Published in final edited form as:

Int J Cardiol. 2018 March 15; 255: 61–68. doi:10.1016/j.ijcard.2017.12.050.

Muscarinic type-1 receptors regulate $I_{K,ACh}$ in human atrial cardiomyocytes and are upregulated in patients with chronic atrial fibrillation

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Abstract

Background—Basal and acetylcholine-gated inward-rectifier K^+ -currents (I_{K1} and $I_{K,ACh}$, respectively) are altered in atrial fibrillation (AF). G_i -protein-coupled muscarinic (M) receptors type-2 are considered the predominant receptors activating $I_{K,ACh}$. Although a role for G_q -coupled non- M_2 -receptor subtypes has been suggested, the precise regulation of $I_{K,ACh}$ by multiple M-receptor subtypes in the human atrium is unknown. Here, we investigated M_1 -receptor-mediated $I_{K,ACh}$ regulation and its remodeling in chronic AF (cAF).

Methods and Results— M_1 -receptor mRNA and protein abundance were increased in atrial cardiomyocyte fractions and atrial homogenates from cAF patients, whereas M_2 -receptor levels were unchanged. The regulation of $I_{K,ACh}$ by M_1 -receptors was investigated in right-atrial cardiomyocytes using two applications of the M-receptor agonist carbachol (CCh, 2 μ M), with pharmacological interventions during the second application. CCh application produced a rapid current increase (Peak- $I_{K,ACh}$), which declined to a quasi-steady-state level (Qss- $I_{K,ACh}$). In sinus rhythm (Ctl) the selective M_1 -receptor antagonists pirenzepine (10 nM) and muscarinic toxin-7 (MT-7, 10 nM) significantly inhibited CCh-activated Peak- $I_{K,ACh}$, whereas in cAF they

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[#]This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation

Disclosures: none (all authors)

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significantly reduced both Peak- and Qss- $I_{K,ACH}$, with no effects on basal inward-rectifier currents in either group. Conversely, the selective M_1 -receptor agonist McN-A-343 (100 μ M) induced a current similar to the CCh-activated current in Ctl atrial cardiomyocytes pretreated with pertussis toxin to inhibit M_2 -receptor-mediated G_i -protein signaling, which was abolished by MT-7. Computational modeling indicated that M_1 - and M_2 -receptors redundantly activate $I_{K,ACH}$ to abbreviate APD, albeit with predominant effects of M_2 -receptors.

Conclusion—Our data suggest that G_q -coupled M_1 -receptors also regulate human atrial $I_{K,ACH}$ and that their relative contribution to $I_{K,ACH}$ activation is increased in cAF patients. We provide novel insights about the role of non- M_2 -receptors in human atrial cardiomyocytes, which may have important implications for understanding AF pathophysiology.

Keywords

atrial fibrillation; inward-rectifier K^+ -channel; muscarinic receptor subtypes

Introduction

The pathogenesis of AF involves a multitude of mechanisms that control the initiation, maintenance and progression of the disease [1, 2] and is strongly modulated by the autonomic nervous system [3, 4]. Vagal nerve activation decreases heart rate, but enhances the susceptibility to AF, largely through shortening of the atrial action potential duration (APD) and effective refractory period (ERP). The acetylcholine-gated inward-rectifier K^+ -current ($I_{K,ACH}$) is the main electrophysiological effector of vagal nerve stimulation in the atrium and contributes to APD shortening [5]. Moreover, in patients with long-standing persistent (chronic) AF (cAF), $I_{K,ACH}$ develops agonist-independent “constitutive” activity, promoting APD shortening even in the absence of vagal stimulation [6, 7]. Because of the latter and its atrial-predominant occurrence [8], $I_{K,ACH}$ is considered a potential target for atrial-selective antiarrhythmic therapies with minimized ventricular proarrhythmic risk [9].

