Upregulation of K_{2p}3.1 K^+ Current Causes Action Potential Shortening in Patients With Chronic Atrial Fibrillation

Constanze Schmidt, MD; Felix Wiedmann, MD; Niels Voigt, MD; Xiao-Bo Zhou, MD; Jordi Heijman, PhD; Siegfried Lang, PhD; Virginia Albert, BSc; Stefan Kallenberger, MD, PhD; Arjang Ruhparwar, MD; Gábor Szabó, MD, PhD; Klaus Kallenbach, MD; Matthias Karck, MD; Martin Borggreve, MD; Peter Biliziki, MD, PhD; Joachim R. Ehrlich, MD; István Baczkó, MD, PhD; Patrick Lugenbiel, MD; Patrick A. Schweizer, MD; Birgit C. Donner, MD, PhD; Hugo A. Katus, MD, PhD; Dobromir Dobrev, MD; Dierk Thomas, MD

Background—Antiarrhythmic management of atrial fibrillation (AF) remains a major clinical challenge. Mechanism-based approaches to AF therapy are sought to increase effectiveness and to provide individualized patient care. K_{2p}3.1 (TASK-1 [tandem of P domains in a weak inward-rectifying K^+ channel–related acid-sensitive K^+ channel-1]) 2-pore-domain K^+ (K_{2p}) channels have been implicated in action potential regulation in animal models. However, their role in the pathophysiology and treatment of paroxysmal and chronic patients with AF is unknown.

Methods and Results—Right and left atrial tissue was obtained from patients with paroxysmal or chronic AF and from control subjects in sinus rhythm. Ion channel expression was analyzed by quantitative real-time polymerase chain reaction and Western blot. Membrane currents and action potentials were recorded using voltage- and current-clamp techniques. K_{2p}3.1 subunits exhibited predominantly atrial expression, and atrial K_{2p}3.1 transcript levels were highest among functional K_{2p} channels. K_{2p}3.1 mRNA and protein levels were increased in chronic AF. Enhancement of corresponding currents in the right atrium resulted in shortened action potential duration at 90% of repolarization (APD_{90}) compared with patients in sinus rhythm. In contrast, K_{2p}3.1 expression was not significantly affected in subjects with paroxysmal AF. Pharmacological K_{2p}3.1 inhibition prolonged APD_{90} in atrial myocytes from patients with chronic AF to values observed among control subjects in sinus rhythm.

Conclusions—Enhancement of atrium-selective K_{2p}3.1 currents contributes to APD shortening in patients with chronic AF, and K_{2p}3.1 channel inhibition reverses AF-related APD shortening. These results highlight the potential of K_{2p}3.1 as a novel drug target for mechanism-based AF therapy. (Circulation. 2015;132:82-92. DOI: 10.1161/CIRCULATIONAHA.114.012657.)

Key Words: arrhythmias, cardiac | atrial fibrillation | electrophysiology

Successful, safe pharmacological treatment of atrial fibrillation (AF) is a primary yet unmet need in cardiovascular medicine. Patients with AF exhibit largely variable disease characteristics and continue to be at high risk for hospitalizations, heart failure, and stroke as a result of the limited effectiveness of unspecific pharmacological or interventional treatment. Patient-tailored therapy is required to improve the outcomes of patients with AF. However, mechanism-based approaches are currently limited by an insufficient understanding of precise molecular remodeling associated with AF. Shortening of action potential (AP) duration (APD) is considered a hallmark of atrial remodeling in AF that promotes re-entry, supporting the perpetuation of the arrhythmia. The therapeutic significance of accelerated atrial repolarization is highlighted by AF suppression through

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Correspondence to Dierk Thomas, MD, FAHA, FESC, FHRS, Department of Cardiology, University of Heidelberg, Im Neuenheimer Feld 410, D-69120 Heidelberg, Germany. E-mail dierk.thomas@med.uni-heidelberg.de

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inhibition of repolarizing K+ currents by class III antiarrhythmic drugs or via targeted gene transfer. Although constitutive \( I_{K_{ACh}} \) activity, increased \( I_{K_{ACh}} \) current, and decreased \( I_{Ca,L} \) have previously been implicated in APD shortening during AF, the contribution of other ion channels is poorly understood.

Two-pore-domain K+ (K_{2p}) channels facilitate AP repolarization, and regulation of K_{2p} currents dynamically determines cellular excitability. Specifically, cardiac K_{2p,3.1} (TASK-1 [tandem of P domains in a weak inward-rectifying K+ channel–related acid-sensitive K+ channel-1]) currents are implicated in AP regulation and may contribute to AF. Inhibition or genetic inactivation of cardiac K_{2p,3.1} channels results in APD prolongation in rodents. In the human heart, K_{2p,3.1} K+ channels are expressed predominantly in the atria and could serve as atrium-specific antiarrhythmic targets for AF therapy. A role for cardiac K_{2p,3.1} channels as drug targets is further supported by their sensitivity to established antiarrhythmic compounds. The aim of this study was to explore the potential contribution of K_{2p,3.1} current dysregulation to AF-related APV abbreviation and to assess the relevance of K_{2p,3.1} inhibition for mechanism-based therapy in patients with paroxysmal AF (pAF) and chronic AF (cAF).

**Methods**

**Study Patients**

A total of 122 patients (mean age, 68±12 years; male/female, 83/39) with sinus rhythm (SR; n=39), pAF (n=39), and cAF (ie, persistent, long-standing persistent, or permanent AF; n=44) undergoing open heart surgery for coronary artery bypass grafting or valve repair/replacement were included. Written informed consent was obtained from all patients, and the study was performed with 1-way ANOVA. The Bonferroni adjustment was used for post hoc testing. A comprehensive expression analysis of all human K_{2p} isoforms identified K_{2p,1.1} and K_{2p,3.1} as predominant K_{2p} subunits in the right and left atria of patients with SR (n=14; Figure 1). K_{2p,3.1} channels were studied in detail in the present study owing to robust atrial expression in combination with pronounced AF-associated remodeling that was unique among K_{2p} channels (Figure 1). In LV tissue samples (n=5), K_{2p,3.1} transcript levels were low compared with the right atrium (16-fold; n=5–10; \( P<0.0001 \)) and left atrium (14-fold; n=4; \( P=0.066 \); Figure 1). For comparison, ion channel genes with established significance in human atrial electrophysiology and arrhythmogenesis were analyzed, revealing that atrial K_{2p,3.1} mRNA expression was similar to K_{4.3} currents conducting the cardiac transient outward K+ current and to inward-rectifier potassium channels K_{2,2} and K_{2,3} (Figure 2).

**Data Acquisition and Statistical Analysis**

Data acquisition was performed with pClamp software (Molecular Devices, Sunnyvale, CA); Origin 6 (OriginLab, Northampton, MA) was used for data analysis. Patient data are expressed as mean±SD. Data obtained from patch-clamp recordings are provided as mean±SEM. Statistical significance between means of continuous variables was evaluated with the Student \( t \) test. Values of \( P<0.05 \) were considered statistically significant. Multiple comparisons were performed with 1-way ANOVA. The Bonferroni adjustment was used for post hoc testing. If a quantity was dependent on 2 attributes (ie, to analyze correlations between channel expression and rhythm or LV function), we performed a 2-factor ANOVA to assess the main effects of the factors and their interaction. Similarly, 2-factor repeated-measures ANOVA was applied when multiple measurements were taken on individual myocytes at different membrane voltages. To test for rank-order correlation, we calculated the Kendall \( \tau \).

**Results**

**K_{2p} Channel Expression in the Human Heart**

A comprehensive expression analysis of all human K_{2p} isoforms identified K_{2p,1.1} and K_{2p,3.1} as predominant K_{2p} subunits in the right and left atria of patients with SR (n=14; Figure 1). K_{2p,3.1} channels were studied in detail in the present study owing to robust atrial expression in combination with pronounced AF-associated remodeling that was unique among K_{2p} channels (Figure 1). In LV tissue samples (n=5), K_{2p,3.1} transcript levels were low compared with the right atrium (16-fold; n=5–10; \( P<0.0001 \)) and left atrium (14-fold; n=4; \( P=0.066 \); Figure 1). For comparison, ion channel genes with established significance in human atrial electrophysiology and arrhythmogenesis were analyzed, revealing that atrial K_{2p,3.1} mRNA expression was similar to K_{4.3} currents conducting the cardiac transient outward K+ current and to inward-rectifier potassium channels K_{2,2} and K_{2,3} (Figure 2).

