Human inward rectifier potassium channels in chronic and postoperative atrial fibrillation

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Abstract

Objective: We showed recently that the 825T allele of the G-protein β3-subunit C825T polymorphism is associated with large inward rectifier K currents $I_{K_1}$ but low acetylcholine-activated K current $I_{K,ACCh}$ amplitudes. During chronic atrial fibrillation (AF), $I_{K_1}$ and $I_{K,ACCh}$ current densities were increased when compared to sinus rhythm (SR). It is unknown whether chronic AF and Gβ3 gene status are independent contributors to atrial K current activity. We measured $I_{K_1}$ and $I_{K,ACCh}$ in tissue from AF patients with different Gβ3 genotypes and assessed the relation between the $I_{K_1}$ and $I_{K,ACCh}$ amplitudes and the incidence of postoperative AF.

Methods: We measured the amplitudes of $I_{K_1}$ and $I_{K,ACCh}$ in atrial myocytes from 26 patients with sinus rhythm (SR) and from 16 patients with chronic AF (>6 months). The K currents were measured with standard patch-clamp techniques. The Gβ3 gene status of the patients was determined by PCR and restriction analysis.

Results: At $-100$ mV, the amplitude of $I_{K_1}$ was larger in AF (10.9 ± 1.0 pA/pF, $n=49/16$, cells/patients) than in SR (6.3 ± 0.6 pA/pF, $n=68/26$, $P<0.05$), whereas the amplitude of $I_{K,ACCh}$ was smaller in chronic AF (2.9 ± 0.7 pA/pF, $n=49/16$) than in SR (6.3 ± 0.7 pA/pF, $n=68/26$, $P<0.05$). These changes were independent of the patient Gβ3 gene status. Eight patients out of 26 in the SR group (31%) developed postoperative AF. When analysed based on incidence of postoperative AF, current amplitudes did not differ significantly.

Conclusion: We provide evidence for up-regulation of $I_{K_1}$ but down-regulation of $I_{K,ACCh}$ in chronic AF which are independent of Gβ3 gene status. Atrial myocytes from patients who are in SR but later develop postoperative AF have no manifestation of altered $I_{K_1}$ and $I_{K,ACCh}$ at the time of cardiac surgery. Our results suggest that the AF-related changes of $I_{K_1}$ and $I_{K,ACCh}$ may be a consequence of or a contributory factor to chronic AF. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Atrial function; Gene expression; K-channel

1. Introduction

Chronic atrial fibrillation (AF) is characterized by shortened effective refractory period (ERP), shortened action potential duration (APD) and by blunted cycle length-dependent rate adaptation ("electrical remodeling"; for review, see Ref. [1]). On a cellular basis, L-type Ca currents are significantly decreased in AF-induced remodeling [2,3], whereas changes in K+ currents are less consistent [2,4–6]. The inward rectifier K+ current $I_{K_1}$ was increased in atria of patients with chronic AF [4], whereas its density remained unchanged in the canine rapid-atrial pacing model [7]. Vagal stimulation causes bradycardia and decreases contractility via activation of another inward rectifier current, the acetylcholine-activated K+ current ($I_{K,ACCh}$). While the amplitude of $I_{K,ACCh}$ was reported to be increased in patients with chronic AF [2], mRNA and protein levels of these channels were decreased [8].

Some patients in SR develop postoperative AF, and patients with the largest atrial L-type Ca$^{2+}$ currents are at the highest risk [3]. This association suggests that a high cellular Ca$^{2+}$ load may contribute to the initiation of AF. Experimentally, AF can be induced by vagal stimulation or

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by direct injection of ACh [9,10]. Vagal stimulation shortens action potential duration and increases the dispersion of refractoriness via cholinergic modulation of repolarization mediated by activation of $I_{K,ACb}$ [11]. Therefore, we investigated the electrophysiological properties of $I_{K1}$ and $I_{K,ACb}$ in chronic AF and assessed the relation between their amplitudes and the incidence of postoperative AF. Since we recently demonstrated that a C825T polymorphism in the $G_{β3}$-protein $β3$-subunit gene affects the $I_{K1}$ and $I_{K,ACb}$ current density [12], all patients were genotyped to investigate whether chronic AF and $Gβ3$ gene status are independent contributors to atrial K$^+$ current activity.

