Biochemical and Biophysical Research Communications 451 (2014) 415–420

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Vernakalant activates human cardiac $K_{2P}17.1$ background K^+ channels

Claudia Seyler, Patrick A. Schweizer, Edgar Zitron, Hugo A. Katus, Dierk Thomas *

Department of Cardiology, Medical University Hospital, Heidelberg, Im Neuenheimer Feld 410, D-69120 Heidelberg, Germany

ARTICLE INFO

Article history:
Received 28 July 2014
Available online 7 August 2014

Keywords:
Antiarrhythmic drug
Atrial fibrillation
Flecainide
K_{2P} channel
Personalized medicine
Vernakalant

ABSTRACT

Atrial fibrillation (AF) contributes significantly to cardiovascular morbidity and mortality. The growing epidemic is associated with cardiac repolarization abnormalities and requires the development of more effective antiarrhythmic strategies. Two-pore-domain K^+ channels stabilize the resting membrane potential and repolarize action potentials. Recently discovered $K_{2P}17.1$ channels are expressed in human atrium and represent potential targets for AF therapy. However, cardiac electropharmacology of $K_{2P}17.1$ channels remains to be investigated. This study was designed to elucidate human $K_{2P}17.1$ regulation by antiarrhythmic drugs.

Two-electrode voltage clamp and whole-cell patch clamp electrophysiology was used to record K_{2P} currents from *Xenopus* oocytes and Chinese hamster ovary (CHO) cells. The class III antiarrhythmic compound vernakalant activated $K_{2P}17.1$ currents in oocytes an in mammalian cells ($EC_{50,CHO}$ = 40 μM) in frequency-dependent manner. $K_{2P}17.1$ channel activation by vernakalant was specific among K_{2P} channel family members. By contrast, vernakalant reduced $K_{2P}4.1$ and $K_{2P}10.1$ currents, in line with $K_{2P}2.1$ blockade reported earlier. $K_{2P}17.1$ open rectification characteristics and current–voltage relationships were not affected by vernakalant. The class I drug flecainide did not significantly modulate K_{2P} currents.

In conclusion, vernakalant activates $K_{2P}17.1$ background potassium channels. Pharmacologic K_{2P} channel activation by cardiovascular drugs has not been reported previously and may be employed for personalized rhythm control in patients with AF-associated reduction of K^+ channel function.

1. Introduction

Effective and safe pharmacological management of atrial fibrillation (AF) constitutes a major challenge in cardiovascular medicine [1]. Two-pore-domain potassium (K_{2P}) channels stabilize the resting membrane potential and facilitate action potential repolarization in excitable cells [2,3]. In the heart, the currents contribute to the cardiac background K^+ conductance [4]. Multimodal K_{2P} channel regulation is a dynamic mechanism for control of cellular excitability [5–13]. Inhibition of cardiac K_{2P} channels has been proposed as novel concept in antiarrhythmic therapy [3,14], with blockade of $K_{2P}2.1$ and $K_{2P}3.1$ K^+ currents by antiarrhythmic drugs serving as proof of this emerging principle [15–21]. Specifically, $K_{2P}3.1$ current reduction results in prolonged action potential duration (APD) [22,23] and may be applied for rhythm control in a subgroup of AF patients mechanistically characterized by atrial action potential shortening. However, other AF subgroups are associated with normal or prolonged atrial refractoriness and require different, individualized therapeutic approaches [24,25]. In particular, $K_{2P}3.1$ loss-of-function mutations have recently been associated with human AF [26]. Furthermore, downregulation of $K_{2P}2.1$ and $K_{2P}3.1$ channels [20,27] was observed in a porcine model of AF and heart failure [28]. In those cases, compensatory activation of repolarizing atrial K_{2P} channels could serve as novel concept for personalized rhythm control.

$K_{2P}17.1$ (tandem of P domains in a weak inward rectifying K^+ channel (TWIK)-related acid sensitive K^+ channel (TASK)-4 or TWIK-related alkaline pH-activated K^+ channel (TALK)-2) K^+ channels are predominantly expressed in human atrium [29–31] and represent potential "atrial-selective" drug targets. Genetic enhancement of $K_{2P}17.1$ currents resulted in action potential shortening in murine atrial myocytes [31]. A mechanistic contribution of $K_{2P}17.1$ to AF is indicated by the association between ischemic stroke and a genetic $K_{2P}17.1$ variant in Caucasians [32].

We sought to identify modulators of $K_{2P}17.1$ currents by screening clinically used antiarrhythmic compounds flecainide and vernakalant (RSD1235). Vernakalant is effective in converting AF to

Abbreviations: AF, atrial fibrillation; APD, action potential duration; CHO, Chinese hamster ovary; K_{2P}, two-pore-domain K^+ channel; SR, sinus rhythm; TALK, TWIK-related alkaline pH-activated K^+ channel 2; 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.

* Corresponding author. Fax: +49 6221 565514.
E-mail address: dierk.thomas@med.uni-heidelberg.de (D. Thomas).

http://dx.doi.org/10.1016/j.bbrc.2014.07.133
0006-291X/

SR [33]. Rate-dependent inhibition of sodium currents and additional blockade of $I_{K,ACh}$ and early I_{to} constitute its main mechanism of action in human atrial myocytes [34]. Flecainide achieves rhythm control in AF primarily by inhibiting cardiac I_{Na}, I_{to}, and Kv11.1 channels [35–38]. The present study reveals that vernakalant activates $K_{2P}17.1$ currents significantly and specifically among K_{2P} channels, whereas flecainide does not affect K_{2P} function.

