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RESEARCH PAPER

Carvedilol targets human $K_{2P}3.1$ (TASK1) K^+ leak channels

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BACKGROUND AND PURPOSE

Human $K_{2P}3.1$ (TASK1) channels represent potential targets for pharmacological management of atrial fibrillation. K_{2P} channels control excitability by stabilizing membrane potential and by expediting repolarization. In the heart, inhibition of K_{2P} currents by class III antiarrhythmic drugs results in action potential prolongation and suppression of electrical automaticity. Carvedilol exerts antiarrhythmic activity and suppresses atrial fibrillation following cardiac surgery or cardioversion. The objective of this study was to investigate acute effects of carvedilol on human $K_{2P}3.1$ ($hK_{2P}3.1$) channels.

EXPERIMENTAL APPROACH

Two-electrode voltage clamp and whole-cell patch clamp electrophysiology was used to record $hK_{2P}3.1$ currents from *Xenopus* oocytes, Chinese hamster ovary (CHO) cells and human pulmonary artery smooth muscle cells (hPASMC).

KEY RESULTS

Carvedilol concentration-dependently inhibited $hK_{2P}3.1$ currents in *Xenopus* oocytes (IC_{50} = 3.8 μM) and in mammalian CHO cells (IC_{50} = 0.83 μM). In addition, carvedilol sensitivity of native $I_{K2P3.1}$ was demonstrated in hPASMC. Channels were blocked in open and closed states in frequency-dependent fashion, resulting in resting membrane potential depolarization by 7.7 mV. Carvedilol shifted the current–voltage (I–V) relationship by –6.9 mV towards hyperpolarized potentials. Open rectification, characteristic of K_{2P} currents, was not affected.

CONCLUSIONS AND IMPLICATIONS

The antiarrhythmic drug carvedilol targets $hK_{2P}3.1$ background channels. We propose that cardiac $hK_{2P}3.1$ current blockade may suppress electrical automaticity, prolong atrial refractoriness and contribute to the class III antiarrhythmic action in patients treated with the drug.

Abbreviations

AF, atrial fibrillation; CHO, Chinese hamster ovary; ERP, effective refractory period; K_{2P}, two-pore-domain K^+ channel; RMP, resting membrane potential; TASK, TWIK-related acid sensitive K^+ channel; TWIK, tandem of P domains in a weak inward rectifying K^+ channel

Introduction

Two-pore-domain background potassium (K_{2P}) channels (nomenclature follows Alexander *et al.*, 2009) stabilize resting membrane potential (RMP) and promote action potential repolarization (Goldstein *et al.*, 2001; Thomas and Goldstein, 2009). Regulation of K_{2P} background currents provides a mechanism for control of cellular excitability (Goldstein

Table 1

Pharmacology of $K_{2P}3.1$ (TASK1) channels

Drug	Effect	IC_{50}/EC_{50}	Reported maximum effect	Reference
A293 (experimental AAD)	Inhibition	0.2 μM (XO)	~95% reduction	Putzke *et al.*, 2007b
Amiodarone (AAD)	Inhibition	0.40 μM (XO)	~58% reduction	Gierten *et al.*, 2010
Anandamide (CB)	Inhibition	NI	~90% reduction (3 μM; MC)	Maingret *et al.*, 2001
Bupivacaine (LA)	Inhibition	41 μM (XO)	~95% reduction	Kindler *et al.*, 1999
Carvedilol (AAD)	Inhibition	3.8 μM (XO) 0.83 μM (MC)	~95% reduction	This study
CP55940 (CB_1/CB_2 agonist)	Inhibition	NI	~50% reduction (10 μM; MC)	Maingret *et al.*, 2001
Diethyl ether (VA)	Inhibition	NI	~45% reduction (0.6 mM; MC)	Patel *et al.*, 1999
Doxapram (ventilatory stimulant)	Inhibition	0.41 μM (XO)	~95% reduction	Cotten *et al.*, 2006
Etidocaine (LA)	Inhibition	39 μM (XO)	~90% reduction	Kindler *et al.*, 1999
Etomidate (IA)	Inhibition	119 μM (XO)	~60% reduction	Putzke *et al.*, 2007a
Genistein (experimental TK inhibitor)	Inhibition	10.7 μM (XO) 12.3 μM (MC)	~90% reduction ~85% reduction	Gierten *et al.*, 2008
Halothane (VA)	Activation	0.3–1.0 mM (MC)	~60% increase (MC) ~30% increase (XO)	Patel *et al.*, 1999; Sirois *et al.*, 2000; Putzke *et al.*, 2007a
Isoflurane (VA)	Activation	NI	~20% increase (2 mM; MC) ~15% increase (1 mM; XO)	Patel *et al.*, 1999; Putzke *et al.*, 2007a
Lidocaine (LA)	Inhibition	222 μM (XO)	~70% reduction	Kindler *et al.*, 1999
Mepivacaine (LA)	Inhibition	709 μM (XO)	~52% reduction	Kindler *et al.*, 1999
Methanandamide (CB)	Inhibition	0.7 μM (MC)	~99% reduction	Maingret *et al.*, 2001
Phenytoin (antiepileptic drug)	Inhibition	NI	53% reduction (200 μM; XO)	Leonoudakis *et al.*, 1998
Quinidine (AAD)	Inhibition	NI	71% reduction (100 μM; XO)	Leonoudakis *et al.*, 1998
R(+)-ropivacaine	Inhibition	51 μM (XO)	~95% reduction	Kindler *et al.*, 1999
S(−)-ropivacaine	Inhibition	53 μM (XO)	~92% reduction	
Sevoflurane (VA)	Activation	NI	~60% increase (1 mM; XO)	Putzke *et al.*, 2007a
SR141716A (CB_1 antagonist)	Inhibition	NI	~25% reduction (10 μM; MC)	Maingret *et al.*, 2001
Tetracaine (LA)	Inhibition	668 μM (XO)	~52% reduction	Kindler *et al.*, 1999
WIN552122 (CB_1/CB_2 agonist)	Inhibition	NI	~85% reduction (10 μM; MC)	Maingret *et al.*, 2001

AAD, antiarrhythmic drug; CB, cannabinoid; CB_1/CB_2, cannabinoid receptor subtypes; IA, intravenous anaesthetic; LA, local anaesthetic; MC, mammalian cells; NI, not investigated; TK, tyrosine kinase; VA, volatile anaesthetic; XO, *Xenopus* oocytes.

et al., 2001; 2005; Patel and Honore, 2001; Bayliss *et al.*, 2003; Thomas *et al.*, 2008). Among K_{2P} family members, cardiac expression of $K_{2P}3.1$ (TASK1) channels is well documented (Enyedi and Czirják, 2010). In humans, expression of $K_{2P}3.1$ channels is mainly restricted to atria whereas, in rodents, these channels were detected in atria and ventricles. Genetic inactivation or pharmacological inhibition of $K_{2P}3.1$ channels resulted in prolongation of cardiac action potentials and QTc intervals in rodent models (Putzke *et al.*, 2007b; Donner *et al.*, 2011), consistent with its proposed physiological role. Prolongation of cardiac effective refractory periods (ERP) and of action potentials is a hallmark of class III antiarrhythmic drugs, reducing membrane excitability and decreasing arrhythmia susceptibility. In particular, prolonged atrial refractoriness prevents electrical re-entry and suppresses atrial fibrillation (AF; Dobrev and Nattel, 2010). Thus, $K_{2P}3.1$ channels may serve as atrial-selective targets for novel pharmacological approaches against AF (Ravens, 2010). Data on cardiovascular pharmacology of $hK_{2P}3.1$ channels, however, are limited (Gierten *et al.*, 2010; Table 1).

