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RESEARCH PAPER

Modulation of drug block of the cardiac potassium channel KCNA5 by the drug transporters OCTN1 and MDR1

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BACKGROUND AND PURPOSE

A common site for drug binding on voltage-gated ion channels is at the interior face of the channel pore. In this study, we tested the hypothesis that the extent of drug block of the human cardiac KCNA5 (K_v1.5) channel underlying the atrial-specific, ultra-rapidly activating, delayed K^+ current (I_{Kur}) is modulated by the drug uptake and efflux transporters encoded by organic cation transporter 1 (OCTN1) and multiple drug-resistant gene 1 (MDR1) and expressed in human heart.

EXPERIMENTAL APPROACH

Drug block of KCNA5 was assessed in Chinese hamster ovary cells transiently transfected with KCNA5 alone or in combination with the OCTN1 or MDR1 transporter construct, as well as in an MDR1 stably expressed cell line.

KEY RESUTLS

Co-expression of OCTN1 significantly facilitated block by quinidine (10 μM), verapamil (20 μM), propafenone (5 μM) and clofilium (30 μM). Further evidence of drug transport modulating drug block was the finding that with OCTN1, block developed faster and only partially washed-out, and that block potentiation was prevented by cimetidine, an inhibitor of OCTN1. MDR1 expression attenuated KCNA5 block by erythromycin (an MDR1 substrate). Block was restored by reversin-205 (10 μM, an MDR1 inhibitor). MDR1 did not affect KCNA5 inhibition by KN-93 (1 μM), a blocker acting on the outer mouth of the channel pore.

CONCLUSIONS AND IMPLICATIONS

The extent of drug block of KCNA5 can be modulated by drug uptake and efflux transporters. These data provide further support for the idea that modifying intracellular drug concentrations could modulate the effects of blocking ion channels in patients.

Abbreviations

4-AP, 4-aminopyridine; ABCB1, multiple drug-resistant gene 1 (MDR1); CHO, Chinese hamster ovary cells; GFP, green fluorescent protein; KCNA5, gene encoding the ultra-rapid outward rectifying K^+ current (I_{Kur}); OCTN1, organic cation transporter 1; P-gp, P-glycoprotein

Introduction

Atrial fibrillation (AF) is the most common sustained cardiac tachyarrhythmia for which drug therapy is prescribed, and affects approximately 2–5 million patients in the United States and developed countries (Hylek *et al.*, 2001; Fuster *et al.*, 2006; Thom *et al.*, 2006). One approach to therapy involves increasing myocardial refractoriness by block of atrial repolarizing currents carried by K^+ channels, such as the delayed repolarizing current I_{Kr} (current and channel nomenclature follows Alexander *et al.*, 2009). However, block of I_{Kr} also prolongs refractoriness in the ventricle leading to the lethal arrhythmia Torsade de pointes. An atrial-specific and ultra-rapidly activated potassium current (I_{Kur}), encoded by the gene *KCNA5* ($K_v1.5$), has been detected in humans (Wang *et al.*, 1993; Li *et al.*, 1996; Nattel *et al.*, 1999). Native cardiac I_{Kur} displays similar biophysical and pharmacological profiles to those observed with heterologous expression of cloned $K_v1.5$ channels: for example, voltage-dependent rapid activation, slow/partial inactivation and sensitivity to several blocking agents, such as quinidine and 4-aminopyridine (4-AP) (Snyders *et al.*, 1993; Yeola *et al.*, 1996). Since I_{Kur} is specifically present in human atrial cells, selective block of I_{Kur} has been suggested as a target for development of drugs against AF (Savelieva and Camm, 2008a; Ehrlich and Nattel, 2009; Milan *et al.*, 2009).

For many ion channels, including KCNA5, the drug-binding site is located on the intracellular face of the channel pore (Mitcheson *et al.*, 2000; Chen *et al.*, 2002; Seebohm *et al.*, 2003; Decher *et al.* 2004). This finding suggests the hypothesis that inter-patient variability in drug block may in part reflect variable drug uptake/influx into or efflux from intracellular molecular effector sites. Studies by us and others have demonstrated that many drug influx and efflux transporters are present in human heart (Grube *et al.*, 2006; Iwata *et al.*, 2008; McBride *et al.*, 2009a,b). We have recently shown that drug block of the KCNH2 potassium channel (responsible for cardiac I_{Kr}) can be modulated by co-expression of the drug uptake transporter organic cation transporter 1 (OCTN1) and variants of multiple drug-resistant gene 1 (MDR1), encoding the efflux transporter P-glycoprotein (P-gp) (McBride *et al.*, 2009a,b). In the present study, we have extended these findings to study the modulating effects of OCTN1 and MDR1 co-expression on the degree of drug block of a second K^+ channel, KCNA5. In addition, we show that the extent of drug block in these settings can be modulated by inhibitors of transporter function.

Methods

FuGENE6-mediated KCNA5 channel expression

Recombinant cDNA for the human cardiac KCNA5 ($K_v1.5$) channel was transiently transfected in Chinese hamster ovary (CHO) cells. The cells lack endogenous outward currents and are thus suitable for K^+ current studies. The cells were grown to confluence in F-12 nutrient mixture (HAM) medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% horse serum, 2 mM L-glutamine and penicillin (50 $U \cdot mL^{-1}$)-streptomycin (50 $\mu g \cdot mL^{-1}$) at 37°C. Cells were transiently transfected with wild-type KCNA5 in a pBK/CMV vector (Stratagene, Santa Clara, CA, USA) with a pIRES-green fluorescent protein (GFP) bi-cistronic expression vector containing either an empty vector site (control), wild-type OCTN1 or MDR1 (isoform ABCB1*7). The ration of Fugene6 to cDNA plasmids was 2 µg:2 µg:12 µL. The cells were subsequently removed from the dish by brief trypsinization, and stored in standard medium for the experiments within the next 12 h. Cells showing green fluorescence by enhanced green fluorescent protein (eGFP) were chosen to identify successfully transfected cells for the voltage-clamp analysis. In some experiments, a pig kidney epithelial cell line, stably expressing MDR1 (L-MDR cells), was also used for transfections with KCNA5-eGFP construct to investigate the effects of endogenous MDR1 and its inhibitor (reversin-205) on KCNA5 block by erythromycin and KN-93.