Stimulation of type-2 muscarinic receptors (M_2) by acetylcholine results in dissociation of G_i proteins in $G\alpha_i$ and $G\beta\gamma$ subunits with the latter activating $I_{K,ACH}$ [10]. Atrial $I_{K,ACH}$ channels are controlled by a complex interplay of regulatory mechanisms, including binding of phosphatidylinositol-4,5-bisphosphate, (de)phosphorylation by various kinases and phosphatases, and regulation by intracellular Mg^{2+} and Na^+ [7, 11, 12]. Since $I_{K,ACH}$ is non-specifically activated by $G\beta\gamma$ subunits, multiple G protein-coupled receptors can activate $I_{K,ACH}$, independent of the exact composition of $G\alpha$ subunits [13–17]. Multiple M-receptor isoforms are expressed in the atrium of large mammals, including humans [18–21] and there is accumulating evidence for a role of non- M_2 receptors in the functional response to vagal stimulation [22, 23]. Moreover, expression and function of different M-receptor isoforms are altered after 1 week of artificially-maintained atrial tachycardia or experimental heart failure in dogs [24, 25]. These findings point to a dynamic remodeling of atrial M-receptor subtypes in cardiac diseases. Although there is evidence for presence of non- M_2 receptors in the human atrium, we are not aware of any studies specifically addressing the contribution of different M-receptor subtypes to the regulation of the human atrial $I_{K,ACH}$ in AF.

The present study was designed to identify functional non-M₂ receptors in human atrial cardiomyocytes, to study whether and how they affect M-receptor-gated I_{K,ACh} and atrial repolarization, and to explore the consequences of AF-related remodeling for non-M₂-receptor function. Our results identify a novel role for M₁-receptor-mediated regulation of I_{K,ACh} in human atrial cardiomyocytes, with upregulation of M₁-receptors in cAF, which may have important consequences for the function of the diseased atrium, particularly in AF.

Methods

An extended description of specific methods is provided in the online data supplement.

Ethical approval

The investigation was approved by the ethics committee of Dresden University of Technology (No: EK114082002). The study conforms with the principles outlined in the Declaration of Helsinki (Cardiovascular Research 1997;35:2–4). Each patient gave written informed consent. Patient data for samples used for molecular biology experiments and electrophysiological recordings are shown in Supplemental Tables 1 and 2, respectively.

Human atrial cardiomyocyte isolation and electrophysiological recordings

Human atrial cardiomyocytes were isolated using a standard protocol [13]. Currents were measured at room temperature using the voltage-clamp technique, as described [6, 26]. Cell capacitance averaged 91.6 ± 2.5 pF ($n=182/63$ cells/patients) and 105.3 ± 4.1 pF ($n=73/25$) in sinus rhythm (Ctl) and cAF patients, respectively ($P<0.01$). Accordingly, current amplitudes were corrected for cell size and expressed as current densities (pA/pF). Agonist-inducible I_{K,ACh} was stimulated with carbachol (CCh, 2 μ M) using two applications (S₁ and S₂) of 2 mins, with 2 mins in between [6, 16]. The contribution of different M-receptor subtypes was analyzed during S₂ using pirenzepine (10 nM) and muscarinic toxin-7 (MT-7, 10 nM) for M₁-receptor inhibition, methoctramine (20 nM) for M₂-receptor inhibition, 4-diphenylacetoxy-N-methylpiperidine (4-DAMP) mustard (1 nM or 10 nM) for M₃-receptor inhibition, or atropine (10 nM) for inhibition of all M-receptors. In some experiments, M₁-receptors were activated with the selective M₁-receptor agonist McN-A-343 (100 μ M).

Biochemistry and molecular biology

Cardiomyocyte-enriched fractions were obtained after collagenase/protease digestion using a 6% bovine serum albumin (BSA) gradient, as described [27]. RNA was isolated using an RNA isolation kit. Gene-specific primer pairs for M₁, M₂, M₃ and M₅ were used as previously reported [18], with primers for M₄ from [21]. Primary antibodies against M₁ and M₂ were used to quantify corresponding proteins in atrial homogenates by immunoblotting [28]. Competition radioligand binding was performed with THRX 182087 as previously described, using [³H]-quinuclidinylbenzylate (QNB) as radioligand [29].