2. Materials and methods

2.1. Molecular biology

Complementary DNAs encoding human $K_{2P}1.1$ K274Q [39] and $hK_{2P}9.1$ (GenBank accession number NM_016601) were kindly provided by Steve Goldstein (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 [5]: $hK_{2P}4.1$ (EU978935), $hK_{2P}5.1$ (EU978936), $hK_{2P}6.1$ (EU978937), $hK_{2P}10.1$ (EU978939), $hK_{2P}13.1$ (EU978942), $hK_{2P}16.1$ (EU978943), and $hK_{2P}17.1$ (EU978944). *In vitro* transcription was performed as published [7]. Complementary RNAs were transcribed after vector linearization using T7 RNA polymerase and the mMessage mMachine kit (Ambion, Austin, TX, USA). RNA transcripts were quantified by spectrophotometry and cRNA integrity was assessed by agarose gel electrophoresis.

2.2. Oocyte preparation and injection

The investigation conforms to the Directive 2010/63/EU of the European Parliament. Approval was granted by the local Animal Welfare Committee (reference number A-38/11). Oocyte preparation has been carried out in accordance with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 1996) as reported earlier in detail [5,9,13]. Complementary RNA (0.5–1.5 ng; 46 nl/oocyte) was injected into defolliculated *Xenopus laevis* oocytes.

2.3. Cell culture

Chinese hamster ovary (CHO) cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal bovine serum (Invitrogen), 100 U/ml penicillin G sodium, and 100 μg/ml streptomycin sulphate in an atmosphere of 95% humidified air and 5% CO_2 at 37 °C. Cells were passaged regularly and subcultured before treatment. Transient transfections of CHO cells were performed using Viromer YELLOW transfection reagent (Lipocalyx, Halle, Germany) according to the manufacturer's instructions.

2.4. Electrophysiology

Macroscopic K_{2P} currents were recorded from *Xenopus* oocytes using two-electrode voltage clamp electrophysiology with an Oocyte Clamp amplifier (Warner Instruments, Hamden, CT, USA) and pCLAMP9 (Axon Instruments, Foster City, CA, USA) software 2–3 days after injection at room temperature (20–22 °C), as described [7,13]. Whole-cell patch clamp recordings from HEK 293 cells were carried out using a RK-400 amplifier (Bio-Logic SAS, Claix, France) as reported [13,21,18].

2.5. Drug administration

Vernakalant (Brinavess®) solution was commercially obtained from Merck Sharp & Dohme (Haar, Germany) and stored in 1 ml aliquots (51.8 mM) at −20 °C. Flecainide (Sigma–Aldrich, St. Louis, MO, USA) was prepared as 100 mM stock solution in dimethyl sulfoxide (DMSO) and stored at −20 °C. On the day of experiments, aliquots of the stock solutions were diluted to the desired concentrations with the bath solution.

2.6. Data analysis and statistics

Origin (OriginLab, Northampton, MA, USA) software was used for data analysis. Data are expressed as mean ± SEM. Concentration–response relationships for drug-induced block were fit with a Hill equation of the following form: $I_{drug}/I_{control} = 1/[1 + (D/EC_{50})^n]$, where I indicates current, D is the drug concentration, n is the Hill coefficient, and EC_{50} is the concentration necessary for 50% maximum activation. We applied paired or unpaired Student's t tests (two-tailed tests) to compare statistical significance of the results where appropriate. Multiple comparisons were performed using one-way ANOVA. If the hypothesis of equal means could be rejected at the 0.05-level, pair wise comparisons of groups were made and the probability values were adjusted for multiple comparisons using the Bonferroni correction. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Activation of human $K_{2P}17.1$ potassium channels expressed in Xenopus oocytes by vernakalant

Vernakalant sensitivity of human $K_{2P}17.1$ channels was assessed in *X. laevis* oocytes. From a holding potential of −80 mV, currents were evoked by hyperpolarizing and depolarizing pulses (500 ms) to voltages between −140 and +60 mV in 20 mV increments. Current amplitudes were measured at the end of the +20 mV-pulse. This protocol was used in all oocyte experiments in this study. Vernakalant (100 μM; 30 min) significantly activated $K_{2P}17.1$ currents by 70.0 ± 8.8% ($n = 28$; $p < 0.001$) (Fig. 1). To assess specificity of vernakalant-induced K_{2P} current activation, functional human K_{2P} channels were studied comprehensively (Fig. 1). Human $K_{2P}1.1$ subunits were previously revealed to produce functional channels when a lysine residue at position 274 is replaced by glutamine [39]. Thus, we expressed $K_{2P}1.1$ K274Q cRNA to achieve significant current levels. Electrophysiological effects of 100 μM vernakalant on K_{2P} family members are summarized in Fig. 1. Vernakalant significantly inhibited human K_{2P} channels $K_{2P}4.1$ (TRAAK; −17.1 ± 7.6%; $n = 10$; $p = 0.001$) and $K_{2P}10.1$ (TREK-2; −19.8 ± 1.3%; $n = 18$; $p < 0.001$) in addition to previously reported block of $K_{2P}2.1$ currents by 21% [21].

3.2. Flecainide does not significantly modulate human K_{2P} channels

Electrophysiological properties of the class IC antiarrhythmic drug flecainide on $K_{2P}17.1$ currents and on remaining K_{2P} subunits were assessed in *Xenopus* oocytes under similar experimental conditions. 100 μM flecainide (30 min) did not exert significant inhibitory or activating effects on any K_{2P} channel studied here (Fig. 2) or in prior work ($K_{2P}2.1$, $K_{2P}3.1$ [21]).