**Increased K_{2p,3.1} Levels Contribute to Atrial Remodeling in Patients With cAF**

Remodeling of ion channel expression is generally believed to constitute the electric substrate that shortens atrial APD, supporting AF-maintaining re-entry. We found that K_{2p,3.1} mRNA expression in the right atrium was elevated by 59.8%.
In patients with cAF (n=10) compared with individuals with SR (n=10; Figure 1). In addition, there was a 27.6% increase of K2P3.1 mRNA levels in left atrial tissue (cAF, n=11 versus SR, n=4) that was not statistically significant (P=0.55; Figure 1). In contrast, K2P3.1 mRNA levels did not change in patients with pAF (n=16) compared with patients in SR (n=14; Figure 1). Alterations of K2P3.1 mRNA expression levels were consistent with K2P3.1 immunoblots (Figure 3 and Figure II in the online-only Data Supplement). cAF was associated with upregulation of K2P3.1 immunoreactivity at 50 to 55 kDa, corresponding to the fully processed membrane protein, in the right atrium by 64.0±17.7% (P=0.030; n=4) compared with patients in SR (Figure 3A–3C). We also observed a moderate increase in K2P3.1 protein expression in pAF (37.4±13.1%; P=0.043; n=4). Of note, K2P3.1 immunosignal intensity at ≈200 kDa, which may reflect channel aggregates, was similarly upregulated in patients with cAF (Figure 3A). Low protein levels were detected by anti-K2P3.1 antibodies in an exemplary ventricular sample, highlighting weak K2P3.1 expression in LV tissue (Figure IIIA in the online-only Data Supplement). However, limited discrimination of K2P3.1 and other cardiac proteins by anti-K2P3.1 antibodies in mice requires cautious attention in the interpretation of human Western blot data (the online-only Data Supplement provides an in-depth appraisal of antibody specificity).

In addition to K2P3.1, K2P channels K2P13.1 and K2P17.1 were significantly affected in patients with cAF, displaying reduced mRNA levels in the right atrium (Figure 1). cAF was further associated with significant upregulation (K ir2.1; KCNQ1) or suppression (sulfonylurea receptor 1, potassium channel-interacting protein 2, Kir3.1, Kir3.4) of additional ion

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Table. Baseline Characteristics of Study Patients

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ACE indicates angiotensin-converting enzyme; AT1, angiotensin receptor-1; AVD, aortic valve disease; CAD, coronary artery disease; cAF, chronic atrial fibrillation; LAA, left atrial appendage; LVEF, left ventricular ejection fraction (normal, ≥55%; mild impairment, 45%–54%; moderate impairment, 30%–44%; severe impairment, <30%; MVD, mitral valve disease; NA, not available; OAC, oral anticoagulation; pAF, paroxysmal atrial fibrillation; RAA, right atrial appendage; and SR, sinus rhythm.
P<0.05 vs SR, †P<0.05 versus corresponding values in the RA from ANOVA followed by Bonferroni multiple-comparisons procedure for continuous variables and from the Fisher exact test for categorical variables.
channels and accessory subunits relevant to atrial electrophysiology (Figure 2). Of note, we did not detect significant electric remodeling in patients with pAF.

**K3.1 Current Enhancement in cAF**

Functional consequences of K3.1 upregulation were studied in right atrial myocytes obtained from patients with SR, pAF, and cAF. K3.1 current was isolated by use of the experimental compound A293, which specifically inhibits the channels at 200 nmol/L (Figure 4A; see also Supplemental Results and Figure IV in the online-only Data Supplement). A293-sensitive K+ currents activated at potentials >−20 mV and showed Goldman-Hodgkin-Katz (open or outward) rectification that is characteristic of K2P channels (Figure 4B–4F). K3.1 current density quantified at 40 mV was increased by 3.1-fold in patients with cAF (n=13 cells obtained from N=5 individuals) compared with SR (n/N=17/6; P=0.002; Figure 4F; see Figure V in the online-only Data Supplement). K3.1 currents tended to be 1.5-fold higher in pAF subjects (n/N=13/6) in relation to SR (n/N=17/6) without statistical significance (P=0.47; Figure 4E and 4G).

**K3.1 Upregulation Is Associated With APD Shortening**

Upregulation of K3.1 mRNA, protein, and corresponding currents in cAF suggest functional relevance in shaping the atrial AP. Atrial APs were studied under current-clamp conditions in human atrial myocytes. APD at 90% of repolarization (APD90) was abbreviated by 42.9% from 213.0±11.1 milliseconds (SR; n/N=9/6) to 121.7±12.6 milliseconds (cAF; n/N=10/6; P<0.0001; Figure 5A, 5C, and 5E) in cAF, consistent with the increase in repolarizing K3.1 currents. In patients with pAF (n/N=9/5), APD90 remained virtually unchanged in relation to SR (P=0.67; Figure 5B, 5D, and 5E). There was no rhythm-dependent modulation of APD at 50% of repolarization (APD50; Figure 5A–5D) or resting membrane potential (Figure V in the online-only Data Supplement) in any group.

**Class III Antiarrhythmic Effects of K3.1 Channel Inhibition in cAF Patients**

The experimental K3.1 inhibitor A293 was used to test the hypothesis that pharmacological K3.1 reduction would reverse APD shortening in cAF. In human atrial myocytes obtained from patients in SR (n/N=9/6), K3.1 block by 200 nmol/L A293 induced only a weak prolongation of APD90 (3.4±1.6%; P=0.012; Figure 5A and 5D–5F). In contrast, APD90 was markedly prolonged by 57.9±10.0% (n/N=10/6) in cAF (200 nmol/L A293; P<0.0001), indicating significant class III antiarrhythmic efficacy in this subset of patients with AF (Figure 5C, 5D, and 5F). A293 also increased APD90 in pAF, albeit to a lesser degree (27.8±6.3%; P=0.003; Figure 5B, 5D, and 5F). A direct
comparison of the A293 effects between patients with SR, pAF, and cAF revealed that specific K2P3.1 blockade had little effect on absolute APD90 in SR and pAF (Figure 5E), whereas in patients with cAF, A293 increased APD90 to APD levels typical for SR subjects (Figure 5E and 5F).

**Computational Analysis of the Effect of K2P3.1 Current on APD in SR and cAF**

The Grandi et al.36 computational model of the human atrial cardiomyocyte was extended with a formulation for the K2P3.1 current based on the experimentally measured I-V relationship (Figure I in the online-only Data Supplement). The SR and cAF versions of the model were adjusted to reproduce the experimental APD50 and APD90 under simulated conditions corresponding to the experimentally used pipette and bath solutions (Figure 6A and 6B). Simulated inhibition of K2p3.1 channels produced a modest prolongation of APD90 in the SR model but a much larger prolongation in the cAF model (Figure 6A and 6C), consistent with experimental results. Moreover, this APD prolongation was observed at all pacing frequencies between 0.2 and 3.3 Hz (Figure 6D). Finally, APD in the cAF model after K2P3.1 channel blockade approached that of the SR model, with a reduction in the APD difference from 93.2 to 28.1 milliseconds (−70%) after K2P3.1 channel blockade compared with SR simulations. Together, these data suggest that, under these conditions, upregulation of K2p3.1 in patients with cAF plays a major role in the proarrhythmic APD shortening.
expression ($F=42.3; P=0.026$) characterized by cAF-associated upregulation ($P=0.022$; Figure 7B). There was no significant correlation between LV function and cardiac rhythm ($F=11.8; P=0.35$; Kendall $\tau=-0.16$) in the patient cohort.

**Discussion**

**Atrial $K_{\text{p}3.1}$ K+ Channels in Humans With SR**

$K_{\text{p}3.1}$ potassium channels conduct repolarizing currents and contribute to the resting membrane voltage in excitable cells. In the present work, we delineated mRNA expression of multiple $K_{\text{p}}$ channels in left and right atria obtained from control subjects with SR. $K_{\text{p}3.1}$ displayed highest transcript levels among $K_{\text{p}}$ family members with confirmed K+ channel function (ie, after exclusion of $K_{\text{p}1.1}, K_{\text{p}7.1}, K_{\text{p}12.1},$ and $K_{\text{p}15.1}$ subunits, which do not produce substantial K+ currents) and was specifically studied. The high ratio of atrial to ventricular $K_{\text{p}3.1}$ transcripts (16:1) highlighted predominantly atrial expression. Inhibition of $K_{\text{p}3.1}$ current produced a tendency toward prolonged APD$_{90}$ by 17% in patients in SR, reflecting class III antiarrhythmic effects. These data indicate that $K_{\text{p}3.1}$ functionally contributes to the atrial AP in subjects with SR and represents an atrium-selective target for antiarrhythmic therapy.