Computational modeling

A model of receptor-dependent I_{K,ACh} regulation [30] was extended with separate populations of M₁- and M₂-receptors with competitive inhibition by antagonists with specific affinities for each receptor. The combined M-receptor/G-protein/I_{K,ACh} model was

integrated in the Grandi et al. human atrial cardiomyocyte model with Na⁺-dependent regulation of I_{K1} and I_{K,ACh} [12]. The model was implemented in Myokit [31] and simulations were performed under voltage-clamp conditions or during steady-state pacing in current-clamp at the indicated frequencies. Application of CCh and M-receptor antagonists was simulated similar to the experimental protocols. A detailed overview of model equations and validation is provided in the online data supplement.

Statistical analysis

Differences between group means for continuous data were compared by unpaired Student's *t*-test or by one-way or two-way ANOVA and Holm-Sidak post-hoc test for data following a Gaussian distribution (D'Agostino-Pearson omnibus normality test), as appropriate. Alternatively, Mann-Whitney or Kruskal-Wallis (with Dunn's multiple comparison) tests were employed. Frequency data were analyzed with χ^2 statistics. Data are shown as mean $\pm$ SEM. *P* < 0.05 was considered statistically significant. N- numbers (n=X/Y) represent number of cells/number of patients. All statistical analyses were performed using Graphpad Prism 6 (GraphPad Software, La Jolla, USA)

Results

Expression of M-receptor subtypes in the human right atrium

Previous studies have identified M₁-M₅ receptors in human right-atrial homogenates [18]. Potential differences in M-receptor expression in atrial cardiomyocytes between Ctl and cAF samples could be obscured by other cell types. Therefore, BSA-gradient filtration was employed to obtain cardiomyocyte-enriched fractions [27]. In these fractions the mRNA levels of M₁, M₃, M₄, and M₅, but not M₂, were significantly increased in cAF patients (Figure 1A). In agreement, M₁-receptor protein levels were significantly upregulated in cAF patients (Figure 1B). In the absence of specific antibodies for Western blot (not shown), M₂-receptor density was estimated through competitive binding experiments [29]. Consistent with the unaltered mRNA expression of M₂ receptors, the estimates of B_{max} were 101 $\pm$ 12 vs. 78 $\pm$ 8 fmol/mg protein in Ctl and cAF patients, respectively (*P*=0.19, Figure 1C).

Recording of M-receptor-gated I_{K,ACh} in human atrial cardiomyocytes

Whole-cell voltage-clamp experiments were performed in cardiomyocytes isolated from right-atrial appendages to analyze I_{K,ACh} as functional readout for the specific contribution of different M-receptor subtypes (Supplemental Figure 1A). In line with previous results [6, 16, 32], application of CCh produced a rapid current increase (Peak-I_{K,ACh}), which declined to a quasi-steady-state level (Qss-I_{K,ACh}) (Supplemental Figure 1B). Basal inward-rectifier current was larger, and both Peak-I_{K,ACh} and Qss-I_{K,ACh} were smaller in cAF compared with Ctl atrial cardiomyocytes, whereas S₂/S₁ ratios were similar in both groups (Supplemental Figure 1C), consistent with previous work [6, 12, 16, 32].

Regulation of I_{K,ACh} by M₂-receptors

Application of the non-selective M-receptor antagonist atropine (10 nM) during the second CCh pulse (S₂) abolished I_{K,ACh} (Supplemental Figure 2A), confirming that it is fully mediated by M-receptors. Since M₂-receptors are considered the predominant cardiac M-

receptor subtype, we first applied the M₂-receptor blocker methoctramine at a specific, but only moderately-effective concentration (20 nM; compare Supplemental Table 3). In Ctl atrial cardiomyocytes, methoctramine caused a borderline-significant reduction in S₂/S₁ ratio of both Peak-I_{K,ACh} and Q_{SS}-I_{K,ACh} (Supplemental Figure 2B, $P=0.055$ and $P=0.070$, respectively), confirming the functional contribution of M₂-receptors.