3.3. Biophysical characteristics of $K_{2P}17.1$ current activation by vernakalant

Drug effects on $K_{2P}17.1$ current voltage (I–V) relationships were investigated under isochronal recording conditions. Currents were recorded as described in Fig. 1. Representative families of $K_{2P}17.1$ current traces are shown for control conditions and after application of 100 μM vernakalant for 30 min (Fig. 3A and B). The current–voltage relationship was not affected by 100 μM vernakalant (Fig. 3C and D). The time course of effect is shown in Fig. 3E, illustrating rapid

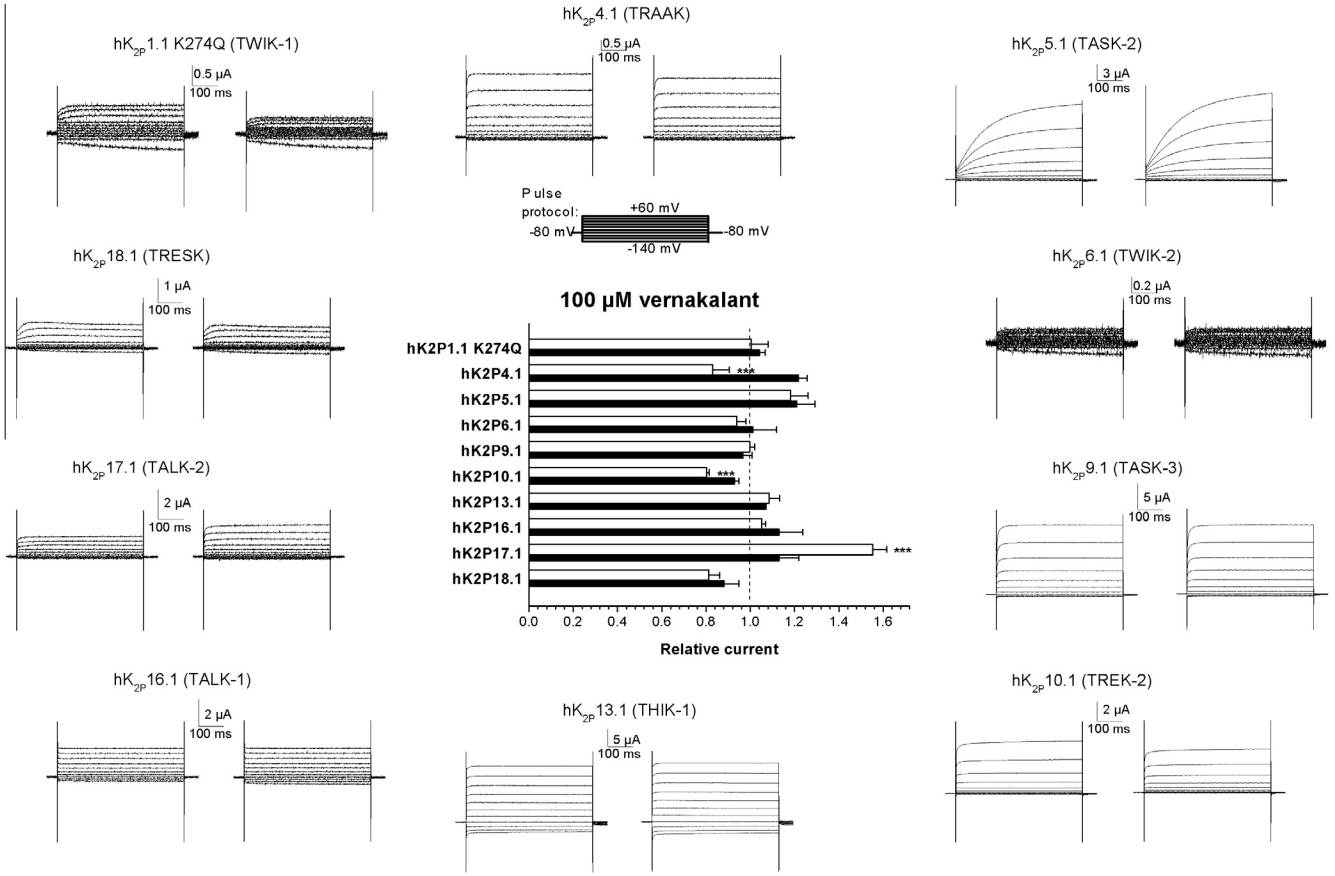

Fig. 1. Effects of vernakalant on human K_{2P} potassium channels expressed in *Xenopus* oocytes. Representative original current traces recorded under control conditions and after application of 100 µM vernakalant (30 min) are displayed for indicated channels. Center, mean current levels quantified at +20 mV membrane voltage. Note significant K_{2P}17.1 channel activation by vernakalant, whereas current reduction was observed with K_{2P}4.1 and K_{2P}10.1 (n = 6–15 cells). Administration of the solvent, H_2O, for 30 min did not significantly modulate K_{2P} channels (filled columns; n = 5–13 cells). Data are given as mean ± SEM; ***$p < 0.001$ *versus* control measurements.

onset of current enhancement. Linear ramp voltage protocols were applied between −140 and +60 mV (500 ms) before and after application of 100 µM vernakalant (30 min) to assess the effects of vernakalant on current rectification (Fig. 3F). K_{2P}17.1 currents exhibited outward (or open) rectification characteristic to a potassium-selective background leak conductance before and after drug application. The degree of activation at +60-mV ramp potential was +91.5 ± 20.9% (n = 5; p = 0.028). Activation of K_{2P}17.1 background K^+ currents by vernakalant (100 µM; 30 min) was associated with changes in resting membrane potential (RMP; Fig. 3G). Vernakalant hyperpolarized the RMP of oocytes expressing K_{2P}17.1 channels by −9.3 ± 0.7 mV to −42.3 ± 3.4 mV compared to control recordings ($RMP_{control}$ = −33.0 ± 2.7 mV; n = 12; p = 0.002). To study frequency-dependence of channel activation, human K_{2P}17.1 channels were rapidly activated by a depolarizing step to +20 mV (500 ms) at intervals of 1, 2 or 5 s, respectively, for 2 min after application of 100 µM vernakalant (30 min). Current increase was plotted relative to respective control current amplitudes (Fig. 3H), revealing that activation of K_{2P}17.1 channels was frequency-dependent with stronger activation at higher rates. The extent of current increase after 30 min was significantly different ($p < 0.001$) between all stimulation rates (0.2 Hz, +159 ± 2.7%, n = 8; 0.5 Hz, +175 ± 3.3%, n = 8; 1 Hz, +202 ± 1.6%, n = 8).