Carvedilol is a non-selective β-adrenoceptor antagonist characterized by multiple pharmacological actions which translate into a wide-spectrum therapeutic potential (Karle *et al.*, 2001; El-Sherif and Turitto, 2005). In heart failure patients, the use of carvedilol is associated with reduced morbidity and mortality compared to the β_1- adrenoceptor selective antagonist, metoprolol (Poole-Wilson *et al.*, 2003; Remme *et al.*, 2007). Furthermore, survival was improved by carvedilol in high-risk patients with heart failure and AF, indicating specific antiarrhythmic properties in AF (Ramaswamy, 2003). Indeed, carvedilol was effective and superior to metoprolol in the prevention of post-operative AF after coronary artery bypass graft surgery or cardiac valve operation (Merritt *et al.*, 2003; Haghjoo *et al.*, 2007; Acikel *et al.*, 2008; Tsuboi *et al.*, 2008). In addition, carvedilol reduced AF recurrence rates after cardioversion (Katritsis

et al., 2003; Kanoupakis *et al.*, 2004). The antiarrhythmic profile of the drug is further characterized by pronounced prolongation of atrial ERP (Kanoupakis *et al.*, 2008).

Based upon these clinical data, we hypothesized that carvedilol exerts antiarrhythmic effects through inhibition of atrial K_{2P} background currents. This study was designed to assess acute effects of carvedilol on human K_{2P}3.1 channels in order to further elucidate the electrophysiological profile of the drug. We reveal inhibition of hK_{2P}3.1 potassium channels by carvedilol at clinically relevant concentrations, which is expected to cause action potential prolongation and a class III antiarrhythmic effect in patients with AF.

Methods

Molecular biology

Human cDNA encoding K_{2P}3.1 (NM_002246) was kindly provided by Dr Steve Goldstein (Chicago, USA). Procedures for *in vitro* transcription and oocyte injection were performed as published previously (Thomas *et al.*, 2008). Briefly, cRNAs were transcribed after vector linearization using T7 RNA polymerase and the mMessage mMachine kit (Ambion, Austin, USA). Transcripts were quantified using a spectrophotometer and by comparison with control samples separated by agarose gel electrophoresis. Stage V–VI-defolliculated *Xenopus* oocytes were injected with 1–2 ng cRNA encoding hK_{2P}3.1.

Cell culture

Chinese hamster ovary (CHO) cells were cultured in minimum essential medium α (MEM α, Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal bovine serum (Invitrogen), 100 U·mL^{-1} penicillin G sodium and 100 µg·mL^{-1} streptomycin sulphate in an atmosphere of 95% humidified air and 5% CO_2 at 37°C. Cells were passaged regularly and subcultured prior to treatment. Transient transfections of CHO cells were performed using Lipofectamine 2000 transfection reagent (Invitrogen) according to the manufacturer's instructions. Human pulmonary artery smooth muscle cells (hPASMC; PromoCell, Heidelberg, Germany) were handled according to the supplier's instructions. Cells were maintained in growth medium (PromoCell) in an atmosphere of 95% humidified air and 5% CO_2 at 37°C and passaged every 24 to 48 h. For electrophysiological recordings, hPASMC were plated on glass coverslips following dissociation.

Oocyte preparation

All animal care and experimental procedures were in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health and were approved by the Animal Use Committee of the State of Baden-Wuerttemberg (Germany). Ovarian lobes were surgically removed with aseptic techniques from female *Xenopus laevis* frogs anesthetized with 1g·L^{-1} tricaine solution (pH = 7.5). Frogs were not fed on the day of surgery to avoid emesis during anaesthesia. After surgery, the frogs were allowed to recover consciousness, followed by at least 2 months' recovery period. Oocyte collection alternated between left and right ovaries, and no more than three collections were made from each frog. After the final collection of oocytes, the anesthetized frog was killed by decerebration and pithing. Following collagenase treatment, stage V–VI-defolliculated oocytes were manually isolated under a stereomicroscope.

Electrophysiology

Two-electrode voltage clamp measurements were performed as described earlier (Thomas *et al.*, 1999). Whole oocyte currents were measured 2 to 3 days after injection with an Oocyte Clamp amplifier (Warner Instruments, Hamden, CT) using pCLAMP (Axon Instruments, Foster City, CA) and Origin 6 (OriginLab, Northampton, MA) software for data acquisition and analysis. Data were sampled at 2 kHz and filtered at 1 kHz. Current recordings from CHO cells or from hPASMC were performed using the whole-cell patch clamp configuration as previously reported (Thomas *et al.*, 2001; Kiesecker *et al.*, 2006; Gierten *et al.*, 2008). Human PASMC were clamped at 0 mV for 5 min at the beginning of each experiment to isolate I_{KN} from voltage-gated K^+ channels as described (Gurney *et al.*, 2003). Application of additional ion channel blockers was not required (Olschewski *et al.*, 2006). All experiments were carried out at room temperature (20–22°C), and no leak subtraction was done during the experiments.

Solutions and drug administration

Two-electrode voltage clamp electrodes were filled with 3 M KCl. The standard physiological extracellular solution contained 96 mM NaCl, 4 mM KCl, 1.1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES (pH 7.4). pH was adjusted to 7.4 with NaOH. For whole-cell patch clamp recordings from CHO cells, electrodes were filled with the following solution (in mM): 100 K-aspartate, 20 KCl, 2.0 $MgCl_2$, 1.0 $CaCl_2$, 10 EGTA, 2 ATP, 10 HEPES (pH adjusted to 7.2 with KOH). The external solution for these experiments contained (in mM): 140 NaCl, 5.0 KCl, 1.0 $MgCl_2$, 1.8 $CaCl_2$, 10 HEPES, 10 glucose (pH adjusted to 7.4 with NaOH). Patch clamp recordings from hPASMC were carried out in external solution containing (in mM): 140.5 NaCl, 5.5 KCl, 1.0 $MgCl_2$, 1.5 $CaCl_2$, 0.5 NaH_2PO_4, 0.5 KH_2PO_4, 10 HEPES, 10 glucose (pH adjusted to 7.3 with NaOH). Electrodes were filled with the following solution (in mM): 135 K-methanesulfonate, 20 KCl, 1.0 $MgCl_2$, 1 EGTA, 2 ATP, 20 HEPES (pH adjusted to 7.2 with KOH).