Regulation of I_{K,ACh} by M₁- and M₃-receptors

To investigate the impact of M₁- and M₃-receptors on CCh-activated I_{K,ACh}, M₁-receptors were blocked with pirenzepine (10 nM) or the highly-selective antagonist MT-7 (10 nM; Supplemental Table 3), and M₃-receptors were inhibited with 4-DAMP (1 nM or 10 nM) during the second CCh application (Figure 2A). Pirenzepine and MT-7 significantly reduced the S₂/S₁ ratio of Peak-I_{K,ACh} in Ctl and cAF atrial cardiomyocytes (Figure 2B, top panel). 4-DAMP (1 nM or 10 nM) similarly reduced the S₂/S₁ ratio of Peak-I_{K,ACh}, although this did not reach statistical significance at 1 nM for Ctl atrial cardiomyocytes (Figure 2B). However, since 4-DAMP was unable to further reduce the S₂/S₁ ratio in the presence of MT-7 (Figure 2B, last column), we interpret these data to indicate that the observed 4-DAMP effect is likely due to unspecific inhibition of M₁-receptors by 4-DAMP (Supplemental Table 3). The effect of M-receptor blockers on Q_{SS}-I_{K,ACh} was qualitatively similar, but smaller, than that on Peak-I_{K,ACh} (Figure 2B, bottom panel), suggesting that M₁-receptor activation predominantly contributes to Peak-I_{K,ACh}. Of note, significant inhibition of Q_{SS}-I_{K,ACh} by pirenzepine and MT-7 were only observed in cAF atrial cardiomyocytes (Figure 2B, bottom panel), suggesting an increased relative contribution of functional M₁ receptors in cAF. Similar results were obtained using a square-pulse voltage-clamp protocol (Supplemental Figure 3).

Effects of M-receptor blockers on basal inward-rectifier K⁺-current

We next assessed potential effects of the M-receptor blockers on the basal current just prior to the second CCh application (S₂). In line with previous results [16], even switching between Tyrode solutions had a small effect on basal current (Supplemental Figure 4). Of all pharmacological interventions tested, only pirenzepine had an effect that was significantly larger than that of Tyrode in both Ctl and cAF atrial cardiomyocytes. However, absolute values remained small (<1.0 pA/pF) compared to control basal-current amplitude (<10%; Supplemental Figure 4).

G_i- and G_q-proteins contribute to M-receptor mediated regulation of I_{K,ACh}

M₂- and M₄-receptors predominantly couple to G_i-proteins, whereas M₁, M₃ and M₅ are considered mainly coupled to G_q-proteins [10]. Inactivating G_i-proteins with pertussis toxin (PTX) abolished I_{K,ACh} in 74% of atrial cardiomyocytes and significantly reduced it in the remaining 26% (Figure 3A), validating the predominance of G_i-coupled M-receptors. MT-7 suppressed the CCh-activated I_{K,ACh} in the PTX pre-treated atrial cardiomyocytes in which I_{K,ACh} current could still be detected (Figure 3B). Moreover, the selective M₁-receptor agonist McN-A-343 elicited a current of comparable magnitude (-2.2 ± 0.2 pA/pF, $n=3/2$) compared to the PTX pre-treated atrial cardiomyocytes with detectable I_{K,ACh} (-2.2 ± 0.5 , $n=8/3$, Figure 3C), pointing to the possibility that this I_{K,ACh} current is mediated by G_q-coupled M₁-receptors. PTX pre-treatment had no effect on basal current (Figure 3D).