Whole-cell voltage clamp

KCNA5 current was recorded in the whole-cell configuration of the patch-clamp technique using an Axopatch 200B amplifier (Axon Instruments Inc, Foster City, CA, USA). Patch pipettes were pulled from thick-wall borosilicate glass (World Precision Instruments, Inc., Sarasota, FL, USA) with a multi-stage P-97 Flaming-Brown micropipette puller (Sutter Instruments Co., Novato, CA, USA) and fire-polished. Pipette resistance was 2–4 mΩ. Whole-cell current traces were filtered at 5 kHz and acquired at 1 to 2 kHz. To obtain current–voltage (I-V) relations, the holding potential was –80 mV. Activating currents were elicited with 500 ms depolarizing pulses from –40 to +60 mV in 10 mV steps, and tail currents for another 500 ms were recorded upon return to –40 mV. Pulses were delivered at every 15 s interval. Other specific voltage-clamp protocols are presented as insets in figures.

After collection of baseline data, individual drugs were added to the perfusate while pulsing continued. Prior to and during drug exposure, KCNA5 current was dynamically monitored by using

500 ms pulses to +50 mV from a holding potential of –80 mV, delivered at a frequency of 5 kHz. Pulse generation, data acquisition and analyses were accomplished using Clampex/Clampfit version 9.2 (Axon Instruments, Inc). Current densities (in pA/pF) were obtained after normalization to cell surface area. The I–V relationships were determined by fitting data to the Boltzmann equation: $I = I_{max}/\{1 + \exp[(V_t - V_{1/2})/k]\}$, where I_{max} was for the maximal current, V_t for the testing potential, $V_{1/2}$ for the membrane potential at which 50% of the channels were activated and k for the slope factor. Time constants for activation and deactivation were obtained by mono-exponentially fitting the currents to a Chebyshev equation with CLAMPFIT software. Furthermore, the concentration–response curves for quinidine block were determined in the absence and presence of OCTN1 to calculate an IC_{50} value, the concentration required to inhibit 50% of the channel current. All experiments were conducted at 22–23°C.

Solutions

To record $K_v1.5$ current, the internal pipette filling solution contained (in mM): KCl 110, K_4BAPTA 5, K_2ATP 5, $MgCl_2$ 1 and HEPES 10. The solution was adjusted to pH 7.2 with KOH, yielding a final $[K^+]_i$ of ~145 mM. The external solution was normal Tyrode's, containing (in mM) NaCl 130, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 10 and glucose 10, and was adjusted to pH 7.35 with NaOH.

Statistical analysis

Data are expressed as mean ± SEM. For comparisons among means of more than two groups, ANOVA was used, with *post hoc* pairwise comparisons by Duncan's test if significant differences among means were detected. If only two groups were being compared, Student's *t*-test was used. A P-value < 0.05 was considered statistically significant.

Materials

All chemicals and drugs in this study were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). The drug concentrations used were close to the published IC_{50} values, and in some experiments, a range of concentrations was used. Drugs used in this study included: quinidine (10 μM), verapamil (20 μM), clofilium (30 μM), propafenone (5 μM), 4-AP (200 μM), erythromycin (200 μM) and KN-93 (1 μM). In experiments with the MDR1 inhibitor reversin-205 (10 μM), the agent was added to cell culture medium for 48 h prior to electrophysiological studies.

Results

The drug influx transporter OCTN1 potentiates block of KCNA5 channel by several drugs

In the absence of any drug transporters, we first tested five known KCNA5 blockers in our system at or near concentrations reported to suppress I_{Kur} by 50%, as described above. Some of these drugs (quinidine and verapamil) have also been tested and found to be OCTN1 substrates (Lee and Kim, 2004). In experiments to assess drug trapping in the channel pore, quinidine was used at a high concentration of 100 μM to obtain a maximal block. The blocking effects of each drug were then compared to those in cells transfected with KCNA5 + OCTN1 plasmid(s).

Figure 1A shows the characteristics of the wild-type KCNA5 current: fast activation, slightly slow inactivation by using a voltage clamp protocol from a holding potential of –80 mV to +60 mV with 500 ms depolarizing pulses at 10 mV steps. In separate experiments in which an equal amount of OCTN1-eGFP plasmid was co-expressed with wild-type KCNA5 channel, the characteristics of KCNA5 current still remained unchanged (Figure 1B). There are no statistically significant differences of the magnitudes of activating and deactivating currents in the two groups of cells (Figure 1C). And the $V_{1/2}$, the membrane voltage at which the KCNA5 channel was activated by 50%, was also unaltered: –12.9 ± 1.6 mV (–OCTN1, n = 7) versus –12.2 ± 1.9 mV (+OCTN1, P = NS, n = 8).