3.4. Characterization of K_{2P}17.1 activation by vernakalant in mammalian cells

To further elucidate vernakalant-induced activation of K_{2P}17.1 currents in a mammalian cell environment, the channels were expressed in Chinese hamster ovary (CHO) cells (Fig. 4). From a holding potential of −80 mV, pulses were applied for 500 ms to voltages between −120 and +80 mV in 20 mV increments (0.2 Hz). Following current equilibration, the degree of activation was determined at +40 mV after reaching steady-state conditions (10 min). Representative families of K_{2P}17.1 current traces are shown for control conditions and after application of 100 µM vernakalant for 30 min (Fig. 4A and B). The onset of activation is displayed in Fig. 4C (n = 12). K_{2P}17.1 currents increased rapidly upon administration of 100 µM vernakalant to 59.1 ± 11.6% (p = 0.001). Vernakalant effects on K_{2P}17.1 current–voltage (I–V) relationships were investigated by evoking families of current traces as described, revealing that the I–V relationship was not affected by 100 µM vernakalant (Fig. 4D and E). The half-maximal effective concentration (EC_{50}) for K_{2P}17.1 channels was 39.9 ± 2.2 µM (Fig. 4F) with a Hill coefficient n_H of 2.6 ± 0.46 (n = 6–12 cells were studied at each concentration). The solvent (H_2O) did not affect K_{2P}17.1 current levels in recorded from CHO cells under similar conditions ($I_{13\ min}/I_{control}$=1.03 ± 0.07; n = 9; p = 0.86).

4. Discussion

4.1. Vernakalant, a novel activator of K_{2P}17.1 background K^+ currents

This work identified vernakalant, a clinically used class III antiarrhythmic drug, as activator of human K_{2P}17.1 potassium channels. Enhancement of K_{2P} currents by cardiovascular drugs is novel, and vernakalant represents the first example of K_{2P}17.1 activation as electropharmacological mechanism of action. By contrast,