Computational analysis of the effects of M-receptor-mediated $I_{K,ACH}$ regulation on APD

We employed computational modeling to analyze the effects of M_1 - and M_2 -receptors on APD. The newly developed model incorporates activation of G-proteins by both M-receptors, competitive antagonism of these receptors by pharmacological compounds with distinct affinities, $G\beta\gamma$ -dependent activation of $I_{K,ACH}$ and its regulation by $[Na^+]_i$ (Supplemental Figure 5A). The model reproduced the experimentally observed current-voltage relationship, Na^+ -dependent regulation and effects of MT-7, methoctramine and atropine for both Ctl and cAF conditions (Supplemental Figures 5–6). Simulated application of CCh produced a rapid abbreviation of APD during steady-state pacing at 1-Hz or 3-Hz, which was larger in Ctl than in cAF (Figure 4), confirming previous experimental results [33]. In the absence of M_1 -receptors, the CCh-mediated shortening of APD was reduced by 9% (Ctl, 1-Hz), confirming a major role for M_2 -receptors. However, in the absence of M_2 receptors, APD shortening was still 72% of control conditions (Figure 4C), indicating that M_1 -receptors influence atrial repolarization and suggesting that different M-receptor subtypes may provide redundancy to ensure an appropriate electrophysiological response to vagal stimulation. Qualitatively similar results were obtained at 3-Hz and for cAF conditions.

Discussion

We evaluated the role of different M-receptor subtypes in the regulation of $I_{K,ACH}$ in human atrial cardiomyocytes from Ctl and cAF patients and identified a significant upregulation of M_1 -receptor expression, but unaltered M_2 -receptor expression in cAF patients. Moreover, we discovered a functional contribution of M_1 -receptors to the CCh-activated Peak- $I_{K,ACH}$ in Ctl atrial cardiomyocytes and a stronger contribution to Peak- and Qss- $I_{K,ACH}$ in cAF. Overall, our data suggest for the first time that the activation of $I_{K,ACH}$ in human atrial cardiomyocytes does not only involve G_i protein- coupled M_2 -receptors but also G_q protein-coupled M_1 -receptors, which act as $G\beta\gamma$ donors to activate $I_{K,ACH}$ and are upregulated in cAF, playing a significant role in the regulation of $I_{K,ACH}$ and atrial repolarization.

M-receptor subtype expression and signaling in the heart

Some studies could detect all five M-receptor subtypes [18, 34], whereas others only identified M_2 - and M_3 -receptors in the human heart [20, 21]. Our results are in general agreement with these studies, with reliable detection of M_2 - and M_3 -receptors, but also evidence for the presence of M_1 , M_4 and M_5 subtypes. We furthermore confirmed the presence of M_1 and M_2 at the protein level using Western blot and radioligand binding assays, respectively. Importantly, most previous studies [18] employed tissue homogenates to identify and quantify different M-receptor subtypes, which could be biased by cell types other than cardiomyocytes. Here, we validate the expression of all M-receptor subtypes in atrial cardiomyocyte-enriched fractions.

Animal studies have identified altered M-receptor expression and function in dogs with atrial tachycardia remodeling [24] and congestive heart failure [25], suggesting complex remodeling of M-receptor subtypes during disease conditions, but little is known about M-receptor remodeling in the human atrium. Here, we identified an upregulation of all M-

receptor subtypes, except M₂, at the mRNA level in cAF patients. Moreover, we confirmed the upregulation of M₁-receptors and the unaltered M₂-receptor expression at the protein level. Our data suggest a switch in the relative contribution of individual M-receptor subtypes in cAF, with increasing contribution of non-M₂-receptors.

Regulation of I_{K,ACh} in Ctl and cAF patients

We and others have shown an increase in basal inward-rectifier K⁺-current and a decrease in CCh-activated I_{K,ACh} in cAF patients [6, 12, 13, 16, 33, 35–37]. The present results are consistent with those observations. Although the changes in both currents are at least in part due to the altered protein expression of channel subunits, there is also evidence for cAF-related remodeling of channel regulation, including constitutive activity [6, 16, 38] and Na⁺-dependent regulation [12]. Here we demonstrated that altered expression and/or regulation of non-M₂ receptors may also contribute to the dysregulation of I_{K,ACh} in cAF.

