INTRODUCTION

A number of different potassium channels contribute to repolarisation of cardiac ventricular action potentials (1). The rapid and slow delayed rectifier K+ currents (denoted 'IKr' and 'IKs' respectively) play important roles in determining repolarisation over the ventricular action potential (AP) plateau phase and, thereby, in determining the QT interval of the electrocardiogram (ECG) (1, 2); subsequent terminal AP repolarisation is mediated predominantly by the inwardly rectifying K+ current, IK1 (1). IKs plays an important role in autonomic regulation of ventricular repolarisation (3, 4); it also provides a ventricular 'repolarisation reserve' that restricts excessive action potential prolongation under conditions of reduced IKr (5, 6). Functional IKs channels are comprised of an α subunit encoded by KCNQ1 and a β subunit encoded by KCNE1 (7, 8) with the gene products also respectively denoted KCNQ1 and KCNE1). Mutations in either gene can lead to clinically significant cardiac repolarisation disorders (1, 2, 9, 10).

The short QT syndrome (SQTS) is a cardiac repolarisation disorder in which patients exhibit shortened QT intervals on the electrocardiogram and an increased incidence of cardiac arrhythmias and sudden death in the absence of structural heart disease (10, 11). Gain-of-function mutations to three K+ channel genes (KCNH2, KCNQ1 and KCNJ2) have been identified in SQTS patients, giving rise to the SQT1, SQT2 and SQT3 variants of the syndrome respectively (12-16). The adult SQT2 variant was identified in a 70 year old male who was resuscitated from an episode of ventricular fibrillation (15). No physical abnormalities were present and in sinus rhythm normal conduction intervals were observed (15). However, marked abbreviation of ventricular repolarisation was observed both on initial presentation (with a rate corrected QT(QTC) interval of 302 ms) and over a three year follow-up period (15). Genetic testing identified a G→C substitution at nucleotide 919 of the KCNQ1 gene, giving rise to a single amino-acid substitution (valine→leucine) at position 307 (V307L) of the KCNQ1 protein (15).

Residue V307 of KCNQ1 is located in the channel's pore helix and the V307L mutation was reported to alter activation time-course and voltage-dependence, giving rise to increased current through co-expressed V307L-KCNQ1+KCNE1 channels in conventional voltage-clamp experiments (15). Although computer simulations have predicted accelerated ventricular repolarisation and shortened refractory periods at cell and tissue
levels as a result of the V307L KCNQ1 mutation (15, 17), to-date there has been no direct characterisation of increased repolarising current during physiological waveforms with this mutation. By contrast, for both the SQT1 KCNH2 and SQT3 KCNJ2 mutations the action potential (AP) voltage-clamp technique has been used to provide direct insight into altered timing and magnitude of repolarising currents during AP waveforms (12, 18-21). In addition, whilst in vitro investigations have identified effective pharmacological inhibitors of SQT1 and SQT3 mutant K+ channels (21-24), no such pharmacological data have yet been reported for channels incorporating SQT2 V307L mutant KCNQ1 subunits. The present study was undertaken to address these issues. Thus we report here, for the first time: (i) AP clamp data for V307L-KCNQ1-KCNE1 channels and (ii) an effective pharmacological inhibitor of these channels.

MATERIALS AND METHODS

Maintenance of cells expressing wild-type (WT) and V307L KCNQ1 together with KCNE1

WT KCNQ1 (in pIRES expression vector) was kindly provided by Dr J Bahranin. Plasmid encoding the common S38 variant of KCNE1 (in pCR3.1) (25;26) was kindly donated by Dr F Toyoda. The V307L mutation was introduced into KCNQ1 using QuikChange® (Stratagene; mutagenesis primer of: 5GCT GTG GTG GGG GCT GGT CAC AGT CAC 3). DNA was sequenced for the full length of the KCNQ1 insert to ensure that only the correct mutation had been made (Eurofins MWG Operon).

Chinese Hamster Ovary (CHO) cells were passaged using a non-enzymatic agent (Enzyme Free, Chemicon®International) and then maintained as described previously. Twenty-four hours after plating cells out, the cells were transiently co-transfected 2:1 with KCNE1 and either WT or V307L-KCNQ1 (0.7 µg of each KCNQ1 construct was used) using Lipofectamine™ LTX (Invitrogen) according to the manufacturer’s instructions. Expression plasmid encoding CD8 was also added (in pRES, donated by Dr I Baro and Dr J Barhanin) to be used as a successful marker of transfection. Cells were plated onto small sterilised collagen-coated glass coverslips 6 hours after transfection and recordings were made after at least 24 hours incubation at 37°C. Successfully transfected cells (positive to CD8) were identified using Dynabeads® (Invitrogen).