Carvedilol, metoprolol and propranolol (all from Sigma) were prepared as 100 mM stock solutions in 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. Human K_{2P}3.1 currents recorded from *Xenopus* oocytes as described in Figure 1A were not significantly altered upon application of 0.1% DMSO (v·v^{-1}; maximum bath concentration) for 20 min ($I_{Control}$, 16.2 ± 1.8 µA; I_{DMSO}, 15.7 ± 1.3 µA; n = 5; P = 0.60).

Data analysis and statistics

Concentration–response relationships for drug-induced block were fit with a Hill equation of the following form: $I_{drug}/I_{control} = 1/[1 + (D/IC_{50})^n]$, where I indicates current, D is the drug concentration, n is the Hill coefficient, and IC_{50} is the concentration necessary for 50% block. Data are expressed as mean ± SEM. We used paired and unpaired Student's *t*-tests (two-tailed tests) to compare the statistical significance of the

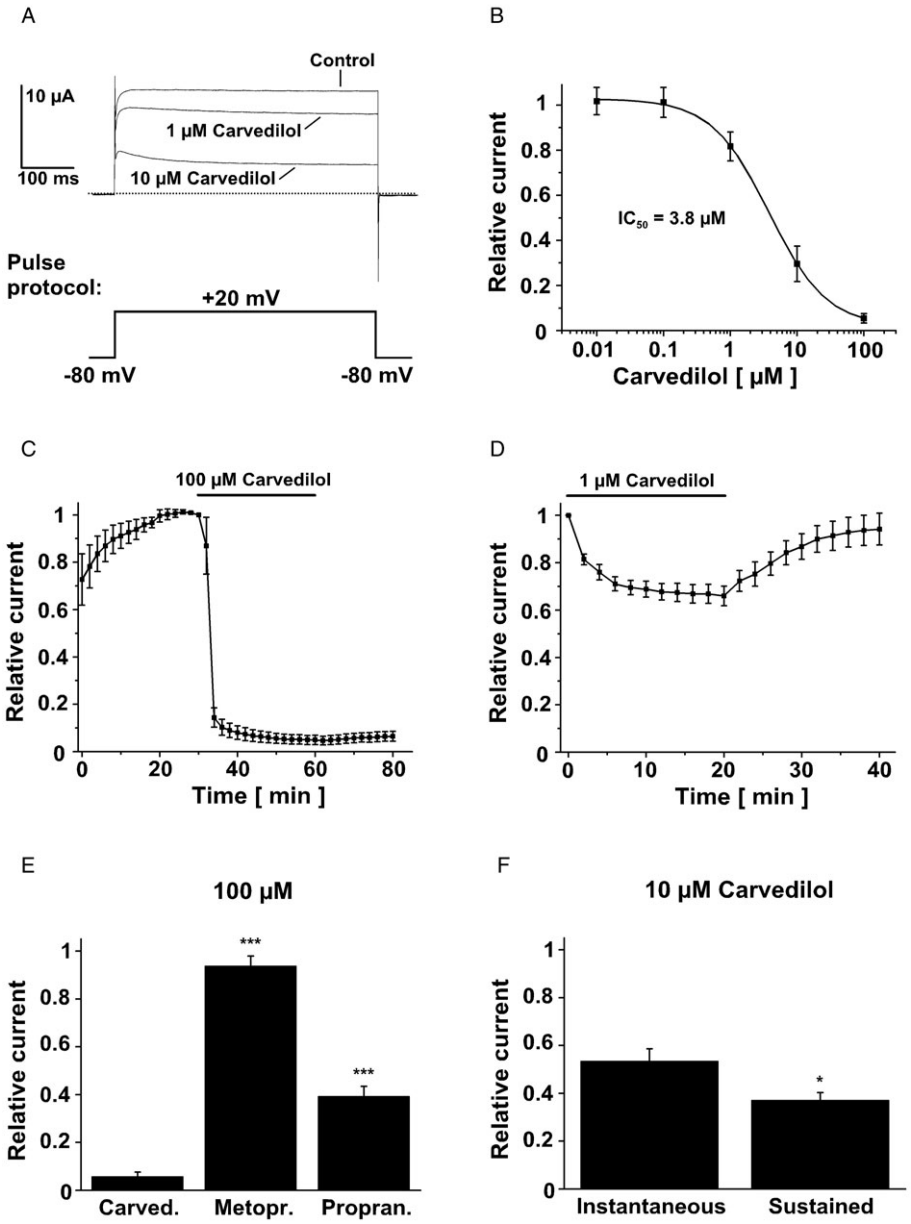

Figure 1

Inhibition of human $K_{2P}3.1$ (TASK1) channels expressed in *Xenopus* oocytes by carvedilol. Representative macroscopic currents recorded under control conditions and after application of carvedilol (20 min) are displayed in panel A. Recordings were performed in 2 min intervals. (B) Concentration–response relationships for the effect of carvedilol on $hK_{2P}3.1$ outward currents measured at the end of the +20 mV voltage step (n = 4 to 5 cells). (C) time course of $hK_{2P}3.1$ current inhibition by 100 µM carvedilol (n = 5). (D) Reversibility of $hK_{2P}3.1$ inhibition by 1 µM carvedilol (n = 5). (E) Effects of metoprolol and propranolol on $hK_{2P}3.1$ currents in *Xenopus* oocytes. Currents were recorded as described in panel A. Mean relative current amplitudes in the presence of carvedilol (Carved; n = 5), metoprolol (Metopr; n = 5) and propranolol (Propran; n = 6) are displayed. Current inhibition induced by carvedilol was significantly stronger compared to propranolol (***P < 0.001 vs. carvedilol). Metoprolol did not significantly inhibit $hK_{2P}3.1$ channels. (F) Carvedilol blockade of the sustained current component of $hK_{2P}3.1$ was more pronounced than that of the instantaneous current (n = 4; *P < 0.05). Data are given as mean ± SEM. Dotted line indicates zero current level.

results. $P < 0.05$ was considered statistically significant. Multiple comparisons were performed using one-way ANOVA. If the hypothesis of equal means could be rejected at the 0.05 level, pairwise comparisons of groups were made and the probability values were adjusted for multiple comparisons using the Bonferroni correction.