The low specificity of Gβγ subunits to effectors [10] suggests that multiple M-receptor subtypes may promote the CCh-induced increase in I_{K,ACh}, with Gβγ subunits released from G_q and G_i protein-coupled M-receptors directly binding and activating the I_{K,ACh} channel. In agreement, inhibition of M₁-receptors with the highly-selective M-receptor antagonist MT-7 significantly reduced Peak-I_{K,ACh}. Since pre-treatment of cardiomyocytes with PTX to inactivate G_i-proteins abolished CCh-activated I_{K,ACh} in the majority of atrial cardiomyocytes and MT-7 eliminated the current in PTX pre-treated cells with detectable I_{K,ACh}, M₁-receptors appear to activate I_{K,ACh} through both G_q- and G_i-proteins, in line with previous results in heterologous expression systems [39]. These data also suggest that other agonists of G_q-coupled receptors (e.g., angiotensin-II, endothelin-1) could similarly regulate I_{K,ACh}. In agreement, endothelins modulate the CCh-activated I_{K,ACh} in guinea pig atrial cardiomyocytes [40]. Moreover, like M₁-receptors, angiotensin-II and endothelin-1 expression are increased in AF patients [41] and AF animal models [42], suggesting a potential larger contribution to the (constitutive) I_{K,ACh} in AF. Thus, regulation of I_{K,ACh} and its impact on atrial repolarization *in vivo* is likely modulated by multiple signaling cascades [11].

Potential limitations

Although we found increased mRNA expression of M₁, M₃, M₄ and M₅ in cAF cardiomyocyte fractions, we could only confirm upregulation at the protein level for M₁-receptors due to limitations of available antibodies, in line with previous observations [43]. Therefore, we employed radioligand binding studies, which demonstrated that cAF is not accompanied by a change in total M₂-receptor density.

Imperfect specificity toward different M-receptor subtypes is an important weakness of using drugs against M-receptor subtypes during functional patch-clamp experiments. Nonetheless, the compounds used in our study represent the best choices available [10] and the effects of the highly-selective toxin MT-7 provide strong evidence for a role of M₁-receptors in the regulation of I_{K,ACh} in human atrial cardiomyocytes from patients with and without AF. The potential contribution of M₃-M₅-receptors to I_{K,ACh} could not be tested due to the lack of subtype-selective inhibitors.

Finally, we were only able to assess M-receptor regulation of $I_{K,ACH}$ in cardiomyocytes from right-atrial appendages from patients undergoing open heart surgery. M-receptor expression and regulation may differ in other parts of the atria [35]. Moreover, these findings may not hold for other cohorts of AF patients and could be modulated by differences in other clinical parameters (see Supplemental Tables 1–2).

Conclusions

We have performed an extensive analysis of the role of different M-receptor subtypes in the human atrium. We provide strong evidence for an important role of M_1 -receptors in the regulation of human atrial $I_{K,ACH}$ and CCh-mediated APD shortening, with upregulation of M_1 -receptor expression in the setting of cAF. These data significantly enhance our understanding of K^+ -channel remodeling and regulation in AF, which might facilitate the discovery of new therapeutic strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant support: This work was supported by the Deutsche Forschungsgemeinschaft (Do 769/1-1-3, to D.D.), National Institutes of Health (R01-HL131517 to D.D.) and the Netherlands Organization for Scientific Research (ZonMW Veni 91616057 to J.H.).

The authors would like to thank Manja Schöne, Annett Opitz and Trautlinde Thurm for outstanding technical assistance, Prof. Dr. Erich Wettwer for expert advice on patch-clamp experiments, and the cardiac surgery team in Dresden for the excellent collaboration.

