model of short-term AF (2 days), the class III drug sotalol lost its efficacy to prolong APD. The discrepancy is probably due to differences in models.

Vernakalant blocks hERG channels expressed in HEK cells with an IC50 of 21 \( \mu \text{M} \). Equivalent concentrations of vernakalant (10–30 \( \mu \text{M} \)) did not at all imitate the strong delay in final repolarization observed with E-4031, thus providing circumstantial evidence for lack of a major contribution of IKr block by vernakalant to the observed AP changes. However, vernakalant also blocked L-type Ca2+ channels with an IC50 of 42 \( \mu \text{M} \), indicating that human atrial ICa,L may be more sensitive to block than its counterpart in rat ventricle. Although block of ICa,L is
expected to be small at clinically relevant concentrations, it could nevertheless increase repolarization reserve and hence attenuate APD prolongation by hERG channel block. 27 Similarly, block of putative late sodium current could counteract the APD-prolonging effect of potassium channel block. However, unlike recently reported35 we have not detected any INa,late in human atrial cardiomyocytes, neither from SR nor AF patients. 24

4.5 IK,ACh, IK1
In SR atrial cardiomyocytes, vernakalant attenuated activation of IK,ACh by carbachol. Similar experiments were not carried out in AF cells because (carbachol-sensitive) IK,ACh is down-regulated in AF.28 Only AF cells exhibited a tertiapin-sensitive component of basal inward rectifier which was interpreted as due to constitutively active IK,ACh,10,40 although the current amplitude was smaller than previously reported. The reason for this discrepancy with our published data remains unclear.

Vernakalant also impaired basal current in SR cells indicating some effect on inward rectifier IK1. Since RMP was not significantly changed (Supplementary material online, Table S1), this small decrease in basal current does not have any functional consequences, but suggests stronger effect on human atrial IK1 than initially reported in guinea pig ventricular myocytes (IC50 > 1 mM).3 In AF cells, decrease of basal current by vernakalant did not reach statistical significance. In any case, attenuation of basal current could be attributed either to block of IK1, constitutively active IK,ACh, or both. Nevertheless, some effects on additional background currents can presently not be ruled out. Single channel measurements are required to distinguish between these possibilities.

4.6 Future perspective
In native cardiomyocytes, it is often difficult to separate drug actions on multiple ion channels because of overlap in potential range of current activation and inactivation or lack of highly selective blockers. As a future perspective, we will try to obtain support for our interpretation from an appropriate computational model which can reproduce the AP data.

4.7 Clinical implications
Vernakalant is intravenously applied for conversion of recent-onset AF and several clinical trials have demonstrated that the drug is effective and safe.13 – 15 Despite profound electrical remodelling in chronic AF,41 we could not detect any differences in efficacy of block of ion channels by vernakalant between SR and AF at the cellular level. Interestingly, vernakalant’s ion channel profile resulted in larger effects on APD in AF where electrical remodelling occurred, than in SR.

Provided that atrial electrophysiological properties in recent-onset AF still resemble those of SR, the antiarrhythmic effect of vernakalant is most likely due to rate-dependent block of Na+ channels rather than IK1 block. In addition, the rapid rates of excitation in AF will enhance the atrial-selective antiarrhythmic potency of vernakalant. At physiological stimulation rates, open channel block of early transient outward currents and IK,ACh could also contribute.

5. Study limitations
RMP was always larger (more negative) in preparations from AF patients compared with SR patients. Although higher expression of inward rectifier K+ channels in AF may serve as a satisfactory
explanation,28 technical reasons cannot be ruled out. Stable impalement of the microelectrode is more easily obtained in AF preparations due to less tissue motion because of AF-induced contractile dysfunction.42

Supplementary material
Supplementary material is available at Cardiovascular Research online.

Acknowledgements
The authors gratefully acknowledge expert technical assistance of Konstanze Fischer and Annegret Hantzschel.

Conflict of interest: U.R. has received research grants and honoraria for consulting and lecturing for Cardiome and Merck, Sharp, and Dohme.

Funding
The study was supported by grants from Merck and Cardiome Pharma Corp. U.R. receives financial support from Fondation Leducq (07 CVD 03, ‘Leducq European-North American Atrial Fibrillation Research Alliance’), the German Federal Ministry of Education and Research (Atrial Fibrillation Competence Network, member of the steering committee); and the EU FP7-Health-2010-single-stage ‘EUTRAF’.

Figure 6 Effects of vernakalant (10 μM, 30 μM) on inward rectifier currents $I_{K1}$ and $I_{KACH}$. (A) Effect of vernakalant, 10 μM. Diary plot of inward current from a SR cardiomyocyte measured at −100 mV during a 1 s long ramp voltage clamp step from −100 to +40 mV, temperature 24°C. $I_{KACH}$ was activated by stimulation of muscarinic receptors of the cells with carbachol (CCh, 2 μM). The cells were exposed twice to carbachol (2 μM) for 2 min with 4 min of wash out between both exposures (S1 and S2). In TMC, less $I_{KACH}$ could be activated during the second exposure due to incomplete recovery from desensitization. (B) Summary of the effects of 10 and 30 μM vernakalant on $I_{KACH}$ activated by carbachol (S2) expressed as a fraction of the first response (S1) in cardiomyocytes from SR patients. Mean values ± SEM from number of cells/number of patients as indicated by the numbers within the columns. (C and D) Basal current in percent of pre-drug control in TMC, after 10 and 30 μM of vernakalant and after 100 nM tertiapin in SR (C) and AF cells (D). (E and F) Reduction of basal current by vernakalant in comparison with the effects of the $I_{KACH}$ channel blocker tertiapin-Q. Note that basal current is significantly reduced by 30 μM vernakalant in SR (E) but fails to reach significance in AF cells (F). In contrast, tertiapin only reduces basal current in AF. Mean values ± SEM from number of cells/number of patients as indicated by the numbers in the columns. Mean capacitance was 76.5 ± 4.2 in SR (n = 58/21) and 93.6 ± 5.3 pF in AF (99/24). Stars indicate statistical significance against TMC. In (F) (100 nM Tertiapin vs. TMC), unpaired t-test with Welch’s correction for different variances was used ($P = 0.0437$).
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