Results

Carvedilol inhibits human $K_{2P}3.1$ (TASK1) background potassium channels

The effects of carvedilol on $hK_{2P}3.1$ channels were studied in *Xenopus laevis* oocytes. Currents were elicited every 2 min by

a 500 ms depolarizing step to +20 mV and measured at the end of the test pulse. The degree of block was determined after 20 min (Figure 1A). The holding potential was –80 mV in all experiments performed in this study. Following current level equilibration, current amplitudes recorded at +20 mV were stable during the drug application period ($I_{0\,min}$, 18.6 ± 2.1 μA; $I_{20\,min}$, 18.6 ± 1.9 μA; n = 9; P = 0.92). In contrast, carvedilol reduced $hK_{2P}3.1$ potassium currents in a concentration-dependent manner (Figure 1A). To study the concentration-dependence of $hK_{2P}3.1$ inhibition by carvedilol, currents in the presence of the drug were normalized to their respective control values and plotted as relative current amplitudes in Figure 1B (n = 4 to 5 cells were investigated at each concentration). Calculation of the half-maximal inhibitory concentration (IC_{50}) for block of $hK_{2P}3.1$ leak channels yielded 3.8 ± 0.3 μM with a Hill coefficient n_H of 1.0 ± 0.1. The onset of block is shown in Figure 1C (n = 5). After a control period of 30 min showing initial current increase during the equilibration period, $hK_{2P}3.1$ current reduction by 100 μM carvedilol developed rapidly. Drug washout (20 min) revealed that inhibitory effects of 100 μM carvedilol on $hK_{2P}3.1$ were virtually irreversible (Figure 1C). Reversibility of block was further analysed using a clinically relevant concentration (Figure 1D). Carvedilol (1 μM) was applied for 20 min, followed by drug washout for 20 min as described above (n = 5). We found that $hK_{2P}3.1$ inhibition by 1 μM carvedilol (34.0 ± 4.1%; P = 0.019) was partially reversible. Currents reached 94.1 ± 6.7% of control levels after removal of the drug.

To assess specificity of the electrophysiological action of carvedilol on $hK_{2P}3.1$, we evaluated the effects of two other β-adrenoceptor antagonists, metoprolol and propranolol, under similar experimental conditions as described above. In contrast to carvedilol, application of the β_1-selective adrenoceptor antagonist metoprolol (100 μM; 20 min) did not significantly affect $hK_{2P}3.1$ currents (P = 0.19; Figure 1E). The non-selective β_1- and β_2-receptor blocker propranolol (100 μM; 20 min) blocked $hK_{2P}3.1$ channels by 61.0 ± 4.4% (n = 6; P = 0.002), albeit with reduced affinity compared to carvedilol (Figure 1E).

$hK_{2P}3.1$ channels activate in two phases (Duprat *et al.*, 1997). The currents activate quickly to approximately 85% of their respective maximum amplitudes within ~50 ms, followed by markedly slower additional activation time course. Macroscopic $hK_{2P}3.1$ currents can be divided into an instantaneous (measured 2 ms after the step to +20 mV) and a time-dependent current component (measured at the end of the 500 ms test pulse) respectively. The instantaneous current was 82.2 ± 3.4% of the fully activated current under control conditions (n = 4). Inhibition of the instantaneous component by 10 μM carvedilol (20 min) (46.7 ± 5.3% current reduction) was weaker compared to the total current (63.1 ± 3.3% inhibition; P = 0.013; Figure 1F).

$hK_{2P}3.1$ currents exhibited electrophysiological characteristics typical for a potassium-selective background leak conductance, that is, a voltage-independent portal showing Goldman-Hodgkin-Katz (open) rectification (Figure 2A). To assess the effects of carvedilol on $hK_{2P}3.1$ rectification, linear ramp voltage protocols were applied between –140 and +60 mV (500 ms) before and after application of 10 μM carvedilol for 20 min (Figure 2A). Outward rectification was

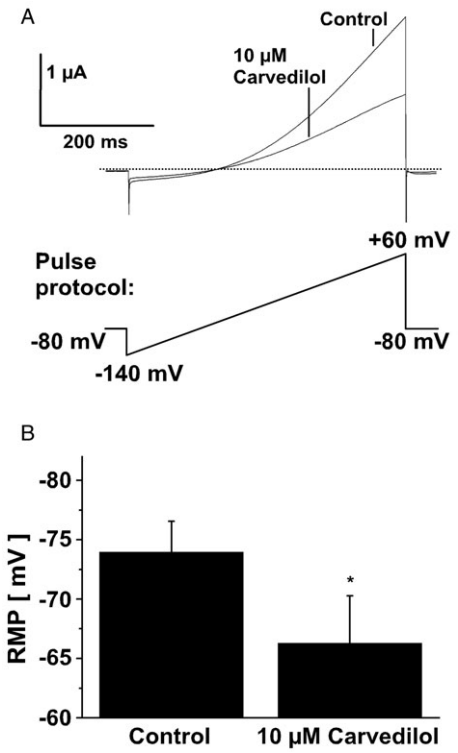

Figure 2

Open rectification of $hK_{2P}3.1$ currents elicited by voltage ramps from –140 to +60 mV. (A) Typical recordings from the same cell in the absence of the drug and after superfusion with 10 μM carvedilol (20 min) are superimposed. Dotted line indicates zero current level. (B) Mean resting membrane potentials (RMP) of *Xenopus* oocytes, measured before and after blockade of $hK_{2P}3.1$ with carvedilol (10 μM; 20 min). Inhibition of the leak current depolarized the cell membrane by 7.7 mV (n = 10). Data are given as mean ± SEM; *P < 0.05.

observed before and after drug application (n = 5). The degree of block determined at +20 mV ramp potential was 49.1 ± 1.7% (n = 5; P = 0.013). Reduction of $hK_{2P}3.1$ leak currents by carvedilol (10 μM; 20 min) was associated with a depolarisation of *Xenopus* oocyte RMP, by 7.7 ± 3.2 mV (Figure 2B; n = 10; P = 0.04).

Carvedilol blocks $hK_{2P}3.1$ channels in the open and closed state

To investigate whether channel sensitivity is state-dependent, we recorded $hK_{2P}3.1$ currents during a single depolarizing step to +20 mV for 7.5 s. This approach is based on voltage-dependent modulation of single channel openings. $hK_{2P}3.1$ currents show increased activation rates (i.e. faster transitions from closed to open states of single $K_{2P}3.1$ channels) at more positive potentials (Lopes *et al.*, 2000). Current traces under control conditions and after application of 10 μM carvedilol for 20 min while holding the cell at –80 mV are shown in Figure 3A. The degree of inhibition during the first 400 ms [i.e. (1 – current in the presence of carvedilol/control current) × 100] after the incubation period is displayed with linear and logarithmic time scales in Figure 3B and C respectively.