Electrophysiological recordings

For perforated patch-clamp recording cells were continuously superfused (at 37°C) with an external solution containing (in mM): 140 NaCl, 4 KCl, 2.5 CaCl2, 1 MgCl2, 10 Glucose and 5

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**Fig. 1.** WT and V307L IkCNQ1-KCNE1 under conventional voltage clamp. (A) Upper traces show records of WT (Ai) and V307L IkCNQ1-KCNE1 (Aii) elicited by 3-s duration voltage clamp commands applied from -80 mV to a range of potentials (lower traces). The numbers adjacent to each of the currents shown in Ai and Aii indicate the command voltage of the corresponding 3-s command used to elicit IkCNQ1-KCNE1. A brief 50 ms pulse to -40 mV from the holding potential of -80 mV was incorporated prior to each test command in order to monitor instantaneous leak current, which can be seen to be negligible in these recordings. Following each test command a 5-s repolarising step to -40 mV was incorporated, enabling observation of deactivating tail currents. Successive steps in the protocol were applied every 10 s. (B) End pulse current-voltage (I-V) relations for WT and V307L IkCNQ1-KCNE1 (n=13 and 9 and denoted by ‘squares’ and ‘triangles’ respectively; *** denotes P<0.001, ** denotes P<0.01, * denotes P<0.05). The protocol used to determine I-V relations is similar to that shown in ‘A’, with successive 10 mV steps from -70 mV to +60 mV (test pulse frequency of one pulse every 10 s). (C) Normalized current-voltage (I-V) relations for peak ‘tail’ amplitudes for WT and V307L IkCNQ1-KCNE1 (n=13 and 9 and denoted by ‘squares’ and ‘triangles’ respectively, voltage protocol as in B). Normalization was carried out as follows: for each cell the amplitude of IkCNQ1-KCNE1 on repolarisation to -40 mV following different test potentials was normalized to the maximum current observed during application of the protocol. The resulting data were fitted with equation 1 to give the V0.5 and k values in the ‘Results and Discussion’ text.
HEPES (titrated to pH 7.45 with NaOH) (21). Patch-pipettes (Corning 7052 glass, AM Systems) were pulled and heat-polished (Narishige MF83) to 2.5-4 MΩ; pipette dialysate contained (in mM): 130 KCl, 1 MgCl₂, 5 EGTA, 5 MgATP, 10 HEPES (titrated to pH 7.2 using KOH) (21) and 225 µg/mL amphotericin B. Recordings of KCNQ1+KCNE1 current (I_{KCNQ1-KCNE1}) were made using an Axopatch 200 amplifier (Axon Instruments) and a CV201 head-stage. Between 70-80% of pipette series resistance was compensated. Voltage-clamp commands were generated using 'WinWCP' (John Dempster, Strathclyde University). Human ventricular and atrial AP waveforms used as voltage commands in AP clamp experiments were generated using established ventricular (27) and atrial (28) cell mathematical models. I_{KCNQ1-KCNE1} elicited under AP clamp was obtained using 'p/4' leak subtraction (cf (29)).

Experimental compounds

Mefloquine-HCl powder (Sigma) was dissolved in DMSO to produce an initial stock solution of 50 mM which was diluted to produce stock solutions ranging down to 100 µM. The mefloquine-HCl stock solutions were diluted at least 1:1000-fold with Tyrode's solution to achieve concentrations stated in the Results. External solutions were applied using a home-built, warmed and rapid solution exchange device. Control solution for experiments with mefloquine contained DMSO (in order to constitute a vehicle control) at a concentration matching that in the mefloquine-containing solutions tested.

Data analysis

Unless otherwise stated in the text data are presented as mean±standard error of the mean (S.E.M.). The voltage dependence of I_{KCNQ1-KCNE1} activation was determined by fitting the values of I_{KCNQ1-KCNE1} tail currents (normalised to peak I_{KCNQ1-KCNE1} tail value and plotted against voltage) with a Boltzmann equation of the form:

\[ I = \frac{I_{\text{MAX}}}{1 + \exp \left( \frac{V_{0.5} - V_m}{k} \right)} \]

where I is the I_{KCNQ1-KCNE1} tail amplitude following test potential V_m, I_{MAX} is the maximal I_{KCNQ1-KCNE1} observed, V_{0.5} is the potential at which I_{KCNQ1-KCNE1} was half-maximally activated, and k is the slope factor for the relationship. The time-course of I_{KCNQ1-KCNE1} activation and deactivation were fitted with standard bi-exponential equations. Concentration-response relations were fitted with a standard Hill equation to obtain half-maximal inhibitory concentration (IC₅₀) and Hill-coefficient (n_H) values. Statistical analysis was performed using analysis of variance or t-tests as appropriate (Graphpad Prism v5). P values of less than 0.05 were taken as statistically significant.