To further test our hypothesis that drug block of the KCNA5 channel could be potentiated by the drug uptake transporter OCTN1, we selected quinidine as a standard KCNA5 blocker to determine the concentration–response curves in the absence and presence of OCTN1. Cells were exposed to quinidine at the concentrations of 1, 3, 10 and 30 μM in order. As shown in Figure 1D and E, superimposed raw current traces recorded with a single 500 ms pulse to +50 mV from a holding potential of –80 mV demonstrate that co-expression of OCTN1 markedly potentiated quinidine block of the KCNA5 current in a concentration-dependent manner. A summary of concentration–response curves is presented in Figure 1F: the IC_{50} values for quinidine block were 7.8 ± 0.9 μM (–OCTN1) versus 4.7 ± 0.3 μM (+OCTN1; n = 4–6 cells, P < 0.01).

Verapamil is an open state blocker of the KCNA5 channel (Rampe *et al.*, 1993), as can be seen in Figure 2B. Verapamil-induced block develops during the depolarizing pulses, especially at more positive potentials where open state probability of the channel is increased. We found that with OCTN1

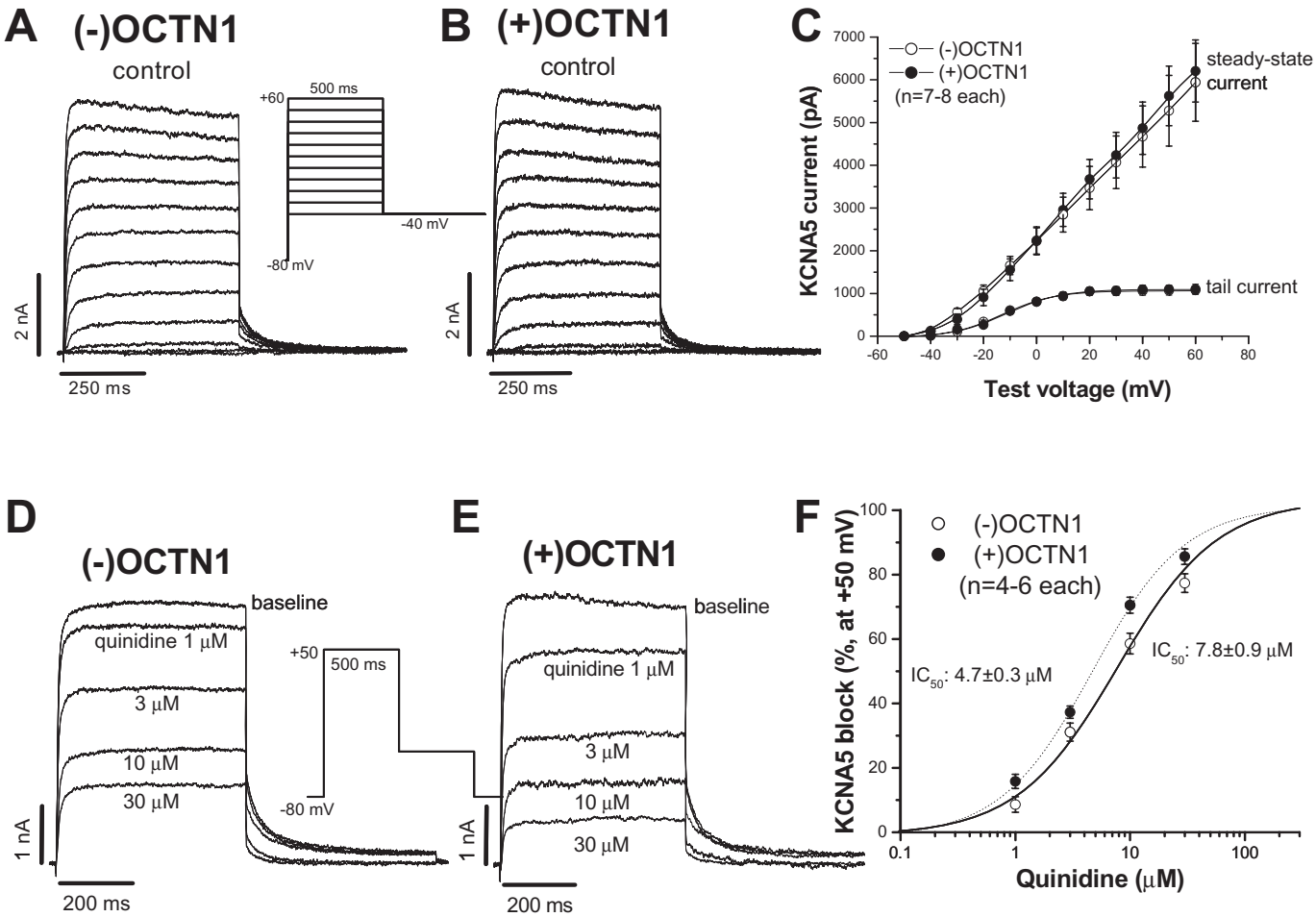

Figure 1

Concentration-dependent block of KCNA5 channel by quinidine in the absence and presence of organic cation transporter 1 (OCNT1). Panel A and B show that co-expression of OCTN1 did not alter the magnitude and gating of the KCNA5 current. Panel C is a summary of activating and deactivating KCNA5 currents in the absence and presence of OCTN1. Panels D and E represent superimposed raw traces at +50 mV and, in F, the concentration–response data for quinidine block of KCNA5 current in the absence and presence of OCTN1 co-expression. The voltage clamp protocols are shown in insets. KCNA5, gene encoding the ultra-rapid outward rectifying K^+ current (I_{Kur}).

expression, verapamil blocked KCNA5 current more rapidly and completely (Figure 2E). Similar results were observed with three other blocking drugs (clofilium, propafenone and 4-AP), and these data are summarized in Figure 2C. These data demonstrate that the drug uptake transporter OCTN1 potentiated the blocking action of several drugs on the cardiac KCNA5 channel.