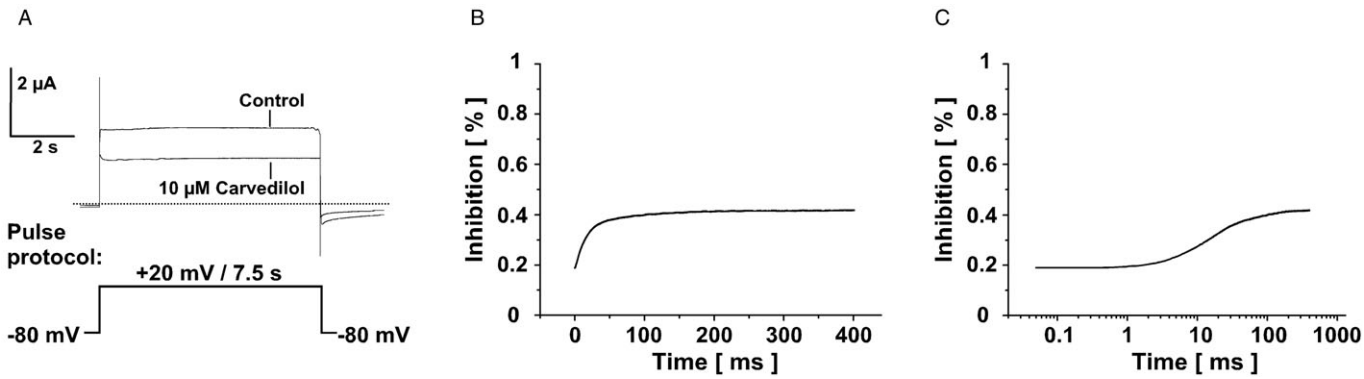

Figure 3

Carvedilol targets open and closed hK$_{2P}$3.1 channels. Currents were activated by a 7.5 s depolarizing voltage step to +20 mV. (A) Control recording and the first pulse measured immediately after administration of 10 µM carvedilol (20 min; dotted line illustrates zero current level). (B) and (C) display the degree of current inhibition during the first 400 ms of the depolarizing voltage step in % (B, linear time scale; C, logarithmic time scale), demonstrating blockade of closed and open hK$_{2P}$3.1 channels. Similar results were obtained from five independent experiments.

During the +20 mV step, more channels are expected to be in the open state compared to –80 mV. Analysis of currents after carvedilol administration revealed that ~20% inhibition of hK$_{2P}$3.1 channels had occurred at –80 mV, as indicated by the level of block at the beginning of the depolarizing pulse (Figure 3B and C). In addition, a second, time-dependent component of block was observed during the +20 mV pulse (block at the end of the test pulse: 42.3 ± 3.9%; n = 5; P = 0.0004).

Effects of carvedilol on hK$_{2P}$3.1 I–V relationship

Drug effects on hK$_{2P}$3.1 current–voltage (I–V) relationship were investigated under isochronal recording conditions. From a holding potential of –80 mV, depolarizing pulses were applied for 500 ms to voltages between –140 and +60 mV in 20 mV increments (0.5 Hz). Representative families of current traces from one cell are shown for control conditions (Figure 4A) and after application of 10 µM carvedilol for 20 min (Figure 4B). The current–voltage relationship was affected by carvedilol administration (Figure 4C and D). Mean half-maximal activation voltage normalized to current amplitudes recorded at +60 mV was shifted by 6.9 ± 1.4 mV towards more negative potentials ($V_{1/2,control}$ = 5.8 ± 1.1 mV; $V_{1/2,carvedilol}$ = –1.1 ± 1.8 mV; n = 7; P = 0.003). Relative inhibition of hK$_{2P}$3.1 currents was plotted as function of the test pulse potential in Figure 4E (n = 7), revealing an apparent increase of current inhibition at more depolarized test potentials. This difference, however, did not reach statistical significance.

Frequency-dependence of carvedilol-induced hK$_{2P}$3.1 block

To study frequency dependence of block, hK$_{2P}$3.1 channels were rapidly activated by a depolarizing step to +20 mV (500 ms) at intervals of 1 or 10 s respectively. Five oocytes were used at each rate, and the development of current reduction in the presence of 10 µM carvedilol was plotted versus time (Figure 5). The degree of inhibition after 20 min was significantly (P = 0.048) higher at 1 Hz stimulation rate (64.4 ± 3.6%; n = 5; P = 0.005) compared to 0.1 Hz (47.0 ± 6.5%; n = 5; P = 0.016), illustrating frequency dependence of hK$_{2P}$3.1 inhibition by carvedilol.

hK$_{2P}$3.1 channels expressed in mammalian cells are blocked by carvedilol

Concentration–response relationships obtained from mammalian expression systems are required to evaluate the clinical relevance of drug effects. We expressed hK$_{2P}$3.1 potassium channels heterologously in CHO cells to demonstrate modulation of hK$_{2P}$3.1 currents in mammalian cells (Figure 6A). From a holding potential of –80 mV, depolarizing pulses were applied for 500 ms to voltages between –120 and +80 mV in 20 mV increments (0.2 Hz). The degree of block was determined at +60 mV after steady-state conditions had been reached (usually within 3–4 min) (Figure 6B and C). The half-maximal inhibitory concentration was then calculated as described in Figure 1 (Figure 6D, n = 3 to 5 cells were investigated at each concentration). The IC$_{50}$ for block of hK$_{2P}$3.1 leak channels in CHO cells yielded 0.83 ± 0.30 µM with a Hill coefficient n_H of 0.88 ± 0.31.

Carvedilol suppresses I$_{K2P3.1}$ in hPASMC

Effects of carvedilol on endogenous human K$_{2P}$3.1 current were studied in hPASMC. This approach allowed for analysis of K$_{2P}$3.1 channels in a native setting, preventing any bias that may be caused by heterologous gene expression. PASMC represent an established system to study endogenous K$_{2P}$3.1 that virtually exclusively accounts for a non-inactivating background K$^+$ current (I_{KN}) in these cells under the given experimental conditions (Gurney *et al.*, 2003; Olschewski *et al.*, 2006). Furthermore, PASMC were used as they allow for more specific assessment of native $I_{K2P3.1}$ compared to cardiac myocytes, where $I_{K2P3.1}$ is less readily recorded owing to the presence of multiple ion currents and to the lack of specific K$_{2P}$3.1 inhibitors. Currents were activated by depolarizing pulses to voltages between –80 and +80 mV in 10 mV increments (500 ms; 0.5 Hz) from a holding potential of –80 mV.