**APD Shortening in cAF Patients: Significance of $K_{\text{p}3.1}$ and Comparison With Previous Studies**

Electric remodeling of human atrial tissue is a hallmark of AF pathophysiology, stabilizing re-entrant circuits via abbreviation of atrial APD. We observed significant shortening of APD$_{90}$ in patients with cAF compared with subjects with SR. In contrast, there was no APD reduction in pAF cardiomyocytes, in accordance with previous data. In addition, the patients’ rhythm status was not associated with atrial resting membrane potential changes in the present study consistent with earlier work. Similarly, inhibition of $K_{\text{p}3.1}$ current had no effect on resting membrane potential. The molecular basis of electric remodeling was further elucidated in a comprehensive approach that included all $K_{\text{p}}$ channels and 21 additional ion channel subunits relevant to atrial electrophysiology. The main finding was a significant upregulation of $K_{\text{p}3.1}$ expression and current levels in patients with cAF but not in patients with pAF, suggesting a mechanistic explanation for the typical APD shortening in patients with cAF. The presence of nonactivating outward K+ currents in patient-derived atrial myocytes after extensive pharmacological block of established potassium channels additionally highlights a significant contribution of $K_{\text{p}3.1}$ conductance to human cardiac electrophysiology.

**Independent Effects of Cardiac Function on Atrial $K_{\text{p}3.1}$ Expression**

To provide a more precise characterization of the patient population likely to benefit from $K_{\text{p}3.1}$ blockade, the correlation of right atrial $K_{\text{p}3.1}$ expression levels with LV function was explored. Patient groups with SR (n=16), pAF (n=12), and cAF (n=11) were analyzed. Study subgroups were not significantly different with respect to sex, body mass index, or medical history. The potential relationship between $K_{\text{p}3.1}$ levels and LV function or rhythm was statistically analyzed via 2-way ANOVA with rhythm status (SR, pAF, cAF) and LV function (normal; mild, moderate, severe reduction) as factors.

**Figure 3.** Western blot analysis of $K_{\text{p}3.1}$ protein in human right atrium. A. Representative immunoblots obtained from patients in sinus rhythm (SR), paroxysmal atrial fibrillation (pAF), or chronic atrial fibrillation (cAF) probed with anti-$K_{\text{p}3.1}$ antibodies. B. Anti-GAPDH antibodies were applied to quantify protein load. C. Means±SEM optical density values normalized to GAPDH expression of indicated patient groups (n=4 subjects per group; *$P<0.05$ vs SR).
Kir3.1, Kir3.4, Kv4.3), which is consistent with previous data and would prolong rather than shorten atrial APD. We conclude that K2P3.1 upregulation, in combination with increased Kir2.1 and KCNQ1 levels, accounts for APD shortening in patients with cAF. AF-related K2P3.1 dysregulation and APD shortening strongly suggest a mechanistic role in cAF perpetuation with implications for patient-tailored antiarrhythmic therapy.

**Therapeutic Implications: K2P3.1 Inhibition Provides Mechanism-Based AF Management**

Atrial selectivity is a desired target in the development of novel compounds for AF. Limiting the electropharmacological action to atrial tissue reduces the risk of proarrhythmic effects in the ventricles. Inhibitors of K2P3.1 channels, which are expressed predominantly in human atria and enhanced in AF, are therefore expected to be particularly effective and safe in AF therapy. In addition, the ability of an antiarrhythmic intervention to prevent AF depends on its capacity to suppress the underlying disease mechanism. Specifically, the reversal of atrial remodeling by targeting substrate development has become a focus of attempts at therapeutic intervention. The present study reveals K2P3.1 current upregulation as a distinct arrhythmogenic substrate in cAF associated with abbreviated APD. Antiarrhythmic drugs with class III characteristics

### Figure 4.

**K2P3.1 current properties in sinus rhythm (SR), paroxysmal atrial fibrillation (pAF), and chronic atrial fibrillation (cAF).**

**A.** Specificity of the K2P3.1 inhibitor A293 assessed in *Xenopus* oocytes (n=4–14 cells were studied; see the online-only Data Supplement for details). Significant current reduction was observed with human K2P3.1 and related, noncardiac K2P9.1 channels. **B through D.** Representative macroscopic currents recorded from human right atrial myocytes using indicated voltage protocols and corresponding mean step current density as a function of the respective test potentials are displayed (top to bottom) for SR (B), pAF (C), and cAF (D). K2P3.1 current was isolated with the use of the specific inhibitor A293. **E and F,** Current-voltage relationships of mean A293-sensitive current density obtained in B through D are depicted compared with SR for patients with pAF (E) and cAF (F). **G,** Mean A293-sensitive current density quantified at 40-mV membrane potential. Data are expressed as mean±SEM. n/N indicates number of myocytes/number of patients. *P<0.05, **P<0.01, ***P<0.001 vs drug-free control conditions (A) or vs SR (E–G).
suppress AF through K⁺ channel inhibition, resulting in prolongation of APD and prevention of electric re-entry. Here, specific \(K_{\text{p-3.1}}\) inhibition by 200 nmol/L A293 prolonged the APD in patients with cAF to achieve levels observed in SR subjects, resulting in functional correction of electric remodeling in this AF subentity. Finally, diminished \(K_{\text{p-3.1}}\) expression in AF subentities with severely reduced LVEF provides a criterion for personalized antiarrhythmic therapy: Clinical efficacy of \(K_{\text{p-3.1}}\) inhibition is expected primarily in patients with cAF and normal or mildly to moderately reduced LVEF. Studies in large animals and humans are required next to further explore this novel antiarrhythmic paradigm in vivo.

Potential Limitations

AF-associated electric remodeling was studied in right and left atrial appendage tissue, revealing a previously unrecognized mechanism of AF pathophysiology. It remains unclear whether the results may be extrapolated to other atrial regions that have not been specifically assessed owing to the limited availability of these samples. Statistically significant \(K_{\text{p-3.1}}\) upregulation was detected in right atrial tissue only (Figure 1). However, there was also a tendency toward increased \(K_{\text{p-3.1}}\) mRNA levels in left atrial tissue obtained from patients with cAF and normal or mildly to moderately reduced LVEF. Studies in large animals and humans are required next to further explore this novel antiarrhythmic paradigm in vivo.

Figure 5. Characteristics of action potentials (APs) and electropharmacological effects of \(K_{\text{p-3.1}}\) current blockade in right atrial myocytes. A through C, Representative APs recorded at 0.2 Hz in the absence or presence of A293 are shown for sinus rhythm (SR; A), paroxysmal atrial fibrillation (pAF; B), and chronic atrial fibrillation (cAF; C). D and E, Corresponding mean AP durations at 50% of repolarization \(\text{APD}_{50}\) and 90% repolarization \(\text{APD}_{90}\). Data are provided as mean±SEM. *P<0.05, ***P<0.001 vs drug-free control conditions; #P<0.05, ##P<0.01, ###P<0.001 vs SR.

Study patients were carefully matched for baseline characteristics, medication, and concomitant heart disease to exclude any bias associated with these conditions. In particular, no patient received class I or class III antiarrhythmic therapy that may have modulated APD. There were minor intergroup differences in age, cardiac function, cardiovascular disease, or medication as potential confounding factors that require consideration in the interpretation of our results. However, \(K_{\text{p-3.1}}\) enhancement may not be attributed to impaired LVEF because we observed a correlation of severely reduced LV function with decreased rather than increased \(K_{\text{p-3.1}}\) levels.

We did not investigate constitutive \(I_{\text{K,ACh}}\) activity that was previously implicated in APD shortening. Given that selective \(K_{\text{p-3.1}}\) inhibition by A293 in patients with cAF fully reconstituted APD, the contribution of constitutive \(I_{\text{K,ACh}}\) activity to APD appears to be minor in the present subentity of patients with cAF. Unspecific antibody detection of cardiac protein observed in knockout mice requires consideration in the interpretation of human \(K_{\text{p-3.1}}\) immunoblot data (Supplemental Results, Table III, and Figure III in the online-only Data Supplement). We cannot fully exclude that available \(K_{\text{p-3.1}}\) antibodies, including those used in this work, which were previously applied to demonstrate cardiac \(K_{\text{p-3.1}}\) expression in mice, rats, and dogs, and humans (Table III in the online-only Data Supplement), may recognize other proteins in humans as well. Therefore, the additional confirmation of increased \(K_{\text{p-3.1}}\) expression at the protein level needs to be interpreted with caution. In human ventricular tissue, low protein levels...
were detected by anti-K<sub>p3.1</sub> antibodies, arguing against relevant cross-reactivity with endogenous human cardiac protein.

Altered ion channel transcript and protein levels analyzed in cardiac tissue may reflect alterations not only in myocytes but also in fibroblasts and other cell types. Importantly, in the present work, electrophysiological recordings provide unequivocal confirmation of K<sub>p3.1</sub> current and APD remodelling in atrial myocytes.

Finally, structural alterations of atrial tissue may contribute to the development and maintenance of AF, in addition to electric remodeling.1,2,31,33 Specifically, atrial fibrosis, which has been implicated in conduction heterogeneity and

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**Figure 6.** Computational analysis of the impact of K<sub>p3.1</sub> channels on action potential duration (APD). 