To further test the idea that this potentiation represents enhanced transport of drug into the cytoplasm, we tested the effects of the H_2-histamine receptor antagonist cimetidine, which is also recognized as an OCTN1 inhibitor (Yabuuchi *et al.*, 1999). We first evaluated the effects of cimetidine (10 μM) on I_{Kur}, and found <5% change in current amplitude (Figure 2F). We next evaluated the effect of cimetidine pretreatment for 30 min on block by quinidine. As described above, 10 μM quinidine suppressed KCNA5 current by ~80% in the presence of OCTN1 (Figure 2F). However, when cells expressing KCNA5 + OCTN1 were pretreated with cimetidine, the same concentration of quinidine reduced current by ~50%, comparable to the effect seen in the absence of OCTN1 co-expression (Figure 2C). These results, summarized in Figure 2F, further support our hypothesis that the transport function of OCTN1 potentiated drug block of KCNA5 channels.

OCTN1 restricts KCNA5 channel recovery from block

Given the results described above, we next examined the time course of recovery from block by quinidine in the absence and presence of the drug uptake transporter OCTN1. We reasoned that when drug block reached a steady state and then drug was

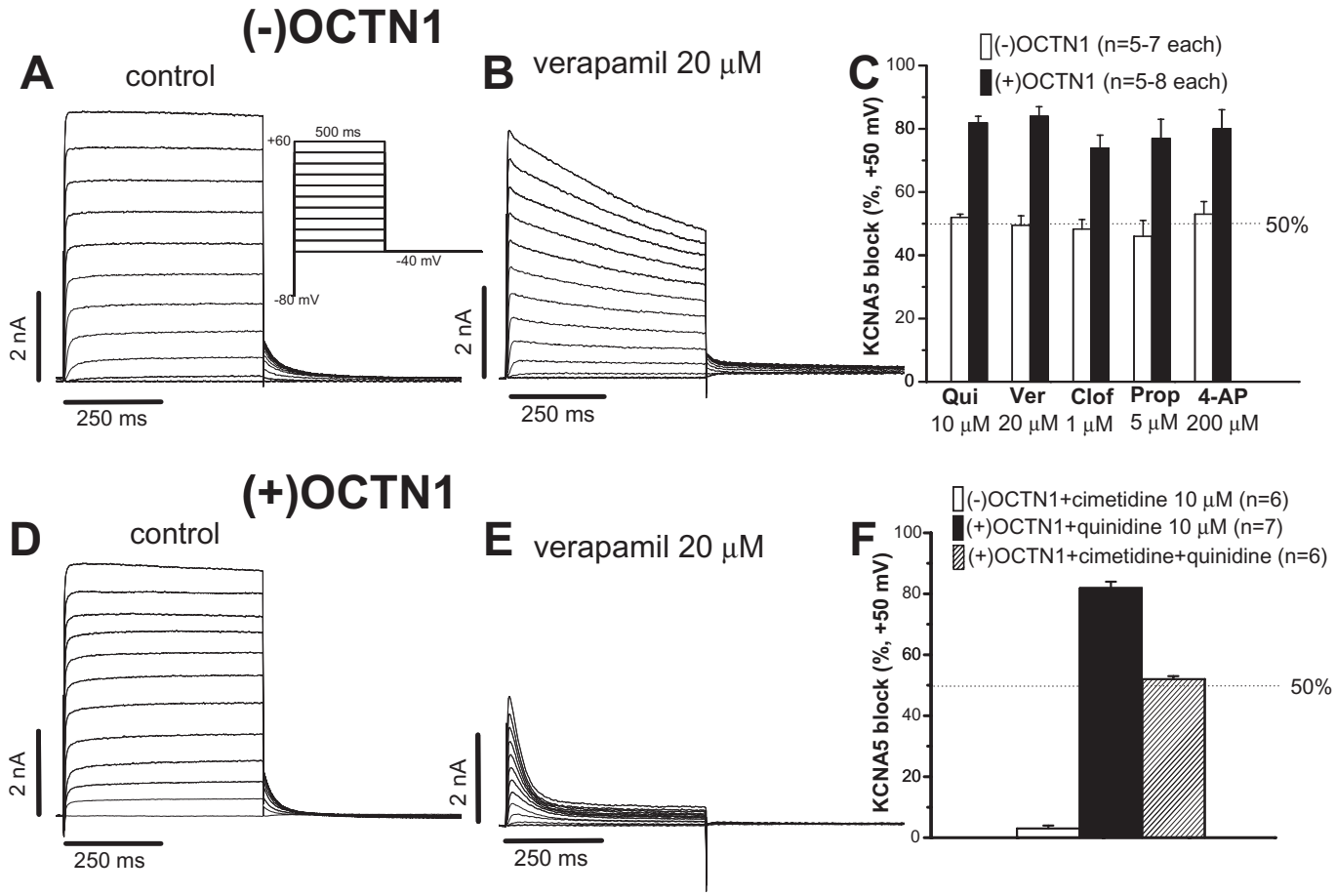

Figure 2

Organic cation transporter 1 (OCTN1) potentiates KCNA5 block by several drugs. Panel A, B, D and E show representative traces prior to and during verapamil (20 μM) in (–) or (+) OCTN1 co-expression for comparison. In the presence of OCNT1, verapamil block was faster in onset and more complete. Panel C summarizes the effects of five KCNA5 blockers (quinidine, Qui; verapamil, Ver; clofilium, Clof; propafenone, Prop; 4-AP) studied in (–) and (+) OCTN1 cells. Panel F shows that the pretreatment with cimetidine (10 μM, an OCTN1 inhibitor) prevented OCTN1 from potentiating quinidine block, whereas cimetidine by itself had no effect on the current. KCNA5, gene encoding the ultra-rapid outward rectifying K^+ current (I_{Kur}).