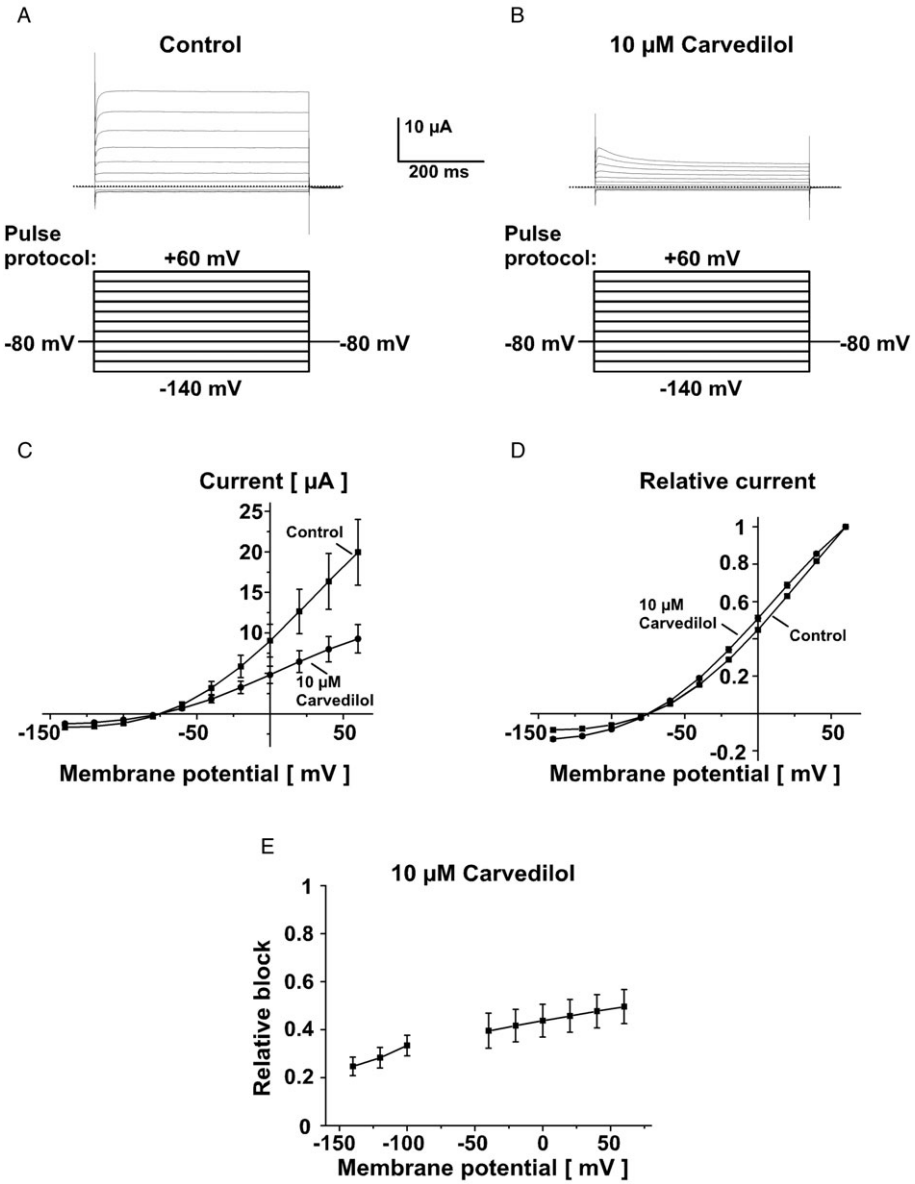

Figure 4

Effects of carvedilol on the voltage-dependence of activation of $hK_{2P}3.1$ channels. Control measurement (A) and the effect of 10 µM carvedilol (20 min; B) are shown in one representative oocyte. Zero current levels are indicated by dotted lines. Panels C and D display activation curves, that is, step current amplitudes as function of test potentials, recorded under isochronal conditions (C, original current amplitudes; D, values normalized to maximum currents) ($n = 7$). (E) Fraction of blocked step currents, plotted as function of the respective test pulse potential. Channel block was not significantly different between potentials tested ($n = 7$ cells). Data are shown as mean ± SEM.

Amplitudes were measured at the end of the 30 mV pulse. After obtaining control recordings (Figure 7A), carvedilol (10 µM) was applied for 10 min, resulting in strong $I_{K2P3.1}$ inhibition by 71.2 ± 9.5% (Figure 7B, D and E, $n = 6$; $P = 0.0007$). Subsequent acidification (pH 6.8) to fully inhibit $hK_{2P}3.1$ channels caused total current reduction of 78.8 ± 11.7% ($n = 6$; Figure 7C–E). The current–voltage relationship of $I_{K2P3.1}$ is displayed in Figure 7D ($n = 3$). The degree of block following acidification was not significantly different from carvedilol-induced inhibition, indicating that the drug caused virtually complete reduction of $I_{K2P3.1}$ in hPASMC.

Discussion

Human $K_{2P}3.1$ background potassium currents were inhibited by carvedilol, yielding IC_{50} values of 3.8 µM in *Xenopus* oocytes and of 0.83 µM in mammalian CHO cells. These findings obtained from heterologous expression systems were extended to native $I_{K2P3.1}$ recorded from hPASMC. Here, carvedilol (10 µM) blocked the acid-sensitive $K_{2P}3.1$ current virtually completely. During therapeutic application of the drug, plasma levels between 0.1 and 0.6 µM have been obtained (Karle *et al.*, 2001), indicating therapeutic relevance

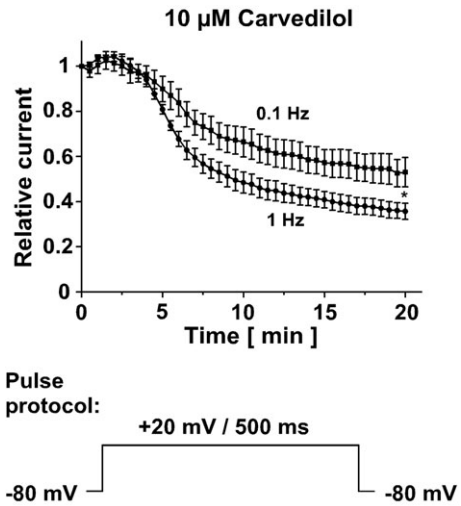

Figure 5

Carvedilol block of $hK_{2P}3.1$ is frequency-dependent. Mean relative $hK_{2P}3.1$ current amplitudes recorded at +20 mV membrane potential (1 and 0.1 Hz stimulation rate) are plotted versus time ($n = 5$ oocytes were studied at each rate; error bars denote SEM; $^*P < 0.05$). For the purpose of clear presentation, not all measurements are displayed.

of $K_{2P}3.1$ current blockade by carvedilol. Pharmacological inhibition of $K_{2P}3.1$ channels has been reported previously for several non-cardiac drugs, including local and volatile anaesthetics and psychotropic compounds (Table 1). In contrast, cardiac agents and particularly antiarrhythmic drugs are less well studied. The present study links inhibition of $K_{2P}3.1$ potassium currents to antiarrhythmic therapy, providing a starting point for establishing cardiac K_{2P} channels as potential antiarrhythmic drug targets.