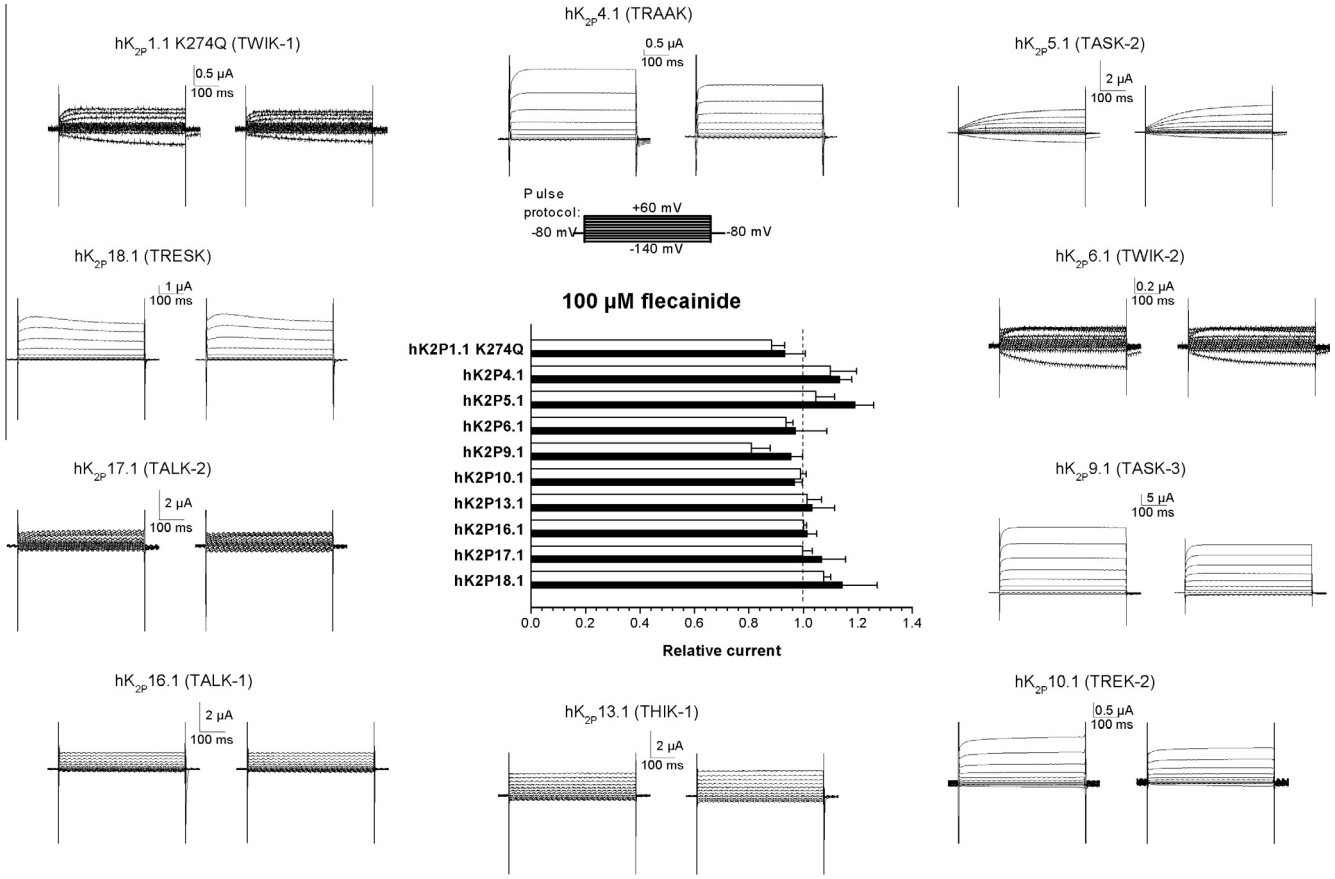

Fig. 2. Flecainide does not target K_{2P} potassium channels. Representative macroscopic currents recorded from *Xenopus* oocytes under control conditions and after application of 100 µM flecainide (30 min) are displayed for indicated channels. Center, mean currents quantified at +20 mV. No significant K_{2P} current modulation was observed (n = 7–12 cells). In addition, K_{2P} channels were not significantly affected by the solvent, DMSO (0.1%, 30 min) (filled columns; n = 7–9 cells). Data are given as mean ± SEM.

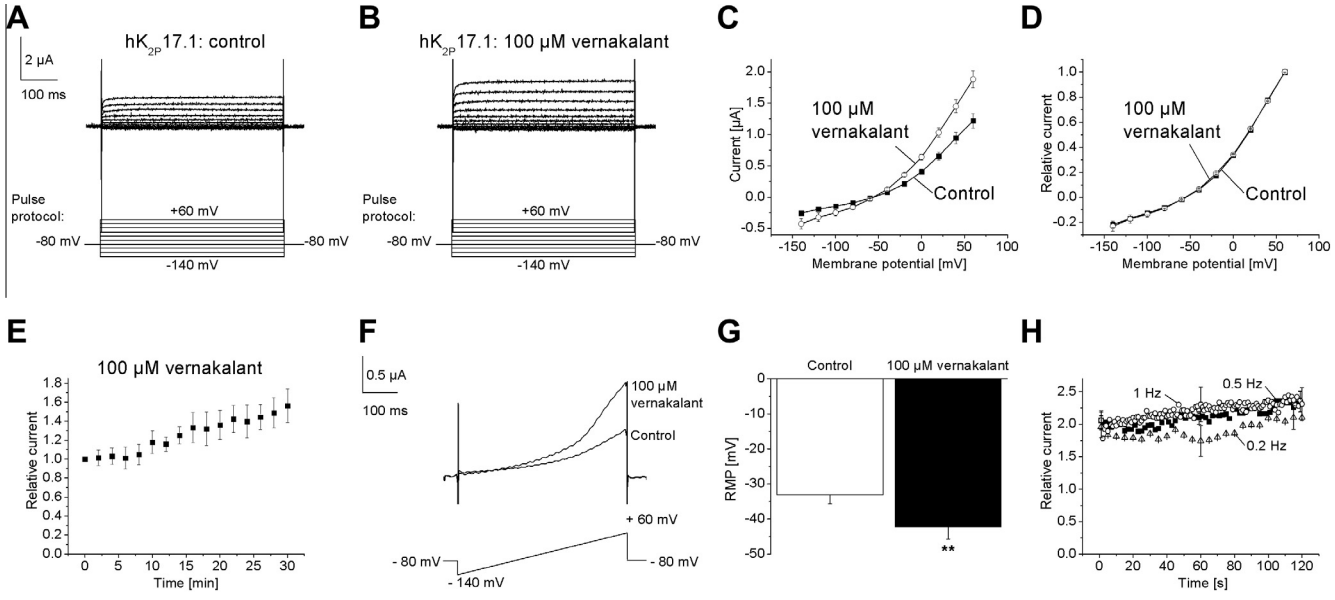

Fig. 3. Biophysical effects of vernakalant on K_{2P}17.1 currents in *Xenopus* oocytes. (A, B) Currents recorded under control conditions (A) and following administration of 100 µM vernakalant (30 min; B). (C, D) *I–V* relationships in the absence and presence of 100 µM vernakalant (C, original current amplitudes; D, values normalized to maximum currents) (n = 28). (E) Time course of current increase (n = 7). (F) Open rectification of K_{2P}17.1 currents elicited by voltage ramps from −140 to +60 mV. Typical recordings from the same cell in the absence of the drug and after superfusion with 100 µM vernakalant (30 min) are superimposed. (G) Mean resting membrane potentials (RMP) of *Xenopus* oocytes, obtained before and after activation of K_{2P}17.1 (n = 12) with 100 µM vernakalant ($^{**}p < 0.01$). (H) Vernakalant activation of K_{2P}17.1 is frequency-dependent. Mean relative K_{2P}17.1 current amplitudes recorded at +20 mV membrane potential (0.2 Hz, 0.5 Hz, and 1 Hz stimulation rates) are plotted *versus* time (n = 8 oocytes were studied at each rate; note that, for the purpose of clear presentation, not all measurements are displayed). Data are provided as mean ± SEM.

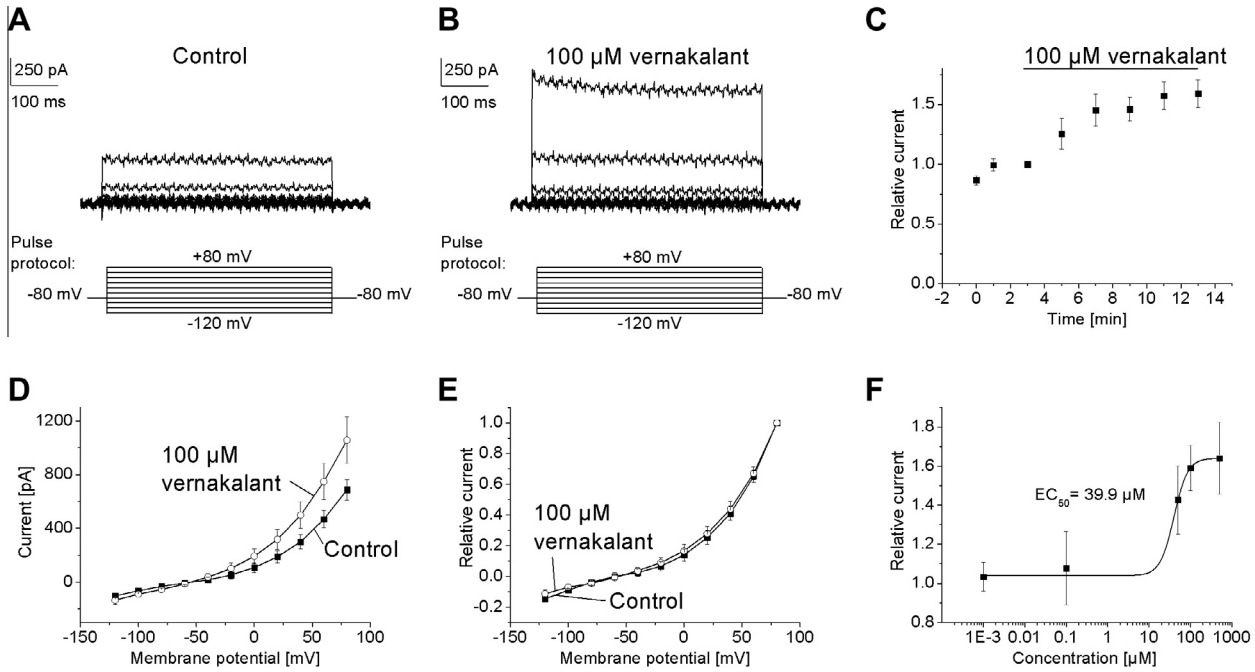

Fig. 4. Pharmacological and biophysical effects of vernakalant on $K_{2P}17.1$ currents in mammalian cells. (A, B) Typical macroscopic currents recorded under control conditions (A) and after application of 100 µM vernakalant (10 min; B). (C) Time course of $K_{2P}17.1$ current activation by 100 µM vernakalant (n = 12). (D, E) Current–voltage relationships (D, original current amplitudes; E, values normalized to maximum currents) in the absence and presence of vernakalant (30 min). (F) Concentration–response relationships for the activating effect of vernakalant on $K_{2P}17.1$ outward currents quantified at +40 mV (n = 6–12 cells). Mean ± SEM data are provided.

vernakalant inhibited $K_{2P}4.1$ and $K_{2P}10.1$ channels, which is in line with blockade of $K_{2P}2.1$ currents that has been reported previously [21]. Of note, the present and a prior study [21] revealed that no functional K_{2P} channel was sensitive to the class I antiarrhythmic compound flecainide. Thus, modulation of K_{2P} channels represents a specific property of vernakalant that distinguishes it from flecainide.

4.2. The biophysical mechanism of $K_{2P}17.1$ channel activation

Biophysical characteristics of $K_{2P}17.1$ activation by vernakalant were studied in more detail. The rapid onset of activation indicates a direct drug–channel interaction and argues against decreased protein turnover or impaired protein degradation as molecular mechanism of action. Frequency-dependence with more pronounced current enhancement at higher rates further reflects direct vernakalant interaction with the channel protein. The drug did not affect open rectification, a biophysical property characteristic to K_{2P} channel function in physiological ionic conditions. Similarly, $K_{2P}17.1$ current–voltage relationships were not altered.

4.3. Pharmacological relevance of $K_{2P}17.1$ during vernakalant therapy

Vernakalant activated $K_{2P}17.1$ channels with an EC_{50} value of 39.9 µM. In AF patients treated with the drug, plasma concentrations ranged from 5 to 20 µM [40]. Vernakalant is bound to plasma proteins by 60%, reducing effective drug concentrations to 2–8 µM. The 5–20-fold difference between EC_{50} and free plasma levels indicates low clinical significance of $K_{2P}17.1$ activation during clinical application of vernakalant with current dosing recommendations. However, impaired vernakalant metabolism may result in significantly higher plasma levels and in modulation of $K_{2P}17.1$ channel function during routine administration of the drug.

4.4. Clinical significance of $K_{2P}17.1$ activation in AF

Reduced electrical conduction velocity and shortening of atrial effective refractory periods are accepted mechanisms that perpetuate AF through promotion of electrical reentry [1,41,42]. However, a more detailed elucidation of molecular mechanisms underlying AF revealed extremely high complexity of the rhythm disorder and pointed towards the existence of multiple subgroups of AF patients [43]. Indeed, several lines of evidence suggest that shortened atrial refractoriness is not uniformly present in all AF patients, and normal or even prolonged repolarization and reduced functional K^+ channel expression may be expected in distinct patient subgroups. The association of $K_{2P}3.1$ loss-of-function mutations and downregulation of $K_{2P}2.1$ and $K_{2P}3.1$ channels with AF [20,26,27] indicates that both inappropriate increase and decrease of potassium currents may destabilize cardiac repolarization and promote AF. A similar proarrhythmic paradigm is already established at the ventricular level for long and short QT syndromes. We propose that the molecular characterization of AF subgroups with respect to K_{2P} remodeling will lead to the identification of patients that benefit from activation (as opposed to class III-antiarrhythmic inhibition) of repolarizing K_{2P} channels. Vernakalant may serve as starting point for the development of $K_{2P}17.1$ activating compounds for personalized antiarrhythmic therapy of subjects with reduced overall (K_{2P}) potassium currents and prolonged refractoriness. Furthermore, drugs acting on targets that are predominantly expressed in human atria such as $K_{2P}17.1$ channels [29–31] are expected to be particularly safe in AF therapy, as limiting the electropharmacological action to atrial tissue reduces the risk of proarrhythmic effects in the ventricles [14].

5. Conclusion

$K_{2P}17.1$ current increase by vernakalant represents a previously unrecognized mode of electropharmacological drug action. K_{2P} channel activation may be useful for personalized rhythm control

in subgroups of AF patients characterized by reduced atrial K^+ current levels.

Conflict of interest

Previous work by DT was supported by a grant from MSD Sharp and Dohme.

Acknowledgments

We thank Jennifer Gütermann, Christine Jeckel, Kai Sona and Bianca Stadler for excellent technical assistance. The present study was supported by grants from the Joachim Siebeneicher Foundation (to DT) and from the DZHK (Deutsches Zentrum für Herz-Kreislauf-Forschung – German Centre for Cardiovascular Research) and the BMBF (German Ministry of Education and Research) (to HAK and DT). The funding sources were not involved in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

References

[1] C. Schmidt, J. Kisselbach, P.A. Schweizer, et al., The pathology and treatment of cardiac arrhythmias: focus on atrial fibrillation, Vasc. Health Risk Manage. 7 (2011) 193–202.
[2] S.A. Goldstein, D. Bockenhauer, I. O'Kelly, et al., Potassium leak channels and the KCNK family of two-P-domain subunits, Nat. Rev. Neurosci. 2 (2001) 175–184.
[3] C. Schmidt, F. Wiedmann, P.A. Schweizer, et al., Inhibition of cardiac two-pore-domain K^+ (K_{2P}) channels – an emerging antiarrhythmic concept, Eur. J. Pharmacol. 738 (2014) 250–255.
[4] E. Marban, Cardiac channelopathies, Nature 415 (2002) 213–218.
[5] J. Gierten, E. Ficker, R. Bloehs, et al., Regulation of two-pore-domain (K_{2P}) potassium leak channels by the tyrosine kinase inhibitor genistein, Br. J. Pharmacol. 154 (2008) 1680–1690.
[6] J. Gierten, D. Hassel, P.A. Schweizer, et al., Identification and functional characterization of zebrafish K_{2P}10.1 (TREK2) two-pore-domain K^+ channels, BBA – Biomembranes 2012 (1818) 33–41.
[7] D. Thomas, L.D. Plant, C.M. Wilkens, et al., Alternative translation initiation in rat brain yields K_{2P}2.1 potassium channels permeable to sodium, Neuron 58 (2008) 859–870.
[8] G. Sandoz, D. Douguet, F. Chatelain, et al., Extracellular acidification exerts opposite actions on TREK1 and TREK2 potassium channels via a single conserved histidine residue, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 14628–16433.
[9] K. Staudacher, I. Baldea, J. Kisselbach, et al., Alternative splicing determines mRNA translation initiation and function of human K_{2P}10.1 K^+ channels, J. Physiol. 589 (2011) 3709–3720.
[10] J. Kisselbach, P.A. Schweizer, R. Gerstberger, et al., Enhancement of K_{2P}2.1 (TREK1) background currents expressed in Xenopus oocytes by voltage-gated K^+ channel β subunits, Life Sci. 91 (2012) 377–383.
[11] A.K. Rahm, J. Gierten, J. Kisselbach, et al., Protein kinase C-dependent activation of human K_{2P}18.1 K^+ channels, Br. J. Pharmacol. 166 (2012) 764–773.
[12] A.K. Rahm, F. Wiedmann, J. Gierten, et al., Functional characterization of zebrafish K_{2P}18.1 (TRESK) two-pore-domain K^+ channels, Naunyn-Schmiedeberg's Arch. Pharmacol. 387 (2014) 291–300.
[13] C. Seyler, E. Duthil-Straub, E. Zitron, et al., TASK1 (K_{2P}3.1) K^+ current inhibition by endothelin-1 is mediated by Rho kinase-dependent channel phosphorylation, Br. J. Pharmacol. 165 (2012) 1467–1475.
[14] U. Ravens, Novel pharmacological approaches for antiarrhythmic therapy, Naunyn-Schmiedeberg's Arch. Pharmacol. 381 (2010) 187–193.
[15] J. Gierten, E. Ficker, R. Bloehs, et al., The human cardiac K_{2P}3.1 (TASK-1) potassium leak channel is a molecular target for the class III antiarrhythmic drug amiodarone, Naunyn-Schmiedeberg's Arch. Pharmacol. 381 (2010) 261–270.
[16] K. Staudacher, I. Staudacher, E. Ficker, et al., Carvedilol targets human K_{2P}3.1 (TASK1) K^+ leak channels, Br. J. Pharmacol. 163 (2011) 1099–1110.
[17] J. Kisselbach, C. Seyler, P.A. Schweizer, et al., Modulation of K_{2P}2.1 and K_{2P}10.1 K^+ channel sensitivity to carvedilol by alternative mRNA translation initiation, Br. J. Pharmacol. (2014), http://dx.doi.org/10.1111/bph.12596.
[18] C. Schmidt, F. Wiedmann, P.A. Schweizer, et al., Novel electrophysiological properties of dronedarone: inhibition of human cardiac two-pore-domain potassium (K_{2P}) channels, Naunyn-Schmiedeberg's Arch. Pharmacol. 385 (2012) 1003–1016.
[19] C. Schmidt, F. Wiedmann, P.A. Schweizer, et al., Class I antiarrhythmic drugs inhibit human cardiac two-pore-domain K^+ (K_{2P}) channels, Eur. J. Pharmacol. 721 (2013) 237–248.
[20] C. Schmidt, F. Wiedmann, C. Langer, et al., Cloning, functional characterization and remodeling of K_{2P}3.1 (TASK-1) potassium channels in a porcine model of atrial fibrillation and heart failure, Heart Rhythm (2014), http://dx.doi.org/10.1016/j.hrthm.2014.06.020.
[21] C. Seyler, J. Li, P.A. Schweizer, et al., Inhibition of cardiac two-pore-domain K^+ (K_{2P}) channels by the antiarrhythmic drug vernakalant-comparison with flecainide, Eur. J. Pharmacol. 724 (2014) 51–57.
[22] S.H. Limberg, N.F. Netter, A. Rolfes, et al., TASK-1 channels may modulate action potential duration of human atrial cardiomyocytes, Cell. Physiol. Biochem. 28 (2011) 613–624.
[23] C. Petric, L. Clasen, C. van Wessel, In vivo electrophysiological characterization of TASK-1 deficient mice, Cell. Physiol. Biochem. 30 (2012) 523–537.
[24] K. Trappe, D. Thomas, O. Bikou, et al., Suppression of persistent atrial fibrillation by genetic knockdown of caspase 3: a pre-clinical pilot study, Eur. Heart J. 34 (2013) 147–157.
[25] N. Voigt, J. Heijman, Q. Wang, et al., Cellular and molecular mechanisms of atrial arrhythmogenesis in patients with paroxysmal atrial fibrillation, Circulation 129 (2014) 145–156.
[26] B. Liang, M. Soka, A.H. Christensen, et al., Genetic variation in the two-pore domain potassium channel, TASK-1, may contribute to an atrial substrate for arrhythmogenesis, J. Mol. Cell. Cardiol. 67 (2014) 69–76.
[27] C. Schmidt, F. Wiedmann, F. Tristram, et al., Cardiac expression and atrial fibrillation-associated remodeling of K_{2P}2.1 (TREK-1) K^+ channels in a porcine model, Life Sci. 97 (2014) 107–115.
[28] P. Lugenbiel, D. Thomas, K. Kelemen, et al., Genetic suppression of Gαs protein provides rate control in atrial fibrillation, Basic Res. Cardiol. 107 (2012) 265.
[29] N. Decher, M. Maier, W. Dittrich, et al., Characterization of TASK-4, a novel member of the pH-sensitive, two-pore domain potassium channel family, FEBS Lett. 492 (2001) 84–89.
[30] C. Girard, F. Duprat, C. Terrenoire, et al., Genomic and functional characteristics of novel human pancreatic 2P domain K(+) channels, Biochem. Biophys. Res. Commun. 282 (2001) 249–256.
[31] C. Friedrich, S. Rinné, S. Zumhagen, et al., Gain-of-function mutation in TASK-4 channels and severe cardiac conduction disorder, EMBO Mol. Med. 6 (2014) 937–951.
[32] S. Domingues-Montanari, I. Fernández-Cadenas, A. Del Río-Espinola, et al., KCNK17 genetic variants in ischemic stroke, Atherosclerosis 208 (2010) 203–209.
[33] D. Dobrev, B. Hamad, P. Kirkpatrick, Vernakalant, Nat. Rev. Drug Discovery 9 (2010) 915–916.
[34] E. Wettwer, T. Christ, S. Endig, et al., The new antiarrhythmic drug vernakalant: ex vivo study of human atrial tissue from sinus rhythm and chronic atrial fibrillation, Cardiovasc. Res. 98 (2013) 145–154.
[35] M.T. Slawsky, N.A. Castle, K^+ channel blocking actions of flecainide compared with those of propafenone and quinidine in adult rat ventricular myocytes, J. Pharmacol. Exp. Ther. 269 (1994) 66–74.
[36] Z. Wang, B. Fermini, S. Nattel, Effects of flecainide, quinidine, and 4-aminopyridine on transient outward and ultrarapid delayed rectifier currents in human atrial myocytes, J. Pharmacol. Exp. Ther. 272 (1995) 184–196.
[37] J.R. Penniman, D.C. Kim, J.J. Salata, et al., Assessing use-dependent inhibition of the cardiac Na^+ current (I(Na)) in the PatchXpress automated patch clamp, J. Pharmacol. Toxicol. Methods 62 (2010) 107–118.
[38] C.Y. Du, A. El Harchi, Y.H. Zhang, et al., Pharmacological inhibition of the hERG potassium channel is modulated by extracellular but not intracellular acidosis, J. Cardiovasc. Electrophysiol. 22 (2011) 1163–1170.
[39] S. Rajan, L.D. Plant, M.L. Rabin, et al., Sumoylation silences the plasma membrane leak K^+ channel K_{2P}1, Cell 121 (2005) 37–47.
[40] D. Roy, B.H. Rowe, I.G. Stiell, et al., A randomized, controlled trial of RSD1235, a novel anti-arrhythmic agent, in the treatment of recent onset atrial fibrillation, J. Am. Coll. Cardiol. 44 (2004) 2355–2361.
[41] O. Bikou, D. Thomas, K. Trappe, et al., Connexin 43 gene therapy prevents persistent atrial fibrillation in a porcine model, Cardiovasc. Res. 92 (2011) 218–225.
[42] R. Soucek, D. Thomas, K. Kelemen, et al., Genetic suppression of atrial fibrillation using a dominant-negative ether-a-go-go-related gene mutant, Heart Rhythm 9 (2012) 265–272.
[43] P. Kirchhof, G. Breithardt, E. Aliot, et al., Personalized management of atrial fibrillation: proceedings from the fourth atrial fibrillation competence NETwork/European Heart Rhythm Association consensus conference, Europace 15 (2013) 1540–1556.