withdrawn from the extracellular solution, the presence of an uptake transporter would result in slower or incomplete loss of drug effect (since drug leaving a cell would be more likely to undergo reuptake). The pulse protocol we used is shown in Figure 3 (inset). The cells were repetitively stimulated with a 500 ms pulse to +50 mV from a holding potential of –80 mV at 1 Hz. In the absence of OCTN1, 10 μM quinidine reduced the steady-state KCNA5 current by ~50% and block was fully reversible during washout (Figure 3A/C). In the presence of OCNT1 co-expression, however, block by quinidine reached >90% and recovery from block was incomplete (~80%) with washout (Figure 3B/C). In addition, the time constant for the onset of drug block in the presence of OCTN1 was shorter than that in the absence of OCTN1: 13 pulses (+OCTN1) versus 34 pulses (–OCTN1). Also, the time constants for washout were 19 pulses (+OCTN1) versus 10 pulses (–OCTN1). These data lend further support to the idea that OTCN1-mediated drug uptake facilitates quinidine block of the KCNA5 channel.

Figure 4 shows a second approach to further probe recovery from drug block. We first applied a pulse to +50 mV to record KCNA5 current and then examined the time course of recovery from block at the holding potential of –80 mV by varying the inter-pulse interval from 1 to 20 s (Δt), followed by a second pulse back to +50 mV for 100 ms to elicit the current for recovery from block (the protocol is shown in Figure 4D). In this way, dynamic changes in drug block over time can be observed to determine whether recovery from block occurs as the time to allow the channel to re-enter the closed state is varied. In these experiments, we used a high concentration of quinidine (100 μM) which caused a

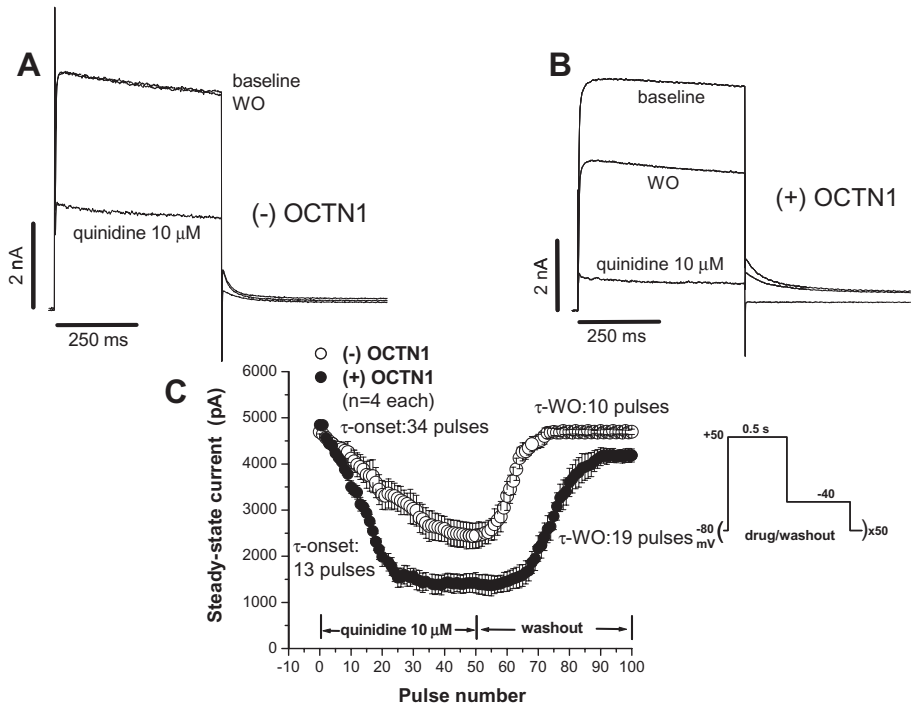

Figure 3

Reversibility of quinidine block in the absence and presence of organic cation transporter 1 (OCTN1) co-expression. These experiments were carried out by using repetitive pulses to +50 mV, shown as an insert. Panels A and B are traces at baseline, during drug exposure and during washout (WO). Panel C shows the current changes over time in two groups of cells (n = 4 each), in which time constants for drug block onset (T onset) and washout (T WO) are indicated.

high degree of block (~80%) even in the absence of OCTN1 (Figure 4A). As expected, when OCTN1 was co-expressed, this concentration of quinidine reduced KCNA5 current >95% (Figure 4B). A summary of the effect of 100 μM quinidine on the KCNA5 current with and without OCTN1 co-expression is shown in Figure 4C: at +50 mV, block was 97.3 ± 2.4% (+OCTN1) versus 83.7 ± 2.1% (–OCTN1, $P < 0.05$, $n = 7$ each). By varying the times at the holding potential (–80 mV), we could observe partial recovery from block in the absence of OCTN1 (Figure 4D): the current gradually increased until it reached a new steady-state level. However, with co-expression of OCTN1, the same pulse protocol revealed almost no recovery from block (Figure 4E). These results, summarized in Figure 4F, imply that the presence of OCTN1 causes an intracellular concentration of quinidine that prevents an observable recovery effect.