Biophysical characteristics of $hK_{2P}3.1$ blockade

Biophysical properties of $hK_{2P}3.1$ inhibition by carvedilol were studied in detail. $hK_{2P}3.1$ channels conduct leak currents and are open across the entire physiological voltage range. Open rectification, a biophysical property characteristic to $hK_{2P}3.1$ function in physiological ionic conditions, was not altered by carvedilol (Figure 2). $K_{2P}3.1$ channels display gating (i.e. opening and closing of single channels), and they show voltage- and time-dependent responses to changes in membrane potential (Lopes *et al.*, 2000) with faster transitions from closed to open states of single channels at more positive membrane potentials. We found that channels were blocked in their closed and open state, as illustrated in Figure 3. Closed state block is reflected by initial current inhibition observed at the beginning of the test pulse, while time-

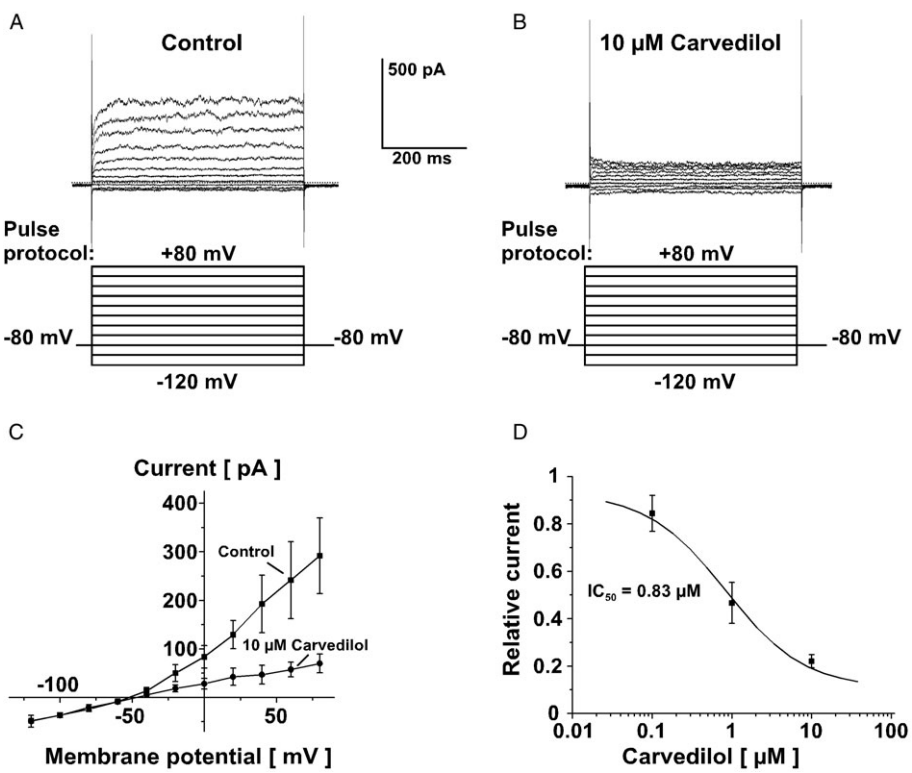

Figure 6

Carvedilol-induced inhibition of human $K_{2P}3.1$ (TASK1) channels expressed in Chinese hamster ovary (CHO) cells. Families of current traces recorded under control conditions and after superfusion with 10 µM carvedilol are displayed in panels A and B respectively. (C) Mean activation curves, obtained under control conditions and after application of 10 µM carvedilol ($n = 3$). (D) Concentration–response relationships for the effect of carvedilol on $hK_{2P}3.1$ currents measured at +60 mV ($n = 3$ to 5 cells; mean ± SEM).