2. Methods

2.1. Patient characteristics

The present investigation conformed with the principles outlined in the Declaration of Helsinki. All patients gave written informed consent, which was approved by the local ethics committee of the Medical Faculty of the Dresden University of Technology (No. EK210/20.07.99). Patients with paroxysmal AF and those treated with antiarrhythmic drugs or having previous cardiac surgery were excluded from the study. A small piece (~1 cm$^2$; 0.1–0.5 g) of the tip of the right atrial appendage was excised from 16 patients with chronic AF (AF >6 months) and from 26 patients without a history of AF as part of the routine atriotomy procedure.

2.2. Voltage-clamp recordings

Atrial myocytes were isolated as previously described in detail [12,13] and were kept in a storage solution containing (in mmol/l) KCl 20, KH$_2$PO$_4$ 10, glucose 10, K-glutamate 70, β-hydroxybutyrate 10, taurine 10, EGTA 10, albumin 1; pH 7.4. Only well-striated, rod-shaped myocytes were used. The single electrode voltage-clamp technique was applied using a List EPC-7 amplifier to measure membrane currents [12,13] and were kept in a storage solution (in mmol/l): NaCl 120, KCl 20, MgATP 5.0, EGTA 2.0, GTP–Tris 0.1, HEPES 10; pH 7.4) the microelectrodes had tip resistances of ~2–4 MΩ. Seal resistances were usually between 4 and 8 GΩ. Cell capacitance ($C_M$) was measured using depolarizing ramps (1 V s$^{-1}$) from −40 to −35 mV and was compensated up to 100 pF. The series resistance was compensated up to 70%. $I_{K,ACb}$ was activated by applying the muscarinic receptor agonist carbachol (2 μmol/l) to the bath solution (composition in mmol/l: NaCl 120, KCl 20, MgCl$_2$ 1, CaCl$_2$ 2.0, glucose 10, HEPES 10, pH 7.4 with NaOH) and analyzed at −100 mV as inward current. The clamp protocol consisted of a 50-ms step to −100 mV from a holding potential of −80 mV, followed by a depolarizing ramp to −10 mV (800 ms), a 100-ms step to −50 mV, and return to the holding potential (pulse frequency 0.5 Hz). All experiments were performed at room temperature.

In all experiments, the inward current at −100 mV and the difference current between the inward current in the absence and presence of the muscarinic receptor agonist carbachol (2 μM) were identified as $I_{K1}$ and $I_{K,ACb}$ by their complete suppression with Ba$^{2+}$ (1 mmol/l, see Fig. 1). The measured $I_{K1}$ and $I_{K,ACb}$ were corrected for linear conductance calculated from the reversal potential of $I_{K,ACb}$ after stimulation with carbachol as previously described [12]. We found that the mean leak conductance of the cells corresponded very well with the residual Ba$^{2+}$-insensitive current obtained after Ba$^{2+}$ application.

2.3. Molecular analysis of the G-protein β3-subunit C825T polymorphism

Genomic DNA (100 ng) extracted from peripheral blood was amplified in a 25-μl standard reaction mix containing GSP (primers: sense, 5'-TGA CCC ACT TGC CAC CCG TGC-3'; antisense, 5'-GCA GCA GCC AGG GCT GCC-3'; accession No: Y12057) and 1.1 mmol/l MgCl$_2$. Cycling conditions were: denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 63.5°C for 45 s, and 72°C for 45 s (final extension for 7 min at 72°C). The Gβ3 gene status at position 825 of the cDNA was determined by digesting PCR products (10 μl) with BseDI (Fermentas, Vilnius, Lithuania). After electrophoresis, digests were ethidium bromide stained and visualized under UV light. The undigested product (TT genotype) has a size of 268 bp; complete digestion (CC genotype) results in bands of 116 and 152 bp, respectively.

2.4. Statistical analysis

Since the number of myocytes investigated varied between the patients, the means of the groups were calculated from average values for each patient. The limited number of cells (two to seven cells per patient) analysed from a single patient were selected by the following criteria: rod shape, clear cross striations, and seal resistance >3 GΩ. Thus we did not pool cells from different patients as a group, because the variability of data from different myocytes for each patient would result in a deviation from the expected mean value if the number of cells is not balanced between patients. In addition, in order to extrapolate the K$^+$ current data to the patient population (selected clinical variables and preoperative
mediation), all statistical analyses concerning the current data were performed with “n” indicating number of patients not cells, unless stated otherwise (mean±S.E.M.). Univariate ANOVA was applied to associate the preoperative variables with electrophysiological data. Independent variables were chronic AF, selected clinical variables and medication (Table 1). To test for interactions between chronic AF and other clinical parameters or the nature of preoperative medication, interaction terms were included in separate two-way ANOVAs. Differences between continuous data from patients were compared by unpaired Student’s t-test. Frequency data were analyzed with likelihood $\chi^2$ statistics (SPSS for Windows, version 10.0). $P<0.05$ was considered statistically significant.

3. Results

The patients’ characteristics are shown in Table I. Statistically significant differences between the patient groups were found with respect to gender, the underlying cardiac disease and size of the left atrium. With univariate analysis, however, chronic AF was the only preoperative clinical variable that significantly associated with density of $I_{k}$ or $I_{k,ACh}$.

Medication was different between the patient groups (Table 1). Use of digitalis and Ca$^{2+}$ channel blockers of non-dihydropyridine type was more prevalent in chronic AF (8% vs. 50%, $P=0.001$ and 0% vs. 31%, $P=0.002$), whereas nitrates were more common in SR (44% vs. 85%, $P=0.010$). However, no significant associations were found between $K^+$ current density and preoperative therapy with either Ca$^{2+}$ channel blockers of non-dihydropyridine type ($P=0.485$) or nitrates ($P=0.965$). Digitalis was the only preoperative medication significantly associated with density of K$^+$ currents ($P=0.016$, see below).

3.1. Influence of human chronic AF on $I_{K1}$ and $I_{K,ACh}$

The resting membrane potential was not corrected for the calculated liquid junction potential ($−12$ mV) of the electrode filling solution [12]. Mean values were more negative in chronic AF than in SR ($−25.4±1.4$ mV, $n=41$, vs. $−21.5±1.1$ mV, $n=58$, respectively; $P=0.034$; statistical analysis on a per cell basis). Accounting for junction potential [12] and assuming [K+]l to be determined mainly by the pipette solution (140 mmol/l), the resting membrane potentials were $−37$ and $−33$ mV, respectively, the deviation from the potassium equilibrium potential of $−49$ mV as calculated with the Nernst equation being explained by a voltage drop resulting from the ratio of the relatively high input resistance of human atrial cells and the seal resistance [14].

The mean cell size of the myocytes in the AF group as measured as $C_M$ was larger than that in SR ($C_M=139±11$ pF, $n=49$, for AF vs. $84±4$ pF, $n=68$, for SR; $P<0.001$;
Table 1
Characteristics of patients in the SR and chronic AF group

<table>
<thead>
<tr>
<th></th>
<th>SR</th>
<th>Chronic AF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>18/8</td>
<td>6/10*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>68±6</td>
<td>65±9</td>
</tr>
<tr>
<td>History AF (months)</td>
<td>–</td>
<td>74±24</td>
</tr>
<tr>
<td>CAD</td>
<td>26</td>
<td>7*</td>
</tr>
<tr>
<td>MVD/AVD</td>
<td>0</td>
<td>9*</td>
</tr>
<tr>
<td>Hypertension</td>
<td>21</td>
<td>7</td>
</tr>
<tr>
<td>Diabetes</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>23</td>
<td>10</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>63±3</td>
<td>54±14</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>47±6</td>
<td>47±15</td>
</tr>
<tr>
<td>LAD (mm)</td>
<td>37±3</td>
<td>49±5*</td>
</tr>
<tr>
<td>Digitalis</td>
<td>2</td>
<td>8*</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>AT1 blockers</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
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<td></td>
</tr>
<tr>
<td>Dihydropyridines</td>
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<td>1</td>
</tr>
<tr>
<td>Non-dihydropyridines</td>
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<td>5*</td>
</tr>
<tr>
<td>Diuretics</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Nitrates</td>
<td>22</td>
<td>7*</td>
</tr>
<tr>
<td>Lipid-lowering drugs</td>
<td>20</td>
<td>9</td>
</tr>
</tbody>
</table>

SR, sinus rhythm; AF, atrial fibrillation; CAD, coronary artery disease; MHD, mitral valve disease requiring valve replacement; AVD, aortic valve disease requiring valve replacement; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LAD, left atrial diameter; ACE, angiotensin-converting enzyme; AT, angiotensin receptor.

*P<0.05 values from non-paired Student’s t-test for continuous variables and from χ² test for categorical variables.

In SR patients, the genotype at position 825 was CC in 12, and CT in 14, and TT in four patients. In contrast, in AF patients the genotype was CC in seven, and CT in nine, but no homozygous 825T allele carriers could be recruited. Therefore, all homozygous 825T allele carriers were excluded from the data analysis in the SR group (see above). As shown in Fig. 3, chronic AF and the G-protein β3-subunit C825T polymorphism appear to be independent contributors to the activity of I_K1 and I_K,ACh, because the statistical analysis on a per cell basis). Therefore, currents were corrected for cell size and are presented in terms of densities (in pA/pF). At −100 mV, mean values for I_K1 were significantly larger in AF (−10.9±1.0 pA/pF, n = 49 cells from 16 hearts (49/16)) than in SR (−6.3±0.6 pA/pF, n = 68/26, Figs. 1 and 2A). In contrast, the amplitude of I_K,ACh, defined as the difference between total current and I_K1, was significantly smaller in AF (−2.9±0.7 pA/pF, n = 49/16) than in SR (−6.3±0.7 pA/pF, n = 68/26), whereas the total current amplitudes (I_K1 + I_K,ACh) were similar in both patient groups (−14.1±1.1 pA/pF, n = 49/16, vs. −12.6±0.8 pA/pF, n = 68/26, for chronic AF and SR, respectively; Figs. 1 and 2A). These differences were also observed at other potentials (data not shown). In addition, when we compared the data on a per patient basis with those on a per cell basis for SR and AF separately, we did not find significant differences in the mean density of the respective currents (data not shown).

In the presence of the non-selective muscarinic receptor antagonist atropine (1 μM), carbachol failed to activate I_K,ACh in both SR and AF cells (data not shown).

Since we recently found that the G protein β3-subunit C825T polymorphism is associated with increased current density of I_K1 and I_K,ACh [12], all patients were genotyped.

In SR patients, the genotype at position 825 was CC in 12, and CT in 14, and TT in four patients. In contrast, in AF patients the genotype was CC in seven, and CT in nine, but no homozygous 825T allele carriers could be recruited. Therefore, all homozygous 825T allele carriers were excluded from the data analysis in the SR group (see above). As shown in Fig. 3, chronic AF and the G-protein β3-subunit C825T polymorphism appear to be independent contributors to the activity of I_K1 and I_K,ACh, because the
AF-related differences in current density are still present in the respective genotype groups.

3.2. Postoperative AF

After open-heart surgery, many patients with SR develop AF in the postoperative period (typically in the first few days after cardiac surgery). Therefore, we reanalysed the current data in the SR group on the basis of the occurrence of postoperative AF. Eight of 26 patients with SR (31%) experienced postoperative AF during the in-hospital recovery period. When separated by the occurrence of postoperative AF, there was no significant difference in $C_M$ (86±7 pF, $n=26$, vs. 83±8 pF, $n=42$, for with and without postoperative AF, respectively; statistical analysis on a per cell basis), resting membrane potential ($-21.6±1.9$ mV, $n=26$, vs. $-21.5±1.1$ mV, $n=42$, for with and without postoperative AF, respectively; statistical analysis on a per cell basis) or clinical characteristics, including medication (data not shown). The only significant difference between the groups was the age of the patient (72±5 vs. 66±5 years, for SR with and SR without postoperative AF, $P<0.01$). In these two groups of patients we detected no differences in the density of $I_{K1}$ ($-5.6±1.2$ pA/pF, $n=26/8$ vs. $-6.5±0.6$ pA/pF, $n=42/18$ for SR with and without postoperative AF, respectively) or $I_{K,ACh}$ ($-6.4±1.5$ pA/pF, $n=26/8$ vs. $-6.2±0.7$ pA/pF, $n=42/18$, Fig. 2).

3.3. Impact of the patients’ medication on AF-related changes in $K^+$ current density

Digitalis was the only medication significantly associated with current density ($P=0.016$, univariate analysis). Myocytes from AF patients on digitalis therapy showed a significantly lower density of $I_{K1}$ ($P=0.037$, Fig. 4). Test of interaction effects between chronic AF and medication with digitalis indicated that these are independently associated with $I_{K1}$, because no significant interaction was found ($P=0.420$).

3.4. Impact of the underlying heart disease on AF-related changes in $K^+$ current density

Valvular heart disease was more common in the chronic AF group (Table 1), which may explain the significantly larger left atrial diameter in the AF group. Thus, current changes during AF may be confounded by atrial dilation. Therefore, the data for current density of $I_{K1}$ and $I_{K,ACh}$ in patients with chronic AF were separated according to the patient’s underlying heart disease (coronary artery disease or valvular heart disease) and only patients with coronary artery disease were compared. Exclusion of all patients with valvular heart disease had no effect on detected differences in density of $I_{K1}$ ($-9.5±1.8$ pA/pF, $n=22/7$ vs. $-6.3±0.6$ pA/pF, $n=68/26$, for AF and SR, respectively; $P=0.031$) and $I_{K,ACh}$ ($-3.0±1.5$ pA/pF, $n=22/7$ vs. $-6.3±0.7$ pA/pF, $n=68/26$; $P=0.045$).

3.5. Relationship between myocyte capacitance and density of $I_{K1}$ and $I_{K,ACh}$

Since the mean cell size as measured as $C_M$ was larger in AF than in SR, we evaluated the impact of cell size on the measured current densities. The densities of $I_{K1}$ and $I_{K,ACh}$ at $-100$ mV were plotted for small (<70 pF), medium-size (70 to 100 pF), and large (>100 pF) atrial myocytes (Table 2). The increased density of $I_{K1}$ in myocytes from AF patients was independent of cell size (although not significant with cells <70 pF). Interestingly,

Table 2

<table>
<thead>
<tr>
<th>Cell capacitance</th>
<th>$I_{K1}$</th>
<th>$I_{K,ACh}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SR</td>
<td>AF</td>
</tr>
<tr>
<td>&lt;70 pF</td>
<td>7.0±0.8 (26)</td>
<td>8.7±3.6 (6)</td>
</tr>
<tr>
<td>70–100 pF</td>
<td>5.5±0.6 (21)</td>
<td>11.7±2.3 (12)*</td>
</tr>
<tr>
<td>&gt;100 pF</td>
<td>6.1±0.8 (21)</td>
<td>12.1±0.9 (31)*</td>
</tr>
</tbody>
</table>

The numbers in parentheses indicate the number of cells. *$P<0.05$ vs. $I_{K1}$ or $I_{K,ACh}$ in the respective SR group.
the AF-related reduction in $I_{K_{ACh}}$ was observed only in myocytes with $C_m$ greater than 100 pF (Table 2).

4. Discussion

Our study demonstrates that chronic human AF is associated with an increased density of $I_{K_1}$ but reduced activity of $I_{K_{ACh}}$. Patients with SR who developed postoperative AF revealed no changes in $K^+$ current density at the time of cardiac surgery. The AF-related changes in $K^+$ current density are independent of G-protein β3-subunit C825T polymorphism and may be a consequence of, or a contributory factor to, chronic AF, providing a potential electrophysiological basis for the self-perpetuating nature of AF.

4.1. Activity of $I_{K_1}$ and $I_{K_{ACh}}$ in chronic AF

The AF-related increase of $I_{K_1}$ confirms previous results reported in the right [2] and left atrium [4]. Interestingly, the $I_{K_1}$ increase in the left atrium was observed only in cells smaller than 75 pF [4], while in the right atrium the increase appears to be present in medium and large cells (present study and Ref. [2]). The reason for these differences is unclear. Since $I_{K_1}$ (and to some extent also $I_{K_{ACh}}$) determines the resting membrane potential, the larger current density of $I_{K_1}$ in AF compared with SR myocytes is expected to hyperpolarize the cell membrane. Indeed, we found more negative resting potentials in AF myocytes. At present, the cause for the increased $I_{K_1}$ is unknown. Two possibilities must be taken into consideration: (i) increased channel density due to enhanced expression or (ii) enhanced single channel activity. Current experiments in our laboratory are directed at elucidating these issues.

Here, we investigated $I_{K_{ACh}}$, because clinical evidence points to an important role of the parasympathetic nervous system in human AF [15,16]. $I_{K_{ACh}}$ was reduced by 54% in myocytes from AF patients, suggesting that vagal tone is most likely involved in electrical remodeling. Our data correspond well with a very recent report on $I_{K_{ACh}}$ reduction by 34% of mRNA and by 62% of protein [8]. The discrepancy between the low (present study) and increased amplitude of $I_{K_{ACh}}$ described by Bosch et al. [2] can be explained by the different populations of myocytes investigated: we found significantly smaller amplitudes of $I_{K_{ACh}}$ only in cells with membrane capacitance >100 pF, while Bosch et al. investigated only cells of <80 pF, in which they found no change. In addition, these authors defined $I_{K_{ACh}}$ as the total current in the presence of acetylcholine, i.e. $I_{K_1}$ plus $I_{K_{ACh}}$. If $I_{K_{ACh}}$ is calculated as the difference between total current and $I_{K_1}$ —as in the present study—there is no change in amplitude of $I_{K_{ACh}}$ in patients with chronic AF [2].

What may be the reasons for the inconsistent results obtained in the present study and Refs. [2,4]? In general, studies in human tissue are limited by various disease states, duration of the disease and drug therapy. We have shown that myocytes from atria of patients receiving digitalis had a significantly lower $I_{K_1}$ density than those from patients without this medication. Atria from patients receiving digitalis also have lower resting membrane potentials (i.e., less negative) than those receiving no digitalis [17]. Thus, our results of a higher $I_{K_1}$ density and resting membrane potential in myocytes from AF patients and the results of Refs. [2,4] may be underestimated. There are also differences when AF is considered as chronic. Both studies defined AF as chronic when persisting for at least 1 month. In our AF group, only patients with AF persisting for more than 6 months were included. Another important difference between the present study and Refs. [2,4] may be the presence of right atrial dilation. In the study of Van Wagoner et al. [4], the diameter of the right atria was not different between the SR and AF groups. Such data are not reported in Ref. [2] and were unavailable in most of our patients. However, since chronic AF leads to enlargement of both atria [18], we cannot exclude the effect of AF-induced dilation of the right atrium in our AF patients. In any case, $I_{K_1}$ was found to remain unaffected in dilated atria without AF [19]. The underlying heart disease may also affect channel activity. Some patients in the AF group had valvular heart disease, but the observed current differences remained the same when these were excluded from the analysis. Reduced functional activity of atrial $I_{K_1}$ and $I_{K_{ACh}}$ has been documented in patients with heart failure [20]. Since there was no evidence for heart failure in our and Bosch’s patients [2], and no data were reported by Van Wagoner et al. [4], it may be speculated that the observed inter-study differences could be due to differences in left ventricular function. Finally, the presence of homozygous 825T allele carriers of the G-3-subunit β3 gene, which exhibit larger $I_{K_1}$ and smaller amplitudes of $I_{K_{ACh}}$ than carriers of the C825 allele, might also affect the data of the two studies [12]. To prevent this genetic influence from confounding the results, our patients were genotyped to examine homozygous 825T allele carriers separately. Interestingly, there was not a single patient of TT genotype with chronic AF, so that patients with TT genotype had to be excluded from the SR group. The AF-related changes are independent of Gβ3 gene status, indicating that Gβ3 genotype and chronic AF are probably independent contributors to atrial inward rectifier $K^+$ channel activity. The lack of homozygous 825T allele carriers in the AF group impeded clarification of any possible interaction effect between the 825T allele and chronic AF on the activity of atrial $K^+$ currents.