The drug efflux transporter MDR1 attenuates drug block of the KCNA5 channel

We also studied the effects of co-expressing MDR1, which encodes the drug efflux transporter P-gp, in this study. We reasoned that a KCNA5 blocker which is also a P-gp substrate would display reduced channel inhibition when the transporter was co-expressed. To test this concept, we used erythromycin, which has been shown to be a P-gp substrate (Sikri *et al.*, 2004; Hariharan *et al.*, 2009) and a KCNA5 blocker (Rampe and Murawsky, 1997).

We first used repetitive 500 ms pulses to +50 mV from a holding potential of –80 mV to define the effects of 200 μM erythromycin (to generate maximal block) with and without co-expression of MDR1. As stated above, erythromycin inhibited KCNA5 current acting as an open-channel blocker. Without MDR1 co-expression, erythromycin reduced the current rapidly and almost completely, within the first 200 ms of pulsing (Figure 5A). Furthermore, block was only partially reversible by washout. When MDR1 was co-expressed, however, block by erythromycin was less and almost completely removed, with recovery of the current to the basal level during washout (Figure 5B).

Figure 5C summarizes the degree of KCNA5 block by erythromycin (200 μM) in the absence and presence of MDR1. MDR1 co-expression did not affect basal KCNA5 current level. Without MDR1 co-expression, erythromycin markedly suppressed the current at all test potentials. With MDR1 expression, however, a portion of the current remained

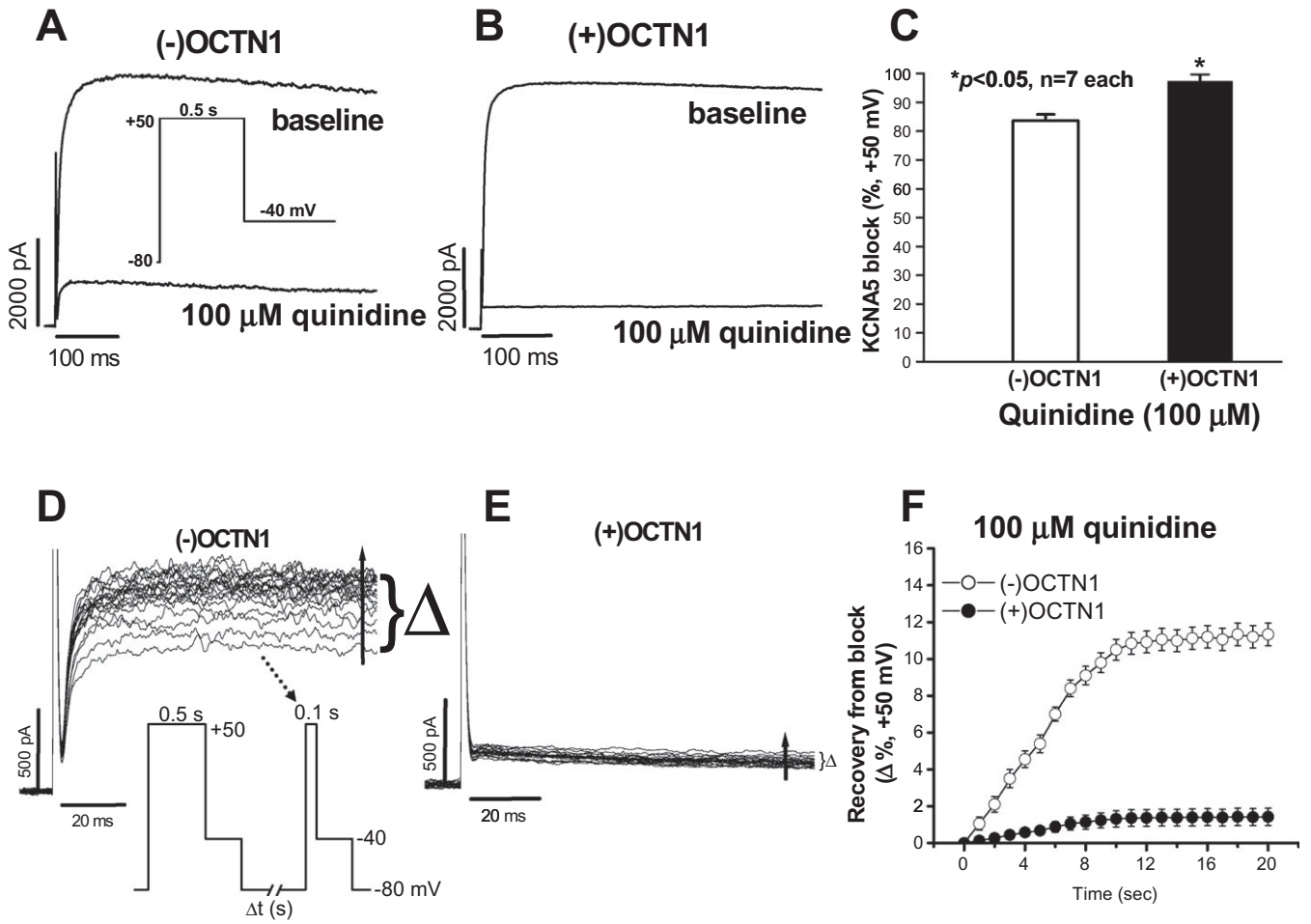

Figure 4

Organic cation transporter 1 (OCTN1) restricted channel recovery from block. (A) With a regular pulse protocol (inset), a high concentration of quinidine suppressed ~85% of KCNA5 current at +50 mV in the absence of OCTN1. (B) Quinidine achieved near complete block of the current at +50 mV in the presence of OCTN1. (C) Summarized data in two groups of cells. (D) Partial recovery from block was seen in the absence of OCTN1 in the experiments with the pulse protocol shown. The dashed arrow indicates the protocol step in which the current is measured. The solid up-arrow represents the direction of the KCNA5 current change (Δ). E, in the presence of OCTN1 the current showed a complete block without recovery from block at 100 µM quinidine. F, a summary of recovery from drug block over time ($n = 4$ each). KCNA5, gene encoding the ultra-rapid outward rectifying K^+ current (I_{Kur}).

unblocked: for example, block at +50 mV was 91 ± 2% (–MDR1) versus 62 ± 3% (+MDR1, $P < 0.01$, $n = 7$ each; Figure 5D). This is consistent with the idea that MDR1 co-expression reduces intracellular drug concentration, thereby attenuating drug block.