A, Action potential (top) and K<sub>p3.1</sub> current (bottom) in the sinus rhythm (SR; left) and chronic atrial fibrillation (cAF; right) models under control conditions (solid lines) or after complete inhibition of K<sub>p3.1</sub> current (dashed lines). Data were obtained at a pacing frequency of 0.2 Hz with intracellular and extracellular ion concentrations based on the experimental pipette and bath solutions. B, Validation of APD at 50% of repolarization (APD<sub>50</sub>; top) and APD at 90% (APD<sub>90</sub>; bottom) in the SR and cAF models under control conditions and after K<sub>p3.1</sub> blockade (solid bars) compared with measurements in isolated human atrial cardiomyocytes from patients with SR and cAF in the absence or presence of 200 nmol/L A293 (open bars). Experimental data are identical to those in Figure 5. C, Validation of the relative prolongation of APD<sub>50</sub> and APD<sub>90</sub> as a result of K<sub>p3.1</sub> channel blockade based on the data from B. D, Rate dependence of APD prolongation after K<sub>p3.1</sub> blockade in the SR (open symbols) and cAF models (solid symbols) with dynamic intracellular ion concentrations.

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**Figure 7.** Correlation of right atrial K<sub>p3.1</sub> mRNA levels with cardiac function. A, K<sub>p3.1</sub> mRNA expression in subjects with normal left ventricular ejection fraction (LVEF) and with mildly, moderately, or severely impaired LVEF. B, Transcript levels in patients with sinus rhythm (SR), paroxysmal atrial fibrillation (pAF), and chronic atrial fibrillation (cAF). Data are expressed as mean±SEM arbitrary units normalized to importin 8 (IPO8). *P<0.05 vs normal/mildly impaired/moderately reduced LVEF (A) or vs SR/pAF (B).
in the promotion of AF, is commonly observed in human AF and in animal models. Structural remodeling was not addressed here because the present study focused on the contribution of \( \text{K}_\text{2P}3.1 \) current dysregulation to electric remodeling only.

**Conclusions**

The data provide novel mechanistic insights into atrial arrhythmogenesis in humans. We detailed increased atrial \( \text{K}_\text{2P}3.1 \) expression and function in patients with cAF that resulted in shortening of AP recorded from patient-derived atrial myocytes. Specific \( \text{K}_\text{2P}3.1 \) inhibition prolonged APD in cardiac myocytes obtained from patients with cAF to reconstitute levels of SR subjects. Functional correction of atrial ionic remodeling through \( \text{K}_\text{2P}3.1 \) channel blockade represents a novel paradigm to optimize and specify AF management.

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**Disclosures**

The experimental compound A293 was kindly provided by Sanofi-Aventis (Frankfurt am Main, Germany). Dr Thomas served on advisory boards for and received honoraria for lectures from Sanofi-Aventis. The other authors report no conflicts.

**References**


**CLINICAL PERSPECTIVE**

Mechanism-based approaches to atrial fibrillation (AF) therapy are sought to increase effectiveness and to provide more individualized patient care. Specifically, the reversal of atrial remodeling by targeting substrate development has become a focus of attempts at therapeutic intervention. Shortening of atrial refractory periods promotes electric re-entry and contributes to maintenance of AF. Outward currents mediated by K2P3.1 (TASK-1) 2-pore-domain potassium (K2P) channels promote atrial electrophysiology in patients with AF, however, is not known. The present work provides novel mechanistic insights into atrial arrhythmogenesis in humans. Cellular electrophysiology, molecular biology, biochemistry, and computational modeling were used to assess the significance of K2P3.1 channels and their remodeling in patients with paroxysmal and persistent, long-standing persistent, or permanent (chronic) AF compared with subjects in sinus rhythm. K2P3.1 subunits exhibited predominant atrial expression. We observed increased K2P3.1 expression and function in patients with chronic AF that resulted in shortening of AP duration in patient-derived atrial myocytes. In patients with paroxysmal AF, K2P3.1 levels were not significantly affected, in line with a lack of AP duration changes. Pharmacological K2P3.1 inhibition prolonged AP duration in cardiac myocytes obtained from patients with chronic AF to reintroduce levels of subjects in sinus rhythm. This work provides the first direct evidence of K2P3.1 dysregulation resulting in AP duration shortening in patients with chronic AF, suggesting a mechanistic role of K2P3.1 in chronic AF perpetuation. Functional correction of atrial ionic remodeling through K2P3.1 blockade represents a novel paradigm to optimize and specify AF management.
Upregulation of K$_{2p3.1}$ K$^+$ Current Causes Action Potential Shortening in Patients With Chronic Atrial Fibrillation

Constanze Schmidt, Felix Wiedmann, Niels Voigt, Xiao-Bo Zhou, Jordi Heijman, Siegfried Lang, Virginia Albert, Stefan Kallenberger, Arjang Ruhiparwar, Gábor Szabó, Klaus Kallenbach, Matthias Karck, Martin Borggrefe, Peter Biliczki, Joachim R. Ehrlich, István Baczkó, Patrick Lugensbiel, Patrick A. Schweizer, Birgit C. Donner, Hugo A. Katus, Dobromir Dobrev and Dierk Thomas

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SUPPLEMENTAL MATERIAL

Upregulation of K$_{2p}3.1$ K$^+$ current causes action potential shortening in patients with chronic atrial fibrillation

Constanze Schmidt, MD$^1$; Felix Wiedmann, MD$^1$; Niels Voigt, MD$^{2,3}$; Xiao-Bo Zhou, MD$^{2,4}$; Jordi Heijman, PhD$^{2,3}$; Siegfried Lang, PhD$^{2,4}$; Virginia Albert, BSc$^1$; Stefan Kallenberger, PhD$^5$; Arjang Ruhparwar, MD$^6$; Gábor Szabó, MD, PhD$^6$; Klaus Kallenbach, MD$^6$; Matthias Karck, MD$^6$; Martin Borggrefe, MD$^4$; Peter Biliczki, MD, PhD$^{7,8}$; Joachim R. Ehrlich, MD$^{6,7}$; István Baczkó, MD, PhD$^9$; Patrick Lugenbiel, MD$^1$; Patrick A. Schweizer, MD$^1$; Birgit C. Donner, MD, PhD$^{10}$; Hugo A. Katus, MD, PhD$^1$; Dobromir Dobrev, MD$^{2,3,*}$; Dierk Thomas, MD$^1,*$

$^1$Department of Cardiology, University of Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany; $^2$Division of Experimental Cardiology, Medical Faculty Mannheim, University of Heidelberg, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany; $^3$Institute of Pharmacology, Faculty of Medicine, University Duisburg-Essen, Hufelandstrasse 55, 45122 Essen, Germany; $^4$First Department of Medicine, University Medical Center Mannheim, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany; $^5$Department for Bioinformatics and Functional Genomics, Division of Theoretical Bioinformatics, German Cancer Research Center (DKFZ), Institute for Pharmacy and Molecular Biotechnology (IPMB) and BioQuant, Heidelberg University, Im Neuenheimer Feld 267, 69120 Heidelberg, Germany;

$^6$Department of Cardiac Surgery, University Hospital Heidelberg, Im Neuenheimer Feld 110, 69120 Heidelberg, Germany; $^7$Department of Cardiology, Internal Medicine III, Goethe University, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany; $^8$Division of Cardiology, Deutsche Klinik für Diagnostik, Aukammallee 33, 65191 Wiesbaden, Germany; $^9$Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged, Dóm tér 12, H-6720 Szeged, Hungary; $^{10}$Department of Cardiology, University of Basel Children’s Hospital, Spitalstrasse 33, 4031 Basel, Switzerland.

*These authors contributed equally to this work.

**Corresponding author:** Dierk Thomas, MD, FAHA, FESC, FHRS; Department of Cardiology, University of Heidelberg, Im Neuenheimer Feld 410, D-69120 Heidelberg, Germany; Tel.: ++49 6221 568855; Fax: ++49 6221 565514; E-Mail: dierk.thomas@med.uni-heidelberg.de
Supplemental Methods

Ethics Statement

This study has been carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health (NIH publication No. 85-23, revised 1985), and the current version of the German Law on the Protection of Animals was followed. The investigation conforms to the Directive 2010/63/EU of the European Parliament.

Quantitative Real-Time PCR

Total RNA was prepared using TRizol- Reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer’s instructions. DNA synthesis was carried out with the Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Fisher Scientific, Waltham, MA, USA) using 3 µg of total RNA. 96 well optical detection plates (Applied Biosystems, Foster City, CA, USA) were then loaded to a total volume of 10 µl per well, consisting of 0.5 µl cDNA, 5 µl TaqMan Fast Universal Master Mix (Applied Biosystems), and 6-carboxyfluorescein (FAM)-labeled TaqMan probes and primers (TaqMan Gene Expression Assays; Applied Biosystems) (Supplemental Table I). In addition, pre-designed primers and probes detecting importin 8 (IPO8) were used for normalization.