RESULTS

Effects of V307L on I_{KCNQ1-KCNE1} under conventional voltage clamp

The functional consequences of the V307L mutation for I_{KCNQ1-KCNE1} were first characterised using conventional whole-cell voltage clamp protocols. Fig. 1A shows representative traces for WT (Fig. 1Ai) and V307L (Fig. 1Aii) I_{KCNQ1-KCNE1} elicited by depolarisation from -80 mV to the test potentials shown. As shown in Fig. 1Ai, a test pulse to -30 mV elicited relatively little WT I_{KCNQ1-KCNE1} whilst, in contrast, markedly larger I_{KCNQ1-KCNE1} was evident for V307L KCNQ1 at this voltage. Mutant I_{KCNQ1-KCNE1} was also markedly larger than WT current at 0 mV. Fig. 1B shows mean end-pulse current-voltage (I-V) relations for WT and V307L I_{KCNQ1-KCNE1}, showing that...
across a range of potentials between -10 and +60 mV V307L I\textsubscript{KCNQ1-KCNE1} was significantly greater than the corresponding WT current. Fig. 1C shows steady-state voltage-dependent activation plots for WT and V307L I\textsubscript{KCNQ1-KCNE1}, with fits to normalized 'tail' current data with equation 1 to derive V\textsubscript{0.5} and k values. There was a marked left-ward shift in activation V\textsubscript{0.5} for V307L I\textsubscript{KCNQ1-KCNE1} (-23.46±0.73 mV; n=9; P<0.001) compared to WT I\textsubscript{KCNQ1-KCNE1} (V\textsubscript{0.5} of +12.52±0.41 mV; n=13), without any significant change to the slope of the relationship (with k values for V307L and WT I\textsubscript{KCNQ1-KCNE1} respectively of 16.49±0.67 mV and 13.69±0.37 mV; P>0.1). This left-ward shift in the voltage-dependence of activation can account for the greater currents at negative voltages for V307L shown in Fig. 1A.

The effects of the V307L mutation on the time-course of I\textsubscript{KCNQ1-KCNE1} activation and deactivation were assessed using bi-exponential curve fitting of the time-course of current development on depolarisation (activation) and decline on repolarisation (deactivation). Similar to Bellocq and colleagues (15), we observed a tendency for both fast and slow activation time-constants to be smaller in magnitude for V307L than for WT I\textsubscript{KCNQ1-KCNE1}, particularly at negative membrane voltages (Fig. 2A, Aii). However, the time-course of activation for WT I\textsubscript{KCNQ1-KCNE1} exhibited considerable heterogeneity in our study, so at the majority of test potentials examined the differences between WT and V307L I\textsubscript{KCNQ1-KCNE1} activation time-constants did not attain statistical significance. By contrast, however, deactivation of V307L I\textsubscript{KCNQ1-KCNE1} was markedly slower than that of WT I\textsubscript{KCNQ1-KCNE1}. This is evident both in the current records shown in Fig. 1A and in the time-constant plots shown in Fig. 2Bi and Bii. The fast time-constant of deactivation was slower for V307L than for WT I\textsubscript{KCNQ1-KCNE1} across all plotted voltages (-60 mV to 0 mV), whilst the slow time constant was also larger for V307L at four of seven test voltages at which deactivation time-course was examined.

**AP clamp of WT and V307L I\textsubscript{KCNQ1-KCNE1}**

The results of AP clamp experiments using an epicardial ventricular AP waveform (19, 21) are shown in Fig. 3. Fig. 3Ai and 3Aii show WT and V307L I\textsubscript{KCNQ1-KCNE1} each overlain on the ventricular AP command. Very little outward current occurred immediately on depolarisation, but current then increased progressively throughout the AP plateau reaching peak amplitude late in the plateau phase. V307L I\textsubscript{KCNQ1-KCNE1} showed a similar profile to that of WT I\textsubscript{KCNQ1-KCNE1}, except that the current
amplitude was markedly larger. Fig. 3Bi and 3Bii show instantaneous I-V plots of current during AP repolarisation for the same cells shown in Figs. 3Ai and 3Aii. For both WT and V307L IKCNQ1-KCNE1 maximal currents occurred between ~0 and +10 mV; WT maximal repolarising current occurred at +5.9±0.9 mV (n=13), whilst V307L maximal repolarising current occurred at +4.8±1.0 mV (n=12; P>0.1 vs. WT). Fig. 3C shows mean plots of the maximal amplitudes of WT and V307L IKCNQ1-KCNE1, demonstrating a significantly greater (>2-fold) maximal repolarising current with the SQT2 mutation.

Although atrial arrhythmia was not reported for the patient in whom the V307L mutation was first identified (15), some SQTS patients do experience atrial fibrillation (10, 11). