Changes in $I_{K,ACh}$ single-channel activity with atrial tachycardia remodelling in canine atrial cardiomyocytes

Niels Voigt, Ange Maguy, Yung-Hsin Yeh, Xiaoyan Qi, Ursula Ravens, Dobromir Dobrev, and Stanley Nattel

Aims Although atrial tachycardia (AT) remodelling promotes agonist-independent, constitutively active, acetylcholine-regulated $K^+$ current ($I_{K,ACh}$) that increases susceptibility to atrial fibrillation (AF), the underlying changes in $I_{K,ACh}$ channel function are unknown. This study aimed to establish how AT remodelling affects $I_{K,ACh}$ single-channel function.

Methods and results $I_{K,ACh}$ single-channel activity was studied via cell-attached patch-clamp in isolated left atrial cardiomyocytes of control and AT (7 days, 400 min) dogs. Atrial tachycardia prolonged the mean duration of induced AF from 44 ± 22 to 413 ± 167 s, and reduced atrial effective refractory period at a 360 ms cycle length from 126 ± 3 to 74 ± 5 ms (n = 9/group, P < 0.001). In the absence of cholinergic stimulation, single-channel openings with typical $I_{K,ACh}$ conductance and rectification properties were sparse under control conditions. Atrial tachycardia induced prominent agonist-independent $I_{K,ACh}$ activity because of increased opening frequency ($f_o$) and open probability ($P_o$); approximately seven- and 10-fold, respectively, vs. control, but did not alter open time-constant, single-channel conductance, and membrane density. With maximum $I_{K,ACh}$ activation (10 μmol/L carbachol), channel $P_o$ was enhanced much more in control cells (~42-fold) than in AT-remodelled myocytes (approximately five-fold). The selective Kir3 current blocker tertiapin-Q (100 nmol/L) reduced $f_o$ and $P_o$ at ~100 mV by 48 and 51%, respectively (P < 0.05 for each), without altering other channel properties, confirming the identity of $I_{K,ACh}$. Atrial tachycardia had no significant effect on mRNA or protein expression of either of the subunits (Kir3.1, Kir3.4) underlying $I_{K,ACh}$.

Conclusion Atrial tachycardia increases agonist-independent constitutive $I_{K,ACh}$, single-channel activity by enhancing spontaneous channel opening, providing a molecular basis for AT effects on macroscopic $I_{K,ACh}$ observed in previous studies, as well as associated refractoriness abbreviation and tertiapin-suppressible AF promotion. These results suggest an important role for constitutive $I_{K,ACh}$ channel opening in AT remodelling and support its interest as a potential target for AF therapy.

1. Introduction

Atrial fibrillation (AF) is the most common clinical arrhythmia, and its treatment remains challenging. Sustained atrial tachycardia (AT) leads to electrical and structural remodelling that promotes AF.1 Dogs subjected to atrial tachypacing at 400 b.p.m., which induces similar remodelling to AF,2 develop a large inwardly rectifying outward $K^+$-current, with properties of the acetylcholine-regulated current $I_{K,ACh}$, that is active in the absence of the agonist acetylcholine.3,4 Atrial cardiomyocytes from patients with long-standing AF similarly show agonist-independent constitutive $I_{K,ACh}$.5 The highly selective $I_{K,ACh}$ blocker tertiapin-Q increases atrial action potential duration (APD) and decreases susceptibility to AF in tachycardia-remodelled atrial preparations, pointing to an important role of constitutive $I_{K,ACh}$ in tachycardia remodelling effects on atrial repolarization and AF susceptibility.5 Traditional antiarrhythmic drug approaches for AF have many limitations, and novel therapies directed at underlying mechanisms may have some advantages.6,7 Since $I_{K,ACh}$ is absent in the ventricles and develops AF-promoting constitutive activity with atrial electrical remodelling,4 it may constitute a novel target for AF therapy lacking ventricular proarrhythmic risk.

In AF patients, constitutive $I_{K,ACh}$ activity results from an increased number of channel openings and open probability without changes in other single-channel properties.5,8 Abnormal phosphorylation-dependent regulation of constitutively

1Department of Pharmacology and Toxicology, Dresden University of Technology, Dresden, Germany and 2Research Center, Montreal Heart Institute and Université de Montréal, 5000 Belanger Street E, Montreal, Quebec H1T 1C8, Canada

Received 28 August 2007; revised 3 October 2007; accepted 21 October 2007; online publish-ahead-of-print 26 October 2007

Time for primary review: 14 days

Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2007.

For permissions please email: journals.permissions@oxfordjournals.org.
active $I_{K,ACH}$ may contribute to increased single-channel function. Because of concomitant cardiac diseases and patient medication, studies in atrial tissue from patients do not allow a clear differentiation between causes and consequences of AF-related changes. The dysregulation of single $I_{K,ACH}$ channels in AF patients may thus result from underlying heart diseases that predispose to AF or may be a consequence of AF-induced remodelling. Furthermore, if $I_{K,ACH}$ channel changes are caused by AF, they may be because of the rapid atrial rate or of some other consequence of AF. Although 7-day atrial tachypacing enhances macroscopic constitutive $I_{K,ACH}$ in canine atrial cardiomyocytes, the underlying mechanism is unknown. The increased current could be caused by increased $I_{K,ACH}$ channel expression without changes in single-channel properties, to increased single $I_{K,ACH}$ channel conductance, or to the activation of another channel that produces macroscopic currents that resemble $I_{K,ACH}$ in current–voltage relations and the response to tertiapin-Q.

Here, we studied the effects of AT-remodelling on single $I_{K,ACH}$ channel function in dog atria and identified prominent agonist-independent constitutive single-channel $I_{K,ACH}$ activity, providing a molecular basis for the previously observed tachycardia-induced enhancement of macroscopic $I_{K,ACH}$, which is associated with APD shortening and tertiapin-suppressible AF promotion. Our findings suggest an important role for increased open probability of constitutively active $I_{K,ACH}$ channels, which have lost their requirement for muscarinic cholinergic receptor agonist binding for activation, in tachycardia-related AF-promoting remodelling.

2. Methods

2.1 Tissue samples, in vivo electrophysiology, and cell isolation

Nine control and nine atrial tachypaced adult mongrel dogs of either sex weighing 20–30 kg were used for this study. The investigation conformed 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). Atrial tachycardia remodelling was induced by 1 week of right atrial pacing at 400 b.p.m. after AV-node ablation as previously described. On study days, dogs were anaesthetized with morphine (2 mg/kg s.c.) and $\alpha$-chloralose (120 mg/kg i.v.) and artificially ventilated. Atrial effective refractory period (ERP) was measured with 10 basic (S1) stimuli at basic cycle lengths (BCLs) of 150, 200, 250, 300, and 360 ms at the RA appendage, followed by an S2 with 5 ms decrements (all pulses twice-threshold, 2 ms). Mean AF duration was determined as an index of susceptibility to AF maintenance. AF was induced with 1–10 s atrial-pacing (10–20 Hz, 4 × threshold, 2 ms pulses), with AF induced 10 times for AF episodes ≤10 min and five times for 10–30 min of AF. If AF ≥30 min occurred, no further AF induction was performed. The mean duration was first calculated in each dog as the average value for all determinations obtained, and then each individual dog mean was used as a single value for statistical analysis. Hearts and adjacent lung tissues were excised via a left thoracotomy and immersed in oxygenated Tyrode solution (in mmol/L: NaCl 136, KCl 5.4, MgCl$_2$ 1, CaCl$_2$ 1, NaH$_2$PO$_4$ 0.33, HEPES 5, dextrose 10; pH 7.35 with NaOH). Atrial cardiomyocytes were isolated as previously described; the proximal left circumflex coronary artery was cannulated and the left atrium was perfused with a solution containing collagenase (100 U/mL$^\text{−1}$, Worthington, type II). Cells were kept in storage solution (in mmol/L: KCl 20, KH$_2$PO$_4$ 10, dextrose 10, mannitol 40, l-glutamic acid 70, β-OH-butyric acid 10, tauroine 20, EGTA 10, and 0.1% bovine serum albumin; pH 7.3 with KOH) at 4°C and were investigated within 12 h.

2.2 Single-channel recording

Single-channel currents were recorded in cell-attached configuration (Axopatch 200A, Axon Instruments) at room temperature. Myocytes were suspended in bath solution (in mmol/L: NaCl 120, KCl 20, MgCl$_2$ 1, CaCl$_2$ 2, glucose 10, HEPES 10, pH = 7.4 with NaOH) in a plexiglass chamber. Patch pipettes were fabricated from 1.0 mm o.d. borosilicate glass, coated at the tip with Sylgard and fire-polished with a microforge. When filled with pipette solution (in mmol/L: KCl 145, MgCl$_2$ 1, CaCl$_2$ 2, HEPES 5; pH 7.4 with KOH) tip-resistances were between 5 and 10 MΩ. Agonist-induced $I_{K,ACH}$ was observed with 10 μmol/L carbacol (CCH) in the pipette solution. Tertiapin-Q (100 nmol/L, Sigma-Aldrich) was added as needed into the bath solution.

Electrical noise was reduced by low-pass filtering at 1 kHz. All potentials are expressed in terms of the cytoplasmic side of the patch relative to the patch pipette (i.e., comparable to standard intracellular voltage/current conventions). Single-channel activity for open probability and histogram analysis was recorded during continuous application of a −100 mV potential to the patch, and current–voltage relationships were obtained with 10 s (ten 1-second tracings), 20 mV steps to voltages between −100 and 0 mV.

2.3 Single-channel property analysis

Data were digitized at 10 kHz and 12 bit bandwidth. Kinetic analyses and amplitude histograms were obtained after leak-current subtraction. The $n_P$ (open probability times number of channels in the patch) was calculated from idealized recordings of twenty 1-second tracings at −100 mV by division of the time integral by the elapsed time. The average $n_P$ per 1 s tracing was used for statistical evaluation. The open probability ($P_o$) was calculated as $n_P$ divided by the estimated number of channels in the patch. Because of a large density of $I_{K,ACH}$ channels in the membrane, patches always contained more than one channel despite the use of very small pipette tips (5–10 MΩ resistance). Therefore, only traces without simultaneous openings were used to obtain open time histograms and to estimate open time-constants. Exponential fits were obtained by nonlinear least-squares regression. Single-channel slope conductances were calculated from individual current–voltage relationships by linear regression.

2.4 Protein extraction and western blot

Fast-frozen tissues [left atrial free wall (LAFW)] from control (n = 4) and AT dogs (n = 4) were pulverized in liquid nitrogen. Chilled protein extraction buffer (Tris 10 mmol/L, NaCl 5 mmol/L, pH = 7.4) containing Complete protease inhibitor cocktail (Roche) was added and tissues were homogenized with a Polytron. Samples were centrifuged 10 min at 3000 r.p.m. (4°C). The supernatant was collected. The pellet was then resuspended, homogenized, and centrifuged (10 min, 3000 rpm, 4°C). The supernatant was collected and pooled with the first. This step was performed three times to improve extraction yield. Pooled supernatants were then centrifuged for 10 min at 14 000 r.p.m. (4°C). The supernatant was then centrifuged at 48 000 r.p.m. (Optima-Max ultracentrifuge, Beckman-Coulter). The pellet corresponding to the membrane fraction was resuspended in 1% Triton X-100 cold extraction buffer. The protein content was quantified (Biorad protein assay). Proteins were separated with sodium dodecyl sulphate-polyacrylamide gel electrophoresis, by loading 20 μg protein samples on 8% polyacrylamide gels, and transferred on a polyvinylidene difluoride membrane. Membranes were hybridized with the following primary antibodies: rabbit anti-GIRK1 (1/2000; Alomone labs), rabbit anti-GIRK4 (1/2000; Cytomix), and mouse anti-GAPDH (1/10 000; RDI). Peroxidase-conjugated goat anti-rabbit (1/10 000; Jackson ImmunoResearch...
Labs) and peroxidase-conjugated anti-mouse IgG (Santa-Cruz Biotechnology) were used as secondary antibodies and visualized by chemiluminescence. Quantification was obtained with Quantity-One software (Biorad).

### 2.5 Real-time PCR

Total RNA was extracted from control \( (n = 5) \) and AT \( (n = 5) \) dog snap-frozen LAFW tissue samples, homogenized in TRIzol Reagent (Invitrogen), and subjected to chloroform extraction and isopropanol precipitation. Genomic DNA was eliminated with DNase I \( (0.1 \mu L, 37^\circ C) \) incubation for 30 min. Phenol–chloroform acid extraction and gel verification were then performed. RNA was quantified spectrophotometrically at 260 nm and integrity confirmed on a denaturing agarose gel. RNA samples were stored in DEPC H\( _2 \)O at \( -80^\circ C \). First-strand cDNA was synthesized by reverse transcription with 2 \( \mu g \) of RNA samples, random primers, and MMLV reverse transcriptase (High Capacity cDNA Archive Kit, Applied Biosystems). Real-time PCR was conducted with a Stratagene Mx3000P QPCR detection system, with Taqman quantitative assay and the following primers and probes: Kir3.1 F: CAGTTGAGATTGTGCATCCTA R: CAGTTGAGATGTGCAGCTTGCA; Kir3.4 F: TTCGAAGTCGTGGTCATTCT AGA R: GCACCTCGATCTCA TGTAGGA Probe: TCATGCCTGTTGCCTCC; 18S rRNA pre-made eukaryote (Applied Biosystems). Each sample was run in duplicate and PCR products were verified with gel electrophoresis. Kir3.1 and Kir3.4 results were normalized to 18S rRNA; internal control data were obtained from the same samples at the same time.

### 2.6 Statistical analysis

Differences between group means for continuous data were compared by unpaired Student’s \( t \)-test (for single comparisons) or by one-way ANOVA and Bonferroni multiple-comparisons procedure. ERPs were compared at each BCL with two-way ANOVA and Bonferroni multiple-comparisons procedure. Data were expressed as mean ± SEM. \( P < 0.05 \) was considered statistically significant. Throughout this report, \( n/N \) refers to the number of myocytes/dogs.

### 3. Results

Atrial tachycardia remodelling did not influence atrial, ventricular, or systemic blood pressure (Table 1). Atrial tachycardia remodelling abbreviated atrial refractoriness and prolonged mean AF duration (Figure 1).

#### 3.1 Constitutively active \( I_{K,ACh} \) channels

Examples of single-channel recordings from control and tachycardia-remodelled canine cardiomyocytes in the absence of cholinergic stimulation are shown in Figures 2A and B respectively. Constitutive \( I_{K,ACh} \) activity was quite apparent in tachycardia-remodelled cells, whereas it occurred only sporadically in control myocytes (for which it was often necessary to obtain prolonged recordings to create amplitude histograms).

Amplitude histograms used to calculate single-channel currents are illustrated in Figures 2C and D. Amplitude histograms at \(-100 \) mV showed discrete peaks at \( \sim 0 \) pA (closed channels) and \( \sim 2.65 \) pA (open channels). Single-channel current–voltage relations were linear at negative potentials, with similar single-channel conductances in both groups (AT: \( 34.7 \pm 1.7 \) pS, \( n = 8/4 \); control: \( 31.6 \pm 2.4 \) pS, \( n = 5/3 \); \( P = NS, \) Figures 2 E and F). Because of the strong inward rectification of \( I_{K,ACh} \) channels, no channel openings could be detected at membrane potentials positive to \(-40 \) mV.

#### 3.2 Carbachol-activated \( I_{K,ACh} \) channels

Inclusion of the non-selective muscarinic-receptor agonist CCh (10 \( \mu \)mol/L) in the pipette solution strongly activated \( I_{K,ACh} \), causing frequent channel openings (Figures 4A and B). The number of channels in each patch was estimated based on the maximum number of simultaneously opening channels observed in each experiment and was similar in control and tachycardia-remodelled cardiomyocytes (tachycardia-remodelled cells: \( 2.8 \pm 0.3 \), \( n = 9/3 \); control channels: \( 2.9 \pm 0.3 \), \( n = 10/4 \); \( P = NS \)).

### Table 1 Haemodynamic parameters of dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CTL</th>
<th>AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mmHg</td>
<td>128 ± 5</td>
<td>132 ± 4</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>79 ± 2</td>
<td>74 ± 4</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>129 ± 6</td>
<td>127 ± 5</td>
</tr>
<tr>
<td>LAP, mmHg</td>
<td>5.0 ± 0.8</td>
<td>5.4 ± 0.6</td>
</tr>
<tr>
<td>RAP, mmHg</td>
<td>4.3 ± 0.8</td>
<td>5.9 ± 0.7</td>
</tr>
</tbody>
</table>

CTL, control myocytes; AT, atrial tachycardia-remodelled myocytes; BP, blood pressure; LVEDP, left ventricular systolic pressure; LAP, left ventricular end-diastolic pressure; RAP, right atrial mean pressure.

The kinetic properties of single-channel activity recorded in the absence of cholinergic stimulation are provided in Figure 3. Atrial tachycardia remodelling increased the open probability of constitutively active \( I_{K,ACh} \) channels by approximately nine-fold (tachycardia-remodelled cells: \( 1.7 \pm 0.3 \%, \) \( n = 9/4 \); control cells: \( 0.2 \pm 0.1 \%, \) \( n = 8/3 \); \( P < 0.001; \) Figure 3A). Kinetic analysis showed that the increase in open probability resulted from a larger opening frequency (tachycardia-remodelled cells: \( 17.6 \pm 3.9 \) Hz, \( n = 9/4 \); control cells: \( 1.8 \pm 0.4 \) Hz, \( n = 8/3 \); \( P < 0.01 \), Figure 3B). To evaluate the duration of single-channel openings, open time histograms were constructed and open time-constants \( (\tau_o) \) calculated from monoexponential fits, as illustrated in Figure 3 C, top. Open time-constants of constitutively active \( I_{K,ACh} \) did not differ between control and tachycardia-remodelled cardiomyocytes (tachycardia-remodelled cells: \( 1.0 \pm 0.1 \) ms, \( n = 9/4 \); control cells: \( 1.1 \pm 0.1 \) ms, \( n = 8/3 \); \( P = NS, \) Figure 3C, bottom). These results suggest that 1 week of atrial tachypacing caused changes facilitating the closed to open transition without altering channel closing kinetics.

![Figure 1](https://academic.oup.com/cardiovascres/article-abstract/77/1/35/463291)

Figure 1  (A) Atrial fibrillation duration; (B) atrial effective refractory period (ERP) at different basic cycle lengths (BCLs) under control (CTL) conditions and with atrial tachycardia (AT) remodelling, \( n = 9 \) dogs/group, ***\( P < 0.001 \).
cells: $3.1 \pm 0.2, n = 15/6; P = \text{NS}$), suggesting comparable $I_{K,ACh}$ channel densities.

In the presence of CCh stimulation, single-channel current–voltage relationships did not differ significantly between control and AT-remodelled myocytes (Figures 4C and D), with unitary conductances being unchanged (tachycardia-remodelled cells: $37.0 \pm 0.5 \text{pS}, n = 9/3$; control cells: $31.5 \pm 3.5 \text{pS}, n = 5/3; P = \text{NS}$, Figure 5A). CCh increased open probability by about 42- and five-fold in control and tachycardia-remodelled myocytes, respectively (Figure 5B). Opening frequency was slightly lower in tachycardia-remodelled vs. control myocytes in the presence of CCh (tachycardia-remodelled cells: $47.5 \pm 6.8 \text{Hz}, n = 9/3$; control cells: $72.7 \pm 4.2 \text{Hz}, n = 15/6; P = \text{NS}$, Figure 5C, right). This difference was offset by a slightly greater open time-constant in remodelled cells (tachycardia-remodelled cells: $1.3 \pm 0.1 \text{ms}, n = 9/3$; control cells: $1.0 \pm 0.0 \text{ms}, n = 15/6; P = \text{NS}$, Figure 5D, right), resulting in similar absolute CCh-activated open probability in both groups (tachycardia-remodelled cells: $9.3 \pm 1.3\%$, $n = 9/3$; control cells: $10.0 \pm 0.9\%$, $n = 15/6; P = \text{NS}$; Figure 5B). The similarity between open time-constants and current–voltage relationships of constitutively active and CCh-activated single-channel activity supports the notion that they are both carried by the same channels. Comparing the kinetic effects of CCh in the absence of tachycardia remodelling with those of tachycardia remodelling in the absence of CCh (both relative to non-remodelled control cells in the absence of CCh, Figure 5), the effects of tachycardia remodelling were qualitatively similar but of lesser magnitude than those of CCh. This result suggests that AT-induced remodelling mimics the effects of CCh on single-channel gating.

### 3.3 Effect of the selective Kir3 subunit channel blocker tertiapin-Q on $I_{K,ACh}$

The current–voltage relations and unitary conductance of single-channel activity both in the absence (Figure 2) and presence (Figure 4) of CCh are typical of $I_{K,ACh}$ and different from $I_{K1}$ and $I_{KATP}$. To confirm channel identity, we applied the selective Kir3-subunit channel blocker tertiapin-Q. Tertiapin-Q was added to the bath solution 30 s after the formation of the Giga-Ohm seal in the presence of maximum $I_{K,ACh}$ activation (10 $\mu$mol/L CCh). Since $I_{K,ACh}$ activity can exhibit a time-dependent decline because of desensitization, parallel experiments were performed with the application of Tyrode’s solution without tertiapin-Q as a time-matched control.
Figure 3. Open state kinetics of constitutive \( \kappa_{\text{ACH}} \) single-channel activity. (A, B) Open probability \( (P_o) \) and opening frequency \( (f_o) \) in control (CTL) and atrial tachycardia (AT) remodelled myocytes (mean ± SEM). (C, top) Histograms of open times of \( \kappa_{\text{ACH}} \) in control (CTL) and atrial tachycardia (AT) remodelled myocytes. Bin-width was 0.4 ms. (C, bottom) Open time-constants \( (\tau_o) \) are expressed as mean ± SEM. (A–C) Numbers within the columns indicate number of myocytes/dogs. **\( P < 0.01 \), ***\( P < 0.001 \) vs. corresponding values of control dogs.

Figure 4. Muscarinic cholinergic receptor-activated \( \kappa_{\text{ACH}} \). (A, B) Original single-channel recordings of control (CTL) and atrial tachycardia (AT) remodelled myocytes. \( \kappa_{\text{ACH}} \) was further stimulated by pipette solution containing 10 \( \mu \)mol/L carbachol. Filled arrowheads indicate the zero current level. Empty arrowheads designate opening levels of either only one channel or simultaneous openings of two different channels, respectively. (C, D) Corresponding current–voltage relations of single-channel currents, calculated from amplitude histograms. Numbers indicate myocytes/dogs. \( G_s \), mean ± SEM of single-channel slope conductance.
control (TMC). Single-channel properties were analysed at baseline and after 10 s, 2 and 4 min of tertiapin-Q application (Figure 6A). Time-matched Tyrode’s-only controls did not reveal statistically significant time-dependent changes in any single-channel parameter over 4 min of observation. Tertiapin-Q did not influence single-channel amplitude (Figure 6B). To reduce variability resulting from varying numbers of channels in individual patches, the effects of tertiapin-Q on opening frequency and open probability were represented as the ratio for each patch between values at each time point and corresponding baseline values (Figures 6C and E). The application of tertiapin-Q significantly reduced the opening frequency without producing any change in open time constant (tO), causing an ~50% decrease in both open probability (Figure 6C) and opening frequency (Figure 6E). The response to tertiapin-Q confirmed the fact that the single-channel activity we studied was attributable to IK,ACh.

3.4 Kir3.1 and 3.4 subunit expression

Figure 7A shows western blots for Kir3.1 and 3.4, as well as GAPDH, from one gel. Several bands were observed for Kir3.1, with bands corresponding to the expected molecular weights of glycosylated and non-glycosylated Kir3.1 seen at 65 and 53 kDa, respectively. Kir3.4 appeared as a single discrete band at 47 kDa. Corresponding mean values normalized to GAPDH (Figure 7B) indicate that there were no significant AT-related changes in Kir3.1 or Kir3.4 protein expression. Figure 7C shows mean mRNA expression values, which were similarly not affected by tachycardia remodelling.

4. Discussion

In this study, we evaluated the effects of AT-remodelling on single IK,ACh channel activity in cell-attached canine atrial myocytes. We found that AT-remodelling enhances constitutive channel activity by increasing the frequency and probability of channel openings in the absence of cholinergic agonists. This discrete change in channel function occurs without any change in other channel properties like unitary conductance and open time, suggesting discrete alterations in the closed to open channel transition, and is associated with a reduced response to CCh in terms of the increment in open probability produced by the cholinergic agonist.

4.1 Comparison with previous studies of inward-rectifier currents and atrial remodelling

The notion that inward-rectifier currents might be altered in AF was first put forward by Van Wagoner et al., who noted increased inward-rectifier activity in left (but not right) atrial cardiomyocytes from AF patients. Subsequent studies confirmed inward-rectifier current activation in right-atrial cells from AF patients. Although the responsible current was initially believed to be the background current, subsequent work showed that AT-remodelling increases constitutive IK,ACh in dog atrium, and that a similar current likely contributes to the inward-current augmentation in AF patients. The present study demonstrates the single-channel mechanism for increases in whole-cell IK,ACh caused by sustained rapid atrial rates, like those occurring in AF.

Constitutive IK,ACh open probability in AT-remodelled cells was less in the present study (~1.7%) than for cardiomyocytes from AF patients (~5%). The discrepancy may be because of species differences (dog vs. man), differences in study conditions, contributions from underlying cardiac disease in human AF subjects, or differences in chronicity of atrial tachyarrhythmia (7-day tachypacing in the present study vs. >6 months of AF in the clinical series). Patients with AF duration <7 days did not show increased constitutive IK,ACh in a recent report, consistent with a role for atrial tachyarrhythmia duration in determining the extent of constitutive IK,ACh activation. In the present study, IK,ACh open probability in non-remodelled and tachycardia-remodelled preparations was similar in the presence of maximal muscarinic-receptor stimulation, in contrast to the much larger single-channel activity for tachycardia-remodelled cells in the absence of cholinergic agonists. This observation parallels relative whole-cell currents under corresponding conditions in previous work and suggests that increased constitutive activity caused by tachycardia remodelling is because of higher basal channel opening with no net change in channel function under maximum stimulation. The reduced increment in single IK,ACh channel open probability resulting from cholinergic stimulation of tachycardia-remodelled cardiomyocytes in the present study corresponds to blunted cholinergic responses of macroscopic IK,ACh of tachycardia-remodelled preparations in previous investigations and indicates that the altered response occurs at the level of channel gating rather than channel subunit expression or conductance per se.

4.2 Potential physiological and clinical significance

To the best of our knowledge, this is the first study to investigate the single-channel mechanism of constitutive IK,ACh
enhancement by AT. Although the work of Dobrev et al. pointed to similar mechanisms in AF patients, the phenomena observed in the clinical study could be because of enhancements in constitutive \( I_{K,ACh} \) function that predispose to AF, rather than being a consequence of the atrial tachyarrhythmia itself. Alternatively, enhanced constitutive \( I_{K,ACh} \) activity in patients could reflect the consequences of underlying clinical conditions that predispose to AF in other ways. The findings in the present study are the first clear indication that a high sustained atrial rate per se alters channel gating to increase single \( I_{K,ACh} \) channel open probability in the absence of muscarinic cholinergic receptor stimulation.

Mathematical modelling studies suggest that increases in inward-rectifier \( K^+ \)-current play a very significant role in AF promotion. Because of their ability to hyperpolarize atrial cardiomyocytes and remove voltage-dependent \( I_{Na} \) inactivation, enhanced inward-rectifier currents are more effective in stabilizing and accelerating AF-sustaining rotors than are changes in other ionic currents (e.g. reduced \( Ca^{2+} \)-currents) that produce a similar degree of APD shortening. Thus, increases in inward-rectifier \( K^+ \)-current resulting from enhancement of constitutive \( I_{K,ACh} \) channel opening by tachycardia remodelling may be of particular importance for AF promotion. Indeed, inhibition of \( I_{K,ACh} \) with tertiapin-Q produces substantial APD prolongation in AT-remodelled preparations (much more than in control atria) and results in atrial tachyarrhythmia suppression.

Figure 6  Effect of tertiapin-Q on single-channel activity of carbachol (CCh)-activated \( I_{K,ACh} \). (A) Original single-channel recordings with pipette solution containing 10 \( \mu \)mol/L carbachol (CCh) at –100 mV. Current amplitude and open kinetics after 10 s, 2 and 4 min of tertiapin-Q application were compared with corresponding control values before application of tertiapin-Q (right panel). In time-matched controls (TMC) Tyrode’s solution only was applied (left panel). (B, D) Single-channel amplitude (B) and open time-constant (D, \( \tau_o \)) under control conditions and after 10 s, 2 and 4 min of tertiapin-Q application compared with corresponding values of TMC. (C) Ratio between \( nP_o \) after tertiapin-Q application and for TMC relative to \( nP_o \) under baseline conditions. (E) Ratio between opening frequency (\( f_o \)) after tertiapin application and in TMC relative to baseline values. (B-E) Mean \( \pm \) SEM. Numbers indicate myocytes/dogs, *\( P < 0.05 \) vs. corresponding values of time matched controls.
mass (47 kDa). (Kir3.1. Anti-Kir3.4 antibody recognized a band at the expected molecular corresponding to glycosylated (Gly-Kir3.1) and non-glycosylated forms of Kir3.4 signals relative to GAPDH. (Expression for control and AT dogs (constitutive-current activation.

potentials, consistent with inward-rectifier K⁺-current activation, has been observed previously in multicellular canine atrial preparations from dogs exposed to long-term atrial tachypacing. Of note, constitutive activity was appreciable with AT-remodelling at −40 mV (Figure 2C), within the repolarization voltage range of the action potential, pointing to the functional relevance of AT-induced constitutive-current activation.

Observations of antiarrhythmic actions of Ik,ACh block in tachycardia-remodelled preparations have led to the idea that suppressing Ik,ACh, particularly the constitutive form, may be an interesting new approach to AF therapy. A prototype Ik,ACh-selective blocker, NIP-151, has been developed and shown to suppress AF in several canine models without affecting ventricular electrophysiological properties. Although Ik,ACh channel pore-blocking drugs may prove to be a safe and effective AF-suppressing approach, they could also have undesirable side effects because of inhibition of parasympathetic nervous system-regulated, Ik,ACh-mediated effects on the sinus node, as well as of Ik,ACh-dependent extracardiac functions like pupillary constriction, bladder function, gastrointestinal activity, etc. The present studies show that tachycardia-induced increases in constitutive Ik,ACh are caused by discrete alterations in channel gating so that binding of the favoured ligands, muscarinic-cholinergic agonists, is no longer needed for channel opening. Identification of the underlying biochemical mechanisms might allow for specific targeting of the abnormalities in constitutive Ik,ACh channel function that result from AT-remodelling, permitting effective therapy that does not interfere with physiological cholinergic agonist-stimulated Ik,ACh function.

The molecular mechanism underlying constitutively active Ik,ACh channels is unknown. The similar changes in single-channel properties of Ik,ACh that occur in both human chronic AF and in the atrial tachypaced dog model studied here suggest a common molecular basis for enhanced constitutive Ik,ACh channel activity. The regulation of Ik,ACh is complex, with several putative mechanisms that could account for abnormal Ik,ACh channel activity. The muscarinic cholinergic receptor antagonist atropine does not abolish constitutively active Ik,ACh, suggesting an agonist-independent mechanism. Increased receptor-independent dissociation of Gαo and Gβγ-subunits appears an unlikely contributor because neither pertussis toxin nor the absence of GTP affected the Ik,ACh-like component of basal current in dogs with AT-remodelling. Because activation of Ik,ACh requires ATP, modified phosphorylation-dependent channel regulation may contribute to the development of constitutive Ik,ACh activity. Accordingly, evidence has been provided to suggest that abnormal PKC function might play an important role in the occurrence of profibrillatory constitutive Ik,ACh activity in clinical AF. Further work will be needed to define the precise molecular-signalling mechanisms that enhance constitutive Ik,ACh channel activity in the present model.

4.3 Study limitations
We used cell-attached patches to study single Ik,ACh channel activity. This approach has the major advantage of preserving cellular integrity and allows for the recording of channel activity under conditions of normal intracellular constitution, metabolism, and regulation. Perhaps because of our use of this relatively physiological system, we were able to obtain results that agree very closely with previous observations of whole-cell current and APD changes in multicellular preparations. A disadvantage of the cell-attached patch approach is that the intracellular milieu is determined by the cell and not controlled by dialysis, and that the extracellular milieu for channels in the patch is controlled by pipette contents (and is therefore not amenable to repeated measurements with different conditions for the same patch). The most effective way to study biochemical mechanisms is with inside-out or outside-out cell-free patches, for which the intra- or extracellular milieu, respectively, can readily be altered while studying the response of the underlying channel(s). Such studies will be necessary to clarify the biochemical mechanisms underlying enhanced single Ik,ACh channel activity in tachycardia-remodelled cells. However, cell-free patches are often associated with substantial run-down and non-physiological behaviours because of loss of key regulating molecules. Slow penetration of tertiapin-Q from the bath through the cell to its extracellular site of action in the patch under the pipette likely explains why we saw only 50% inhibition of
constitutive $I_{K,ACh}$ channel activity with concentrations about 10 times the drug’s IC$_{50}$ for $I_{K,ACh}$ during four minutes of exposure. It will be important in future studies to assess the role of regulatory changes like altered phosphorylation state of constitutive $I_{K,ACh}$ enhancement.

In the present study we investigated $I_{K,ACh}$ behaviour in vitro, with conditions (ion concentrations, temperature, etc.) that are different from those in vivo. We recorded $I_{K,ACh}$ at room temperature only, and it must be kept in mind that its regulation may differ at physiological temperature. Thus, our data should be extrapolated with caution to in vivo conditions.

5. Conclusions

Atrial tachycardia remodelling importantly alters single $I_{K,ACh}$ channel function in canine atrial myocytes. Channel opening frequency in the absence of cholinergic agonists is greatly enhanced by an increase in open probability, without changes in $I_{K,ACh}$ channel conductance, open time, or membrane density. On the other hand, $I_{K,ACh}$ channel opening induced by muscarinic cholinergic stimulation is reduced. These changes in the properties of $I_{K,ACh}$ channels provide a mechanistic explanation for previously observed tachycardia-induced alterations in macroscopic $I_{K,ACh}$ and have significant implications for AF pathophysiology and therapy.

Acknowledgements

The authors thank Nathalie L’Heureux and Chantal St-Cyr for technical support and France Thériault for secretarial help with the manuscript.

Conflict of interest: S. Nattel is listed as co-inventor on a patent for acetylcholine-dependent potassium current as a target for atrial fibrillation therapy, ownership of which belongs to the Montreal Heart Institute and University of Montreal.

Funding

Supported by the Canadian Institutes of Health Research, the Quebec Heart and Stroke Foundation, the MITACS Network of Centers of Excellence, the German Federal Ministry of Education and Research through the Atrial Fibrillation Competence Network (grant 01Gi0204; project C4 to D.D.), and the German Research Foundation (DFG; Do 769/1–1, 2 to D.D.).

References