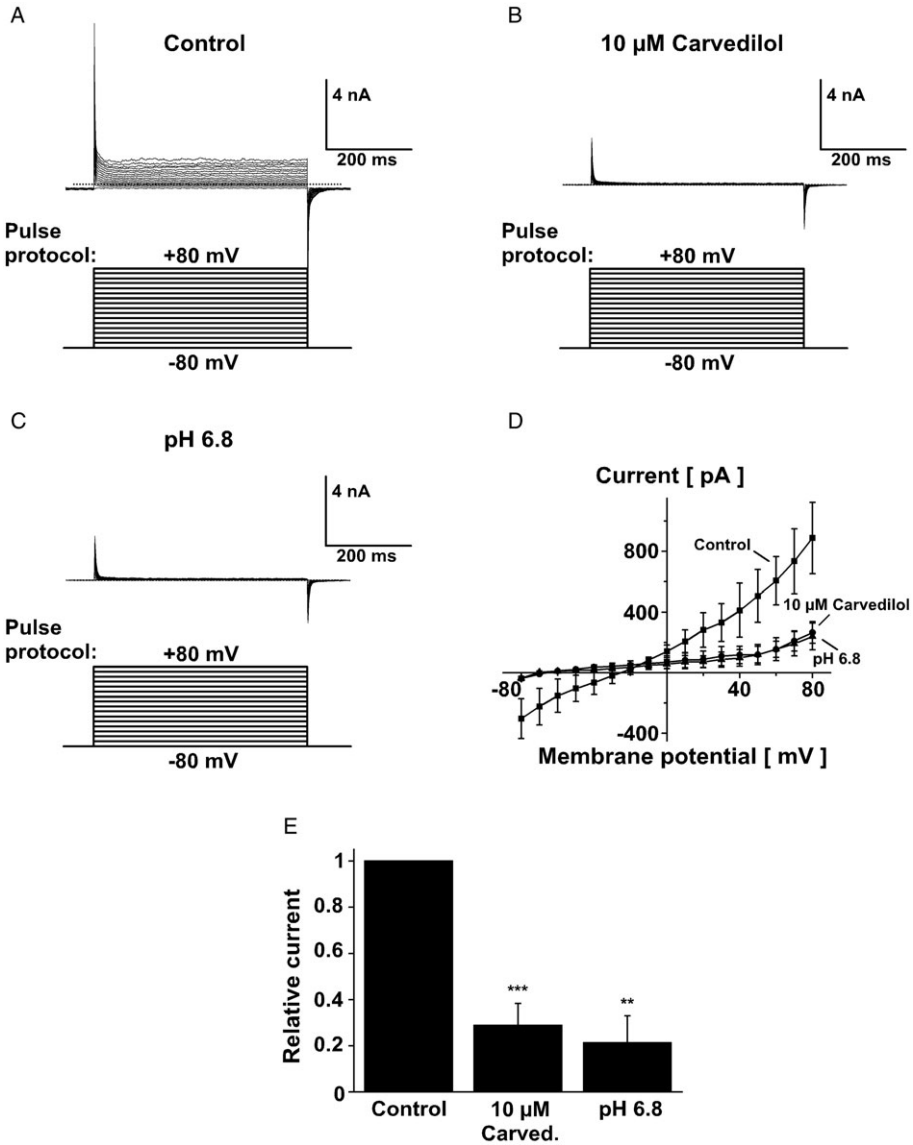

Figure 7

Modulation of endogenous $I_{K2P3.1}$ in cultured human pulmonary artery smooth muscle cells (PASMC) by carvedilol. Representative recordings under control conditions (A), after carvedilol administration (B; 10 µM carvedilol; 10 min) and following additional acidification (C; pH 6.8; 5 min) to completely block $I_{K2P3.1}$ at the end of each experiment are shown. Panel D displays corresponding mean current–voltage relationships ($n = 3$). (E) Mean relative K^+ current amplitudes under indicated experimental conditions (±SEM; $n = 6$ cells studied; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. controls).

dependent blockade indicates open state block. Open channel block is further supported by the observation that block of sustained $hK_{2P}3.1$ current was more pronounced compared to reduction of instantaneous currents (Figure 1F). In addition to a model of closed and open channels, this observation could be attributed to dual mechanisms of open channel block as well. Initial channel block could reflect instantaneous block, while the time-dependent component could be caused by steady-state block with slower kinetics. We did not observe significant voltage-dependence of block which could have served as an explanation for different drug affinities at –80 mV compared to +20 mV. Rather, instantaneous and time-dependent K_{2P} current components may be attributed to transitions of different closed channel states (C1 and C2 respectively) to the open conformation, as suggested previously for $K_{2P}2.1$ (Honore *et al.*, 2002). If this hypothesis is true and may be extended to $K_{2P}3.1$ channels, the proposed closed states C1 and C2 would be blocked with reduced affinity by carvedilol compared to the open state. Limitations arise from the fact that we cannot distinguish precisely between open and closed states because the channels may open over the entire voltage range, albeit with different open probabilities.

Under conditions of repetitive channel activation during drug application (10 µM carvedilol), maximum inhibition of $K_{2P}3.1$ currents was 63% (Figure 1F). In contrast, currents were reduced by 42% when carvedilol was applied while holding the cell at –80 mV (Figure 3), indicating use depen-

dence of block. Open channel block and relatively slow unblocking kinetics (Figure 1C and D) are expected to result in frequency-dependent accumulation of block. Indeed, steady-state inhibition was more pronounced at 1 Hz stimulation rate compared to 0.1 Hz (Figure 5). Frequency-dependent inhibition of atrial-selective potassium currents is clinically beneficial, as prolongation of refractoriness is required primarily during atrial tachycardia or AF, resulting in interruption of electrical re-entry (see below). There was no significant voltage-dependence of block. However, the current–voltage relationship of $hK_{2P}3.1$ was shifted by –6.9 mV towards more negative potentials (Figure 4). This finding in isolation causes current increase which was not observed here owing to pronounced channel blockade. Furthermore, the activation shift confirms a direct drug–channel interaction.

Molecular electrophysiology of carvedilol: focus on AF

Atrial fibrillation may result from a wide range of pathophysiological processes, ultimately leading to electrical and structural remodelling (Dobrev and Nattel, 2010). The generation of substrates that support slow conduction, shortening of atrial refractory periods and electrical re-entry is particularly critical as it provides the basis for maintenance of persistent AF (Nattel *et al.*, 2008; Ravens, 2010). AF suppression may either be achieved by elimination of electrical triggers (e.g. by pulmonary vein isolation) or by prevention of electrical re-entry (Dobrev and Nattel, 2010). Antiarrhythmic pharmacotherapy to treat AF primarily aims at prolonging atrial action potential duration and refractoriness in order to suppress re-entry. Drugs that specifically act on atrial-selective targets are expected to be particularly effective while exhibiting less potential to induce ventricular arrhythmias. In human heart, $hK_{2P}3.1$ represents an atrial ion channel with dual physiological function. First, K_{2P} leak channels stabilize the membrane potential and suppress excitability during RMP at negative voltages. Pharmacological inhibition of $K_{2P}3.1$ potassium currents leads to membrane potential depolarization, as observed in this study (Figure 2B). Second, outward potassium currents mediated by K_{2P} channels contribute to repolarization of cardiomyocytes during the cardiac action potential. Consequently, inhibition of cardiac $K_{2P}3.1$ channels has been shown to prolong refractoriness and action potential duration (Putzke *et al.*, 2007b; Donner *et al.*, 2011).

We propose that $hK_{2P}3.1$ current blockade by carvedilol prolongs atrial refractoriness and contributes to the class III antiarrhythmic action in AF patients. Prolongation of ERP by carvedilol was more pronounced in atrium compared to ventricle in humans (Kanoupakis *et al.*, 2008), illustrating the atrial-selective actions of the drug. Additional clinical studies indicate that, unlike the β_1-adrenoceptor selective antagonist metoprolol, carvedilol has additional benefits for AF management (Merritt *et al.*, 2003; Haghjoo *et al.*, 2007; Acikel *et al.*, 2008; Tsuboi *et al.*, 2008). The superiority of carvedilol to metoprolol has previously been attributed to broad anti-adrenergic properties and unique antioxidant effects of carvedilol. Based on the present work we hypothesize that blockade of $hK_{2P}3.1$ channels contributes, at least in part, to the efficacy of carvedilol in AF patients. Furthermore, metoprolol did not significantly affect $hK_{2P}3.1$ at high concentrations, providing a mechanistic explanation for the favourable clinical outcome associated with carvedilol (Figure 1E). In addition to its anti-adrenergic properties (Ruffolo and Feuerstein, 1997) and its effects on $hK_{2P}3.1$ (this study), carvedilol is a multi-channel blocker with reported inhibition of delayed rectifier potassium currents (I_{Kr}, I_{Ks}), I_{to}, I_{Kur}, pacemaker current (I_f) and L-type calcium current respectively (Cheng *et al.*, 1999; Karle *et al.*, 2001; Kawakami *et al.*, 2006; Deng *et al.*, 2007; Yokoyama *et al.*, 2007). Thus, the overall antiarrhythmic efficacy of carvedilol in AF may result from a combination of multiple electrophysiological actions in human atrium.

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Conflict of interest

None.

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