MDR1 inhibition restored block of KCNA5 channel by erythromycin, but not by KN-93

KN-93 is a channel blocker acting on the outer mouth of the KCNA5 channel (Rezazadeh *et al.*, 2006). This compound also acts as a non-substrate inhibitor of P-gp (Riganti *et al.*, 2009). In the next set of experiments, we examined the effects of erythromycin and KN-93 on KCNA5 channels with and without 48 h pretreatment by reversin-205 (10 µM), a membrane-permeable MDR1 inhibitor (Sharom *et al.*, 1999), in the absence and presence of MDR1 co-expression. These experiments were conducted in both CHO and L-MDR1 cells, stably expressing MDR1. Block by erythromycin in L-MDR1 cells was identical to that in CHO cells with MDR1 co-expression (Figure 5D). In CHO cells transfected with MDR1, reversin-205 pretreatment completely restored erythromycin block to the level seen without MDR1 co-expression (Figure 5D). Similarly, when L-MDR1 cells were pretreated with reversin-205, the extent of erythromycin block of the current was very similar to those observed in (–)MDR1 and (+)MDR1 + reversin in CHO cells (Figure 5D).

By contrast, KCNA5 block by KN-93 (1 µM), which acts on KCNA5 channels from the extracellular aspect, was unaffected despite the dual inhibi-

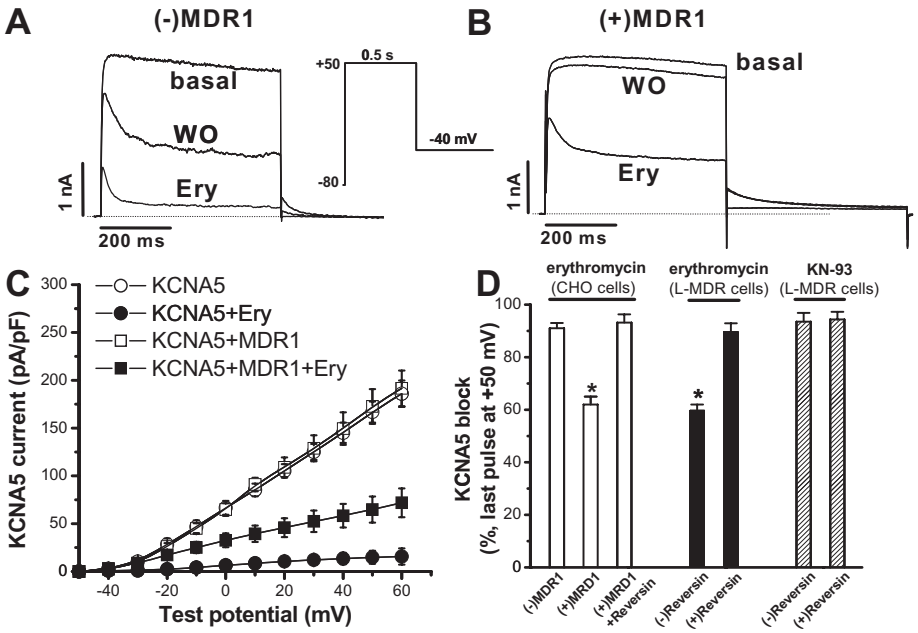

Figure 5

Multiple drug-resistant gene 1 (MDR1) attenuated erythromycin (Ery) block of KCNA5 current. (A) Representative traces pre-/post-drug (Ery at 200 μM) in the absence of MDR1 co-expression. (B) Representative traces pre-/post-drug in the presence of MDR1 co-expression. (C) Summarized data in four groups of cells ($n = 7$ each) with/without drug in the absence and presence of MDR1 co-expression. The voltage clamp protocol is shown in the inset. (D) comparison of KCNA5 block by two drugs (200 μM erythromycin and 1 μM KN-93) in two types of cells [Chinese hamster ovary cells (CHO) and L-MDR1] in the absence and presence of the MDR1 inhibitor reversin-205 (10 μM). *$P < 0.01$ versus (–)MDR1 or (+)reversin. KCNA5, gene encoding the ultra-rapid outward rectifying K^+ current (I_{Kur}).

tion of MDR1 by reversin-205 and KN-93 itself (Figure 5D). Taken together, these data support the idea that the efflux transporter P-gp decreases intracellular drug concentration and thereby reduces the extent of block by drugs acting at intracellular sites of action.

Discussion and conclusions

We and others have previously shown that drug transporter proteins are expressed in heart and we have shown that co-expression of influx or efflux transporters modulates block of the rapid component of the cardiac delayed rectifier channel (encoded by *KCNH2*) in heterologous expression systems. The present study extends this work to show that drug uptake and efflux transporters can similarly modulate block of the KCNA5 channel, which – like KCNH2 – includes a drug-binding/blocking site on its intracellular aspect.