Cardiomyocyte Isolation

Right atrial samples were placed into chilled Ca\textsuperscript{2+}-free solution (100 mM NaCl, 10 mM KCl, 1.2 mM KH\textsubscript{2}PO\textsubscript{4}, 5 mM MgSO\textsubscript{4}, 50 mM taurine, 5 mM 3-(N-...
morpholino)propanesulfonic acid (MOPS), and 20 mM glucose, pH 7.0 with NaOH) supplemented with 2,3-butanedione monoxime (BDM, 30 mM; Sigma-Aldrich, St. Louis, MO, USA), cut into small pieces, and rinsed 3 times for 3 minutes with Ca\(^{2+}\)-free Tyrode’s solution. The solutions were oxygenated with 100% O\(_2\) at 37°C. Tissue aliquots were transferred into Ca\(^{2+}\)-free Tyrode’s solution and digested with 288 U/ml collagenase type I (Worthington Biochemical Corporation, Lakewood, NJ, USA) and 5 mg/ml protease type XXIV (Sigma-Aldrich) for 15 minutes. The Ca\(^{2+}\) concentration was then increased to 0.2 mM, and the tissue was stirred for additional 35 minutes in protease-free solution to release rod-shaped single myocytes. Finally, the suspension was centrifuged, and myocytes were resuspended in storage solution (20 mM KCl, 10 mM KH\(_2\)PO\(_4\), 10 mM glucose, 70 mM K glutamate, 10 mM β-hydroxybutyrate, 10 mM taurine, 10 mM ethylene glycol tetraacetic acid (EGTA), 1% albumin) prior to use at room temperature.

**Patch Clamp Electrophysiology**

For patch clamp recordings, glass pipettes (1B120F-4; World Precision Instruments, Berlin, Germany) with tip resistances ranging from 3 to 4 M\(\Omega\) were filled with the following solution: 60 mM KCl, 65 mM K glutamate, 3 mM K\(_2\)ATP, 0.2 mM Na\(_2\)GTP, 2 mM MgCl\(_2\), 5 mM EGTA, 5 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) (pH adjusted to 7.2 with KOH). Cardiomyocytes were constantly superfused with extracellular solution containing: 140 mM NaCl, 5.4 mM KCl, 1 mM MgCl\(_2\), 1 mM CaCl\(_2\), 0.33 mM NaH\(_2\)PO\(_4\), 5 mM HEPES, 10 mM glucose (pH adjusted to 7.4 with NaOH). Seal resistances yielded 4 to 8 G\(\Omega\). Series resistance and cell capacitance were compensated. Data were not corrected for liquid junction potentials. Membrane currents were evoked by application of voltage steps between
-60 and +60 mV in 10 mV-increments (duration, 300 ms; holding potential, -50 mV).

Patch pipettes for cardiac action potential recordings were back-filled with 134 mM K gluconate, 6 mM NaCl, 1.2 mM MgCl$_2$, 1 mM MgATP, 10 mM HEPES (pH adjusted to 7.2 with KOH). Extracellular Tyrode’s solution was composed of 137 mM NaCl, 5.4 mM KCl, 2 mM CaCl$_2$, 1 mM MgSO$_4$, 10 mM glucose and 10 mM HEPES (pH 7.3 with NaOH).

**Murine Cardiac Samples**

Whole hearts were obtained from C57BL/6 $Kcnk3^{-/-}$ mice$^{1,2}$ and from C57BL/6N $Kcnk3^{+/+}$ animals. Specific approval is not required for experiments solely involving the killing of animals according to the German Law on the Protection of Animals.

**Molecular Biology**

Complementary DNAs encoding human $K_{2p}1.1$ K274Q,$^3$ $K_{2p}2.1$ (GenBank accession number, EF165334), $K_{2p}3.1$ (NM_002246), $K_{2p}9.1$ (NM_016601), KCNQ1 (NM_000218), and KCNE1 (NM_000219) were kindly provided by Steve Goldstein (Brandeis University, Waltham, MA, USA), and $hK_{2p}18.1$ cDNA (NM_181840) was obtained from C. Spencer Yost (San Francisco, CA, USA). Amplification of the following human cDNAs was previously described: $hK_{2p}4.1$ (EU978935), $K_{2p}5.1$ (EU978936), $K_{2p}6.1$ (EU978937), $K_{2p}10.1$ (EU978939), $K_{2p}13.1$ (EU978942), $K_{2p}16.1$ (EU978943), and $K_{2p}17.1$ (EU978944).$^4$ Human ether-a-go-go-related gene (hERG; NM_000238) DNA was kindly supplied by Mark T. Keating (Boston, MA, USA). Human $K_v1.5$ (NM_002234), h$K_v4.3$ (NM_031739), and h$K_{ir}2.2$ (NM_001957) clones were provided by Barbara A. Wible (Cleveland, OH, USA), and h$K_{ir}2.1$ (U12507) and
hK\textsubscript{2.3} (U07364) DNAs were donated by Carol A. Vandenberg (Santa Barbara, CA, USA). \textit{In vitro} transcription was performed as published\textsuperscript{5}. Complementary RNAs were transcribed after vector linearization using T7 or SP6 RNA polymerase and the mMessage mMachne kit (Ambion, Austin, TX, USA). Transcripts were quantified by spectrophotometry, and cRNA integrity was assessed by agarose gel electrophoresis.

**Xenopus Laevis Oocyte Preparation**

\textit{Xenopus laevis} oocytes were surgically removed from adult females, manually dissected, and subjected to collagenase treatment as reported earlier\textsuperscript{6}. After the final collection of oocytes, the anesthetized frog was killed by decerebration and pithing. Approval was granted by the local Animal Welfare Committee (institutional approval numbers A-38/11 and G-221/12).

**Two-Electrode Voltage Clamp Electrophysiology**

Complementary RNA (0.25 - 23 ng; 46 nl/oocyte) was injected into stage V–VI defolliculated \textit{Xenopus} oocytes. Two-electrode voltage clamp (TEVC) measurements on \textit{Xenopus laevis} oocytes were performed one to three days after oocyte injection as described\textsuperscript{5,7}. Macroscopic currents were measured with an OC-725C amplifier (Warner Instruments, Hamden, CT, USA). Pipettes were pulled from borosilicate glass (1B120F-4; World Precision Instruments) using a Flaming/Brown P-87 micropipette puller (Sutter Instruments, Novato, CA, USA). Two-electrode voltage clamp electrodes were filled with 3 M KCl. The standard extracellular solution for oocytes recordings contained 96 mM NaCl, 4 mM KCl, 1.1 mM CaCl\textsubscript{2}, 1 mM MgCl\textsubscript{2}, 5
mM HEPES (pH 7.4). The extracellular pH was adjusted to 8.5 to activate $K_{2P}16.1$ and $K_{2P}17.1$ channels. A293 was dissolved in dimethyl sulfoxide (DMSO) to a stock solution of 10 mM and stored at -20°C. On the day of experiments, aliquots of the stock solution were diluted to the desired concentration with external solution. All experiments were carried out at room temperature (20-22°C), and leak currents were not subtracted.

The holding potential was -80 mV in all oocyte experiments performed in this study, with the exception of h$K_v$4.3 (-100 mV). $K_{2P}$ currents were activated using depolarizing pulses (500 ms) to voltages between -140 mV and +60 mV in 20 mV increments, and current amplitudes were measured at the end of the +20 mV-pulse. $K_v1.5$ currents were elicited with a double step voltage protocol consisting of a variable first voltage step ranging from -90 mV to +50 mV (10 mV increments; 1250 ms) that was followed by a constant second voltage step to +50 mV (125 ms). Maximum currents at +30 mV were measured for quantification. $K_v4.3$ channels were analysed using the following protocol: From a holding potential of −100 mV, cells were depolarized to voltages between −100 and +40 mV (1,000 ms, 10 mV increments). Peak current amplitudes recorded at +20 mV were quantified. In addition, voltage pulses were applied for 400 ms to voltages between −120 mV and +80 mV in 20 mV increments to activate hERG channels, and tail currents were recorded during a constant repolarizing step to −60 mV for 400 ms. Activating hERG currents were quantified at +40 mV. KCNQ1/KCNE1 currents were activated using depolarizing steps to potentials ranging from −60 mV to +120 mV (2 s; currents were measured at +80 mV), and tail currents were recorded at −60 mV (1 s). Finally, inward rectifier currents were recorded from oocytes expressing $K_{ir}$ channel subunits.
during test pulses from −120 mV to +40 mV in 10 mV increments (400 ms). Current amplitudes at −100 mV were determined to quantify drug effects.