4.2. Activity of $I_{K_1}$ and $I_{K_{ACh}}$ in postoperative AF

One frequent complication following cardiac surgery is development of postoperative AF [21]. In myocytes from these patients, the L-type Ca$^{2+}$ current is larger than in
patients without postoperative AF [3]. Thus, Ca\textsuperscript{2+} overload of the myocytes may be an important contributor to the onset of postoperative AF. The relationship between \( I_{K,ACb} \) as an effector of vagal activity and postoperative AF is less clear. In the present study, there were no differences in the density of \( I_{K,ACb} \) between patients with and without postoperative AF. Thus, changes in vagal tone may not play a major role in the initiation of postoperative AF.

4.3. Study limitations

The AF-related decrease of \( I_{K,ACb} \) may also be a consequence of altered density of muscarinic receptors and/or inhibitory (G\textsubscript{i}) G-protein levels. To the best of our knowledge, the density of muscarinic receptors has not been investigated in atria from AF patients and G\textsubscript{i}-protein expression is unaffected by AF [22]. Thus, the observed changes in response to carbachol are probably due to down-regulation of \( I_{K,ACb} \) density.

The incidence of the TT genotype in the Caucasian population is 5–10% [23]. Therefore, the absence of the TT genotype among the AF group may indicate that we have studied too few patients and could possibly encounter homozygous 825T allele carriers when more AF patients are recruited in future studies.

Valvular heart disease was frequently present in the AF group, but was absent in the SR group. Subgroup analysis, however, showed that the underlying heart disease had no effect on electrophysiological data. Digitalis and \( \text{Ca}^{2+} \) channel blockers of non-dihydropyridine type were more frequently prescribed in AF, whereas \( \beta \)-blockers, nitrates, and ACE inhibitors were more common in the SR group. Thus, we cannot exclude that these drugs altered the current density. However, among these drugs, only digitalis was significantly associated with K\textsuperscript{-} current density \( (I_{K_1}) \). Physiologically, the \( \text{Na}^{+}–\text{K}^{+} \) pump creates a current in the outward direction [24]. Thus, inhibition of this pump current by digitalis would reduce its contribution to the background conductance, i.e. a larger \( I_{K_1} \) density in patients treated with digitalis should be expected. Since the digitalis-related changes were in the opposite direction, i.e. the mean \( I_{K_1} \) current density was smaller in patients treated with digitalis, the difference between SR and AF is expected to be even larger without this confounding factor.

4.4. Conclusions

\( I_{K_1} \) and \( I_{K,ACb} \) are the main K\textsuperscript{-} currents contributing to the late phase of repolarization of the atrial action potential [11]. Thus, these currents may play a key role in the frequency-dependent modulation of APD and ERP. Parasympathetic stimulation shortens APD and ERP and increases ERP dispersion, which may contribute to the perpetuation of AF [25]. Therefore, reduction of \( I_{K,ACb} \) might serve as a compensatory mechanism for the AF-related decrease in APD and ERP. Catheter ablation of cardiac parasympathetic nerves in dogs prevents vagal atrial fibrillation [26], suggesting that, at least in some patients with AF, inhibition of the parasympathetic nervous system may have an antiarrhythmic effect.

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