Atrial arrhythmias (AF and flutter) are important cardiac rhythm disorders in humans (Savelieva and Camm, 2008b). Drug treatment is widely used, and inhibition of atrial repolarizing potassium current(s) is a common approach to suppress arrhythmias by increasing effective refractoriness (Ehrlich and Nattel, 2009). In human atrial cells, an important and tissue-specific potassium current is the ultra-rapidly activating outward potassium current (I_{Kur}), which is encoded by KCNA5 ($K_v1.5$) (Wang *et al.*, 1993; Li *et al.*, 1996; Nattel *et al.*, 1999). Numerous studies have demonstrated that the native cardiac atrial-specific I_{Kur} and cloned human cardiac $K_v1.5$-generated current share common biophysical and pharmacological features (Snyders *et al.*, 1993; Yeola *et al.*, 1996). Accordingly, KCNA5 is considered a target for drugs used in the treatment of AF, so our findings imply that blocking drugs that are also transporter substrates may display variable efficacy because of variable transporter function or expression. A number of drugs can produce the non-selective potent blocking action on the KCNA5 channel, besides other (non)cardiac effects, and we have used some of those drugs here to test the role of drug transporters, such as quinidine, verapamil, clofilium, propafenone, 4-AP, erythromycin and KN-93.

Evidence that KCNA5 block is mediated by drug interactions with an intracellular site

Atrial repolarization is accomplished through activation of multiple K^+ currents, including I_{TO} (encoded by *KCND3*), ultra-rapid I_{Kur} (encoded by

KCNA5), I_{Kr} (encoded by *KCNH2*) and I_{Ks} (encoded by *KCNQ1* + *KCNE1*). Like all voltage-gated potassium channels, the KCNA5 protein complex carrying I_{Kur} consists of four α subunits, each with six transmembrane segments. Inactivation of I_{Kur} occurs via an 'N-type' mechanism in which a β-subunit protein ($K_v\beta1.3$) occludes the pore formed by the tetrameric channel complex (Decher *et al.*, 2005). The interaction of $K_v\beta1.3$ with KCNA5 α-subunits has been shown to competitively attenuate the effect of KCNA5 antagonists by competitively mimicking N-type inactivation-induced blockade of the ion channel pore at the intracellular face (Khumar *et al.*, 2004; Decher *et al.*, 2006; Arias *et al.*, 2007).

Most K^+ channel blockers inhibit current by binding to intracellular site(s), such as internal mouth of the ion channel pore. In the KCNH2 channel, specific aromatic amino acid residues lining the intracellular face of the S6 transmembrane domain are critical for drug binding and block by methanesulfonanilide and other KCNH2 inhibitors (Mitcheson *et al.*, 2000; Chen *et al.*, 2002; Perry *et al.*, 2004). Similarly, in the KCNA5 channel, residues of the S6 segment facing towards the channel pore cavity are critical for block by quinidine and other blocking drugs (Decher *et al.*, 2004; 2006; Strutz-Seebohm *et al.*, 2007). However, KN-93 suppresses the current through binding to the external mouth of the KCNA5 channel pore.

Evidence that drug influx and efflux transporters are present in heart

Variable drug metabolism is a commonly invoked mechanism underlying variable drug actions. Given the recognition of specific families of drug uptake and efflux transporters, extensive studies have demonstrated that variable transporter function also contributes to variable drug actions (Kim, 2002; 2006; Lee and Kim, 2004). Such variability may arise from variable transporter expression, variable function due to polymorphism generating variable primary amino acid sequences, or drug interactions inhibiting or enhancing transport function.

We and others have shown that drug transporters, including OCTN1 and MDR1, are expressed on the surface of cardiomyocytes. Iwata *et al.* (2008) reported that the drug influx transporters OCTN1 and OCTN2 localize on the plasma membrane of cardiomyocytes from both atrium and ventricle in mouse heart and mediate uptake of radioactively labelled quinidine. Since drug blocking sites on cardiac ion channels are often located intracellularly (as discussed further below), these data provide support for testing the hypothesis that the effects of drugs that block ion channels may be modulated by transport molecules for which they are also substrates.

Modulation of KCNA5 block by OCTN1 and MDR1

We propose two possible mechanisms for the effect of OCTN1 to potentiate drug block. First, we propose that OCTN1 accelerates and increases intracellular drug accumulation to facilitate drug access to the binding site(s) in the inner mouth of the channel pore, thereby strengthening drug block. Second, intracellular drug accumulation would also be predicted to slow dissociation of drug from its binding site(s) in the channel and thereby further lead to prolonged block.

For the experiments to test the effect of the efflux transporter MDR1 on KCNA5 drug block, we used the antibiotic erythromycin which is both a channel blocker and an MDR1 substrate. As predicted, the strong KCNA5 inhibition that we observed with erythromycin was markedly inhibited by MDR1. This result is in good agreement with our previous study in which MDR1 attenuated inhibition of HERG channel by a potent I_{Kr} blocker ibutilide (McBride *et al.*, 2009b). In these experiments, we also used the MDR1 inhibitor reversin-205 to suppress MDR1 activity before testing the block by erythromycin, and found that block was restored to the level similar to that seen in the absence of MDR1 expression. Finally, when we tested the effect of KN-93, an MDR1 substrate that blocks KCNA5 from the outside of the cell, we found no change in the extent of block regardless of MDR1 expression. This finding supports the concept that transporter function does not modulate the effects of a blocker acting at the external aspect of the channel.

In conclusion, in this study, we have shown that drug-induced block of the atrial-specific KCNA5 channel, responsible for carrying I_{Kur}, can be modulated by drug influx and efflux transporters. Accordingly, variability in the action of such drugs may reflect not only traditionally recognized factors (such as variable absorption or elimination) but also variability in these transport processes.

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Conflict of interest

None.

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