**Concentration-Response Data Analysis**

PCLAMP (Axon Instruments, Foster City, CA, USA) and Origin 8.5 (OriginLab, Northampton, MA, USA) software was used for data acquisition and analysis. Concentration-response relationships for drug-induced block were fit with a Hill equation of the following form: \( \frac{I_{\text{drug}}}{I_{\text{control}}} = \frac{1}{1 + (D/IC_{50})^n} \), where \( I \) indicates current, \( D \) is the drug concentration, \( n \) is the Hill coefficient, and \( IC_{50} \) is the concentration necessary for 50% block.

**Western Blot Analysis**

Protein immunodetection was performed by sodium dodecyl sulfate (SDS) gel electrophoresis and Western blotting as described.\(^8\,9\) Tissue sections obtained from indicated samples were rinsed in phosphate buffered saline (PBS), rapidly frozen in liquid nitrogen and stored at −80°C. Tissue samples were homogenized (TissueRuptor, QIAGEN, Hilden, Germany) in a radioimmunoprecipitation (RIPA) lysis buffer containing 50 mM Tris-HCl (pH 7.4), 0.5% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM Na\(_3\)VO\(_4\), 1 mM NaF, and protease inhibitors (Complete; Roche, Indianapolis, IN, USA). The protein concentration was determined using the bicinchoninic acid (BCA) protein assay (Thermo Scientific, Rockford, IL, USA), and equal amounts of protein were separated on SDS polyacrylamide gels. Nitrocellulose membranes were developed by sequential exposure to blocking reagent containing 3% bovine serum albumin and 5% dry milk,
different primary antibodies directed against K$_{2P}$3.1 (please see Supplemental Table III for details), and appropriate HRP-conjugated secondary antibodies (1:3,000; AB6721; Chemicon). Signals were developed using the enhanced chemiluminescence assay (ECL Western Blotting Reagents, GE Healthcare, Buckinghamshire, UK) and quantified with ImageJ 1.41 Software (National Institutes of Health, Bethesda, MD, USA). Protein content was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using anti-GAPDH primary antibodies (1:40,000; G8140-11; US Biological, Swampscott, MA, USA) and corresponding secondary antibodies (1:40,000; sc-2005; Santa Cruz Biotechnology) for quantification of optical density.

Computational Modeling

The Grandi et al. computational model of the human atrial cardiomyocyte$^{10}$ with our recent update including Na$^+$-dependent regulation of $I_{K_1}$ and $I_{K_{Ach}}$$^{11}$ was extended with a formulation for the K$_{2P}$ current (Supplemental Figure IA). K$_{2P}$3.1 channels were modeled using a two-state (open/closed) Hodgkin-Huxley model with fast activation and deactivation kinetics. Steady-state voltage-dependence of activation was incorporated for the sinus rhythm (SR) and chronic atrial fibrillation (cAF) models based on the A293-sensitive currents recorded in isolated human atrial cardiomyocytes (Supplemental Table II).

For the SR model:

$$I_{K_{2P}} = g_{K_{2P}} \times o_{K_{2P}} \times (v_{M} - E_{K})$$
For the cAF model:

\[ G_{K_{2P}} = 0.005 \text{ nS/pF} \]

\[ E_K = \frac{R \times T}{P} \times \log \left( \frac{[K^+]_2}{[K^+]_1} \right) \]

\[ K_{2P,\infty} = 0.2 + \frac{0.6}{1 + \exp \left( -\frac{V_M - 10}{14} \right)} \]

\[ \tau_{K_{2P}} = 2.0 + \frac{40}{1 + \exp \left( \frac{(V_M + 20)^2}{60} \right)} \]

\[ \frac{dO_{K_{2P}}}{dt} = \frac{K_{2P,\infty} - O_{K_{2P}}}{\tau_{K_{2P}}} \]

For the cAF model:

\[ I_{K_{2P}} = G_{K_{2P}} \times O_{K_{2P}} \times (V_M - E_K) \]

\[ G_{K_{2P}} = 0.0145 \text{ nS/pF} \]

\[ E_K = \frac{R \times T}{P} \times \log \left( \frac{[K^+]_2}{[K^+]_1} \right) \]

\[ K_{2P,\infty} = 0.2 + \frac{0.6}{1 + \exp \left( -\frac{V_M + 5}{14} \right)} \]

\[ \tau_{K_{2P}} = 2.0 + \frac{40}{1 + \exp \left( \frac{(V_M + 20)^2}{60} \right)} \]

\[ \frac{dO_{K_{2P}}}{dt} = \frac{K_{2P,\infty} - O_{K_{2P}}}{\tau_{K_{2P}}} \]
The model was adjusted by scaling a number of selected currents to reproduce experimentally observed APD at 50% and 90% repolarization (APD\text{50}, APD\text{90}) under the given experimental conditions (extracellular Na\textsuperscript{+}, K\textsuperscript{+}, and Ca\textsuperscript{2+} concentrations, and intracellular Na\textsuperscript{+} and K\textsuperscript{+} levels). Changes compared to the values in the original model\textsuperscript{11} are shown in Supplemental Table II below.
Supplemental Results

Effects of A293 on Human Atrial K⁺ Channels Expressed in Xenopus Oocytes

A293 specificity was assessed among all functional K₂P channels using the Xenopus laevis oocyte expression system. In addition, human atrial K⁺ channels Kv1.5, Kv4.3, hERG (Kv11.1), KCNQ1 (Kv7.1)/KCNE1, Kir2.1, Kir2.2, and Kir2.3 were studied. K₂P1.1 subunits were previously revealed to produce functional channels when a lysine residue at position 274 is replaced by glutamine.³,¹² Thus, we expressed K₂P1.1 K274Q cRNA to achieve significant current levels. Electrophysiological effects of 1 µM A293 (30 min application) on potassium channels are summarized in Supplemental Figure IVA. A293 significantly inhibited human K₂P3.1 channels (-69.3 ± 1.1%; n=14; P<0.0001). The IC₅₀ value for blockade of K₂P3.1 channels by A293 yielded 245.2±113.5 nM (Hill coefficient n_H=0.8±0.17; n=4–14 cells; Supplemental Figure IVB). These data are consistent with previously published work, reporting an IC₅₀ value of 222 nM.¹³ In addition, closely related K₂P9.1 channels were blocked as well (-60.5 ± 2.4%; n=12; P<0.0001). However, these channels exhibited negligible cardiac expression (Figure 1), ruling out any relevant electrophysiologic actions of K₂P9.1 inhibition in human atrial tissue. Human K₂P5.1 K⁺ channels were activated (+27.6±6.3%; n=3; P=0.011). Among the remaining cardiac K⁺ channels investigated here, A293 did not significantly affect current amplitudes or caused current modulation by less than ±10%, respectively. Taken together, 1 µM A293 induced pronounced inhibition of K₂P3.1 currents that was specific among cardiac K⁺ channels tested.

K₂P3.1 Protein Expression in Human Atrial Tissue
Immunoblotting was performed to investigate K$_{2p}$3.1 upregulation in cAF patients at protein level. Right atrial tissue obtained from patients exhibiting cAF (n=4) was studied in comparison with pAF patients (n=4) and SR subjects (n=4). Analyses were performed in triplicate using three different anti-K$_{2p}$3.1 antibodies (Supplemental Figure IIA, Alomone, APC-024; Supplemental Figure IIB, Chemicon, AB5250; Supplemental Figure IIC, Biomol, WA-AG1140). This approach yielded consistent findings between the antibodies used. Protein detected at 50-55 kDa was increased in cAF patients compared to SR subjects by $64.0\pm17.7\%$ ($P=0.025; n=4; \text{Alomone}$), $94.6\pm24.1\%$ ($P=0.036; n=4; \text{Chemicon}$), and by $325\pm106\%$ ($P=0.008; n=4; \text{Biomol}$), respectively. There was a tendency towards increased K$_{2p}$3.1 signal in pAF patients that did not reach statistical significance (with the exception of antibodies obtained from Alomone).

**Immunodetection of K$_{2p}$3.1 Protein in Mice**

Specific immunodetection of K$_{2p}$3.1 protein by anti-K$_{2p}$3.1 antibodies has been indicated previously, e.g. by demonstrating lack of immunoreactivity after application of K$_{2p}$3.1 antigen peptide, following K$_{2p}$3.1 protein knockdown, or in controls cells not expressing K$_{2p}$3.1. In addition, antibody specificity was suggested by comparison of native K$_{2p}$3.1 with epitope-tagged K$_{2p}$3.1 protein. Please see Supplemental Table III for detailed summary. However, it is important to note that commercially available antibodies may not specifically recognize murine K$_{2p}$3.1, as protein was previously detected in both $Kcnk3^{+/+}$ and in $Kcnk3^{-/-}$ animals.$^2$ We confirmed non-specific immunodetection of cardiac protein using multiple antibodies in whole hearts of $Kcnk3^{-/-}$ and $Kcnk3^{+/+}$ mice (Supplemental Table III, Supplemental Figure IIIB). Whether the apparent lack of specificity in knockout mice applies to cardiac protein in
large mammals and humans cannot be definitively determined. In summary, we suggest that careful and critical evaluation of Western blot data is warranted despite the widespread and in some cases scientifically validated prior use of commercially available anti-K_{2P}3.1 antibodies (Supplemental Table III). In particular, we cannot fully exclude recognition of proteins other than K_{2P}3.1 by antibodies in humans as well.
**Supplemental Tables**

**Supplemental Table I.** Primers used for real-time quantitative polymerase chain reactions

<table>
<thead>
<tr>
<th>Human target gene</th>
<th>Human target protein</th>
<th>Primer accession number (TaqMan assay)</th>
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<td>K_{2P}12.1/THIK-2</td>
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<td>NCX</td>
<td></td>
</tr>
<tr>
<td>ABCC8</td>
<td>SUR1</td>
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</tr>
</tbody>
</table>

ANP, atrial natriuretic peptide; Cx40, connexin 40; Cx43, connexin 43; hERG, human ether-a-go-go-related gene; KChIP, potassium channel-interacting protein; minK, minimal K⁺ channel; MiRP, minK-related peptide; NCX, sodium-calcium exchanger; SUR, sulfonylurea receptor; TALK, TWIK-related alkaline pH activated K⁺ channel; TASK, TWIK-related acid-sensitive K⁺ channel; THIK, tandem pore domain halothane-inhibited K⁺ channel; TRAAK, TWIK-related arachidonic acid-stimulated K⁺ channel; TREK, TWIK-related K⁺ channel; TRESK, TWIK-related spinal cord K⁺ channel; TWIK, tandem of P domains in a weak inward rectifying K⁺ channel.
**Supplemental Table II.** Changes in ion currents for the SR and cAF models.

<table>
<thead>
<tr>
<th>Current</th>
<th>Change in SR model</th>
<th>Change in cAF model</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{\text{Ca,L}}$</td>
<td>$\text{Amplitude: -35%}$</td>
<td>$\text{Amplitude: -35%}$</td>
</tr>
<tr>
<td>$I_{K1}$</td>
<td>+45%</td>
<td>+70%</td>
</tr>
<tr>
<td>$I_{\text{Kur}}$</td>
<td>0%</td>
<td>+20%</td>
</tr>
<tr>
<td>$I_{\text{NaK}}$</td>
<td>+25%</td>
<td>+25%</td>
</tr>
<tr>
<td>$I_{fo}$</td>
<td>-60%</td>
<td>-60%</td>
</tr>
</tbody>
</table>

The model was paced to steady state at pacing frequencies between 0.2 and 3.3 Hz and blockade of $K_{2P}$ channels was simulated by setting $I_{K2P}$ to zero.
**Supplemental Table III. Commercially available anti-K_{2P}3.1 / TASK-1 antibodies**

<table>
<thead>
<tr>
<th>Distributor (catalog number)</th>
<th>Type and epitope (according to the distributor)</th>
<th>Previous reports of specific K_{2P}3.1 detection</th>
<th>Prior data indicating limited specificity in kcnk3^{+/-} mice</th>
<th>Data obtained with kcnk3^{+/-} mice in present work*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abcam (ab49433)</td>
<td>Rabbit polyclonal; 325IPMIIPRDLS TSDTCVEQSH SSPGGGGGRYS DTPSRRLCLCS GAPRSAISSV^{374} (C terminus of human K_{2P}3.1)</td>
<td>Not available</td>
<td>Not available</td>
<td>Similar protein detection in wild type and kcnk3^{+/-} mouse heart</td>
</tr>
<tr>
<td>Abcam (ab83725)</td>
<td>Rabbit polyclonal; undisclosed peptide from the C terminal region of human K_{2P}3.1</td>
<td>Not available</td>
<td>Not available</td>
<td>Similar protein detection in wild type and kcnk3^{+/-} mouse heart</td>
</tr>
<tr>
<td>Abcam (ab135883)</td>
<td>Rabbit polyclonal; undisclosed peptide from the C terminal region of human K&lt;sub&gt;2P&lt;/sub&gt;3.1</td>
<td>Not available</td>
<td>Not available</td>
<td>Not investigated</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>--------------</td>
<td>-------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Abcam (ab186352)</td>
<td>Mouse monoclonal; 251\text{AEDEKRDAEH RALLTHNGQA GGLGGLSCLS GSLGDGVRPR DPVTC\text{aaaaAG GMGVGVG\text{vGG SGFRN\text{VYAE GMGVGVG\text{vGG SGFRN\text{VYAE LHFQSMCSCL WYKSREKLQY SIPMIIPRDL}}}}</td>
<td>Not available</td>
<td>Not available</td>
<td>Please see Biomol / Stressmarq (SMC-473D)</td>
</tr>
<tr>
<td>Source</td>
<td>Description</td>
<td></td>
<td></td>
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<tr>
<td>-----------------------------</td>
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<td></td>
</tr>
<tr>
<td>Alomone Labs (APC-024)</td>
<td>Rabbit polyclonal; 252EDEKRDAEHR ALLTRNGQ269 (C terminus of human K₂P3.1)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat neurons; 14 rat brain, rat spinal cord; 15 mouse heart; 16 H146 lung cells; 17 rat heart, rat brain; 18 CHO cells, rat heart; 19 lamb vascular smooth muscle cells; 20 rabbit vascular smooth muscle cells; 21 HEK293 cells, human lymphocytes; 22 rat brain, rat vascular smooth muscle cells; 23 rat brain; 24 frog retina; 25 human uterus, rat brain; 26 HEK293 cells; 27 murine neurons; 28 rat neurons; 29 rat brain; 30 human vascular smooth muscle cells; 31 HEK293, rat adrenal gland; 32 HEK293, human breast cancer cells, bovine sperm, bovine oocytes, bovine blastocysts; 33 mouse neuronal cells, mouse oocyte and embryo; 34 A549 cells; 35 Similar protein detection in wild type and kcnk3⁻/⁻ mouse brain, 1 and mouse heart. 36 Similar protein detection in wild type and kcnk3⁻/⁻ mouse heart.</td>
<td></td>
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<tr>
<td>Antibodies Online (ABIN1453980)</td>
<td>Rabbit polyclonal; undisclosed peptide from the N terminal region of human K2P3.1</td>
<td>Not available</td>
<td>Not available</td>
<td>Not investigated</td>
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<tr>
<td>---------------------------------</td>
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<td>Antibodies Online (ABIN202419)</td>
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<td>Not available</td>
<td>Not investigated</td>
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<tr>
<td>Antibodies Online (ABIN719771)</td>
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<td>Not available</td>
<td>Not available</td>
<td>Not investigated</td>
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<tr>
<td>Aviva Systems Biology (OAAF05983)</td>
<td>Rabbit polyclonal; 47ELRARYNLSQ GGYEELEVV LRLKPHKAGV QWRFAGSFYF AITVITTIGY&lt;sup&gt;96&lt;/sup&gt; (N terminus of human K2P3.1)</td>
<td>Not available</td>
<td>Mouse heart&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Biomol / Abgent (WA-AG1140)</td>
<td>Rabbit polyclonal; 252EDEKRDAEHR ALLTRNGQ&lt;sup&gt;269&lt;/sup&gt; (C terminus of human K2P3.1)</td>
<td>Dog heart&lt;sup&gt;37&lt;/sup&gt;</td>
<td>Not available</td>
<td>Similar protein detection in wild type and kcnk3&lt;sup&gt;−/−&lt;/sup&gt; mouse heart</td>
</tr>
<tr>
<td>Biomol / Stressmarq (SMC-473D)</td>
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<td>251AEDEKRDAEH</td>
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<td>LHFQSMCSCL</td>
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<td>WYKSREKLOQ Y SIPMIIPRDL</td>
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<td>SDTPSHPCLE SGTQRSAISS</td>
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<td>VSTGLHSAT FRGLMKRRSS</td>
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<tr>
<td></td>
<td>V^{411} (fusion protein; C terminus</td>
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Similar protein detection in wild type and *kcnk3*^{−/−} mouse heart.
<table>
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<tr>
<th>Company</th>
<th>Antigen Description</th>
<th>Species/Location</th>
<th>Detection Information</th>
<th>Protein Detection Information</th>
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<tbody>
<tr>
<td>Chemicon / Merck Millipore</td>
<td>Rabbit polyclonal; 252EDEKRDAEHR ALLTRNGQ&lt;sup&gt;269&lt;/sup&gt;</td>
<td>Mouse heart, chicken heart&lt;sup&gt;1,38&lt;/sup&gt; rat neurons&lt;sup&gt;1,39&lt;/sup&gt; gerbil hippocampus&lt;sup&gt;40&lt;/sup&gt;</td>
<td>Similar protein detection in wild type and &lt;i&gt;kcnk3&lt;/i&gt;&lt;sup&gt;-/-&lt;/sup&gt; mouse heart&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Similar protein detection in wild type and &lt;i&gt;kcnk3&lt;/i&gt;&lt;sup&gt;-/-&lt;/sup&gt; mouse heart</td>
</tr>
<tr>
<td>Enzo Life Sciences</td>
<td>Rabbit polyclonal; 252EDEKRDAEHR ALLTRNGQ&lt;sup&gt;269&lt;/sup&gt;</td>
<td>Not available</td>
<td>Not available</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Genway Biotech (GWB-892ACE); Antibodies Online (ABIN183130)</td>
<td>Rabbit polyclonal; 338TCVEQSHSSP GGGGRYSDTPT SRRCLCSGAP RSAISSVSTGLHSLSTFRGL&lt;sup&gt;387&lt;/sup&gt; (C terminus of human K&lt;sub&gt;2P3.1&lt;/sub&gt;)</td>
<td>Not available</td>
<td>Not available</td>
<td>Not investigated</td>
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(C terminus of human K<sub>2P3.1</sub>)
of human K2P3.1)

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<th>Availability</th>
<th>Note</th>
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<td>Rat neurons, HEK293 cells</td>
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<td>undisclosed “epitope mapping within a cytoplasmic domain” of human K2P3.1</td>
<td>human lymphocytes</td>
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<td>295GFRNVYAEVL</td>
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<td>Santa Cruz Biotechnology (N-15/sc-32067)</td>
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<td>Kits/Protein Databases</td>
<td>Antibody Type</td>
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<td>Tissues</td>
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<td>Santa Cruz Biotechnology (E-13/sc-32065)</td>
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<td>Mouse carotid body; mouse neurons</td>
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<tr>
<td>Sigma-Aldrich (P0981)</td>
<td>Rabbit polyclonal;</td>
<td>(C terminus of human K2P3.1)</td>
<td>Mouse brain, human lymphocytes</td>
<td>Similar protein detection in wild type and kcnk3−/− mouse brain and mouse heart (kcnk3−/− mice)</td>
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</table>

K2P3.1 rat aorta, HEK293 cells\(^1\), human vascular smooth muscle cells,\(^3^1\) rat neurons\(^4^1\)
<table>
<thead>
<tr>
<th>Sigma-Aldrich / Atlas Antibodies</th>
<th>Rabbit polyclonal;</th>
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<tr>
<td><strong>AV35090</strong></td>
<td>$^{324}$SIPMIIPRDL STSDTCVEQS HSSPGGGGR YSDTPSRRCLE CSGAPRSAIS S$^{374}$</td>
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<tr>
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<td>(C terminus of human K$_{2P3.1}$)</td>
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<tr>
<td><strong>HPA026658</strong></td>
<td>$^{24}$AVFDALESEP ELIERQRLLELRQQELRARYNLSQGGYEELE RVVLRLKPHK AGVQ$^{78}$</td>
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<td>(N terminus of human K$_{2P3.1}$)</td>
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</table>
*Mouse heart homogenate obtained from *kcnk3*−/− mice and from control animals was subjected to Western blot analysis using indicated antibodies. Human right atrial appendage (hRAA) tissue from sinus rhythm (SR) patients (n=2) and human left ventricular (hLV) protein (n=2) was analyzed for comparison.

†Antibody specificity was indicated by lack of immunoreactivity after application of K2P3.1 antigen peptide.

‡Antibody specificity was indicated by lack of immunoreactivity after K2P3.1 protein knockdown.

§Antibody specificity was indicated by lack of immunoreactivity in control cells not expressing K2P3.1.

‖Antibody specificity was indicated by comparison of native K2P3.1 with K2P3.1 protein carrying a protein tag, probed with antibodies against the tag and K2P3.1, respectively.

# No detection of protein with predicted mass in rat brain.
Supplemental References


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Physiol Regul Integr Comp Physiol.* 2006;291:R518-R529.


Supplemental Figure Legends

Supplemental Figure I. A, Schematic overview of the Grandi et al. model of the human atrial myocyte\(^9\), our recent extension with Na\(^+\)-dependent regulation of \(I_{K1}\) and \(I_{K_{Ach}}\)^10, and the \(K_{2P3.1}\) current (\(I_{K2P}\)) formulation included in the present study. B, Simulated \(K_{2P3.1}\) current during 300 ms depolarizing pules from -60 to +60 mV from a holding potential of -50 mV in the sinus rhythm (SR) and chronic atrial fibrillation (cAF) models. Right panel shows validation of voltage dependence of \(K_{2P3.1}\) current activation in SR (black) and cAF (red) models compared to measurements in isolated human atrial cardiomyocytes (symbols).

Supplemental Figure II. Western blot analysis of \(K_{2P3.1}\) protein in human right atrium. A representative membrane is shown, corresponding to sequential immunoblots using anti-\(K_{2P3.1}\) antibodies purchased from Alomone (A, APC-024), Chemicon (B, AB5250), and Biomol (C, WA-AG1140), respectively. Protein samples were obtained from patients in sinus rhythm (SR), paroxysmal atrial fibrillation (pAF), or chronic AF (cAF). Primary and secondary antibodies were removed prior to application of subsequent antibodies. Arrows indicate location of protein with expected molecular weight (50-55 kDa). Mean (±SEM) optical density values normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression (D) of indicated patient groups are displayed (*\(P<0.05\), **\(P<0.01\) vs SR).

Supplemental Figure III. Assessment of anti-\(K_{2P3.1}\) antibody specificity. A, Immunoblot of a representative human ventricular sample in comparison with two right atrial samples, probed with anti-\(K_{2P3.1}\) antibodies. B, Immunoreactivity of anti-
K_{2P}3.1 antibodies in whole murine heart. Protein was similarly recognized by antibodies purchased from Chemicon (AB5250) in samples obtained from representative \textit{Kcnk3}^{+/+} and \textit{Kcnk3}^{-/-} mice, suggesting non-specific binding to murine cardiac protein.

Supplemental Figure IV. Electropharmacologic characterization of the drug A293 in \textit{Xenopus} oocytes. \textbf{A}, Representative macroscopic currents recorded under control conditions and after application of 1 \textmu M A293 (30 min) are displayed for all functional human K_{2P} channels and for atrial K^+ channels hK_{v}1.5, hK_{v}4.3, hERG (human ether-a-go-go-related gene, K_{v}11.1), KCNQ1/KCNE1, hK_{ir}2.1, hK_{ir}2.2., and hK_{ir}2.3, respectively. Currents were elicited using indicated voltage protocols. \textbf{B}, Concentration-response relationships for the effect of A293 on hK_{2P}3.1 currents were measured at +20 mV membrane potential (n=4-14 cells; mean±SEM). The IC_{50} value yielded 245 nM. Insert, skeletal formula of A293.

Supplemental Figure V. K_{2P}3.1 current characteristics and resting membrane potentials (RMP) in sinus rhythm (SR), paroxysmal atrial fibrillation (pAF) and chronic AF (cAF). \textbf{A}, \textbf{B}, Current-voltage relationships of mean A293-sensitive currents (absolute values) obtained in Figure 4B–4D are shown in comparison to SR for patients with pAF (\textbf{A}) and cAF (\textbf{B}). \textbf{C}, Mean A293-sensitive currents prior to normalization to cell capacitance, quantified at +40 mV membrane potential. \textbf{D}, Mean capacitance values of cells analyzed in panels A-C. \textbf{E}, Resting membrane potentials of atrial myocytes recorded before (Control) and after application of 200 nM A293.
Data are expressed as mean±SEM; n/N, number of myocytes/patients; *$P<0.05$ vs SR.
Figure II

A Human RAA K3.1 immunoblot

B Human RAA K3.1 immunoblot

C Human RAA K3.1 immunoblot

D GAPDH loading control
Figure III

A  
K<sub>3.1</sub> immunoblot

B  
K<sub>3.1</sub> immunoblot

Whole heart

Ventricular sample (human)

Right atrial sample (human)

Right atrial sample (human)

Whole heart

Ventricular sample (human)

Right atrial sample (human)

GAPDH

K<sub>3.1</sub>  
55 kDa  
40 kDa  
5 kDa  
130 kDa  
50 kDa  
80 kDa  
40 kDa  
35 kDa

K<sub>3.1</sub>  
35 kDa

GAPDH
Figure V

A Absolute A293 sensitive current [pA]

B Absolute A293 sensitive current [pA]

C Absolute A293 sensitive current (SR, pAF, cAF)

D Membrane capacity [pF]

E Membrane potential [mV]