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Highlights

- The exact role of multiple muscarinic (M)-receptor subtypes in the human atrium is unknown
- M₁-receptors contribute to I_{K,ACh} in human atrial cardiomyocytes
- The expression of M₁-receptors is increased in atrial fibrillation patients
- M₁-receptor-mediated I_{K,ACh} regulation is enhanced in atrial fibrillation patients
- M₁- and M₂-receptors redundantly activate I_{K,ACh} to abbreviate repolarization duration

Figure 1. Upregulation of M₁-receptors but unchanged M₂-receptors in cAF patients
A, Representative ethidium-bromide-stained agarose gels (left) and M-receptor mRNA expression normalized to GAPDH (right, relative to Ctl) in cardiomyocyte-enriched fractions of right-atrial appendages from Ctl or cAF patients. **B**, Representative Western blot and group data of M₁-receptor protein expression normalized to calsequestrin (CSQ). **C**, Competition of the M₂-selective antagonist THR 182087 for QNB binding in atrial membranes from Ctl (open circles) or cAF patients (filled squares) and estimates of M₂-receptor density in Ctl and cAF samples (relative to Ctl). Numbers in bars indicate number of patients. * $P < 0.05$ vs. Ctl.

Figure 2. Regulation of $I_{K,ACh}$ by M_1 - and M_3 -receptors in human atrial cardiomyocytes
A, Representative time course of inward-rectifier K^+ -currents with the M_1 -receptor blocker pirenzepine, the selective M_1 -receptor toxin MT-7 or the putative M_3 -receptor blocker 4-DAMP applied during the second CCh stimulation (S_2) in Ctl (left) or cAF (right). **B**, Quantification of S_2/S_1 ratio of CCh-activated Peak- $I_{K,ACh}$ (top) or QSS- $I_{K,ACh}$ (bottom) in the absence of M-receptor blocker (Tyrode), or in the presence of pirenzepine, MT-7, or 4-DAMP, or the combination of 4-DAMP and MT-7 in Ctl (white bars) or cAF (black bars) atrial cardiomyocytes. Numbers in bars indicate number of cardiomyocytes/number of patients. * $P < 0.05$ vs. Tyrode for a given rhythm type (2-way ANOVA with Holm-Sidak's multiple comparison test). N.D.: not determined.

Figure 3. Regulation of $I_{K,ACh}$ through G_i - and G_q -proteins

A, CCh-activated Peak- $I_{K,ACh}$ and Qss- $I_{K,ACh}$ in Ctl atrial cardiomyocytes incubated in normal Tyrode solution or with 5 μ g/ml PTX for 90 min, divided in cardiomyocytes in which $I_{K,ACh}$ could or could not be activated by CCh. * $P < 0.05$ vs. corresponding Peak- $I_{K,ACh}$. **B**, Representative example of basal current and CCh-activated $I_{K,ACh}$ in a PTX pre-treated atrial cardiomyocyte from a Ctl patient in the absence or presence of MT-7. **C**, Peak- and Qss- $I_{K,ACh}$ current induced by the M_1 -receptor agonist McN-A-343 (100 μ M) observed in 37% of Ctl cardiomyocytes (no effect in 63%). **D**, Effect of PTX pre-treatment on basal current. Numbers in bars indicate number of cardiomyocytes/number of patients.

Figure 4. Computational modeling of M-receptor-mediated, $I_{K,ACh}$ -dependent regulation of repolarization

A, APD at 1-Hz (top) and 3-Hz (bottom) pacing in Ctl (left) and cAF (right) models prior to and during CCh stimulation with M_1 - and M_2 - receptors enabled (solid black), M_2 only (dashed blue), M_1 only (dash-dotted red) or no M-receptors (dash-dot-dotted purple line). **B**, Membrane potential and underlying inward-rectifier K^+ -current during maximal CCh effect for the models and conditions from panel A. **C**, Relative CCh-induced change in APD with both M-receptors, M_2 only or M_1 only, based on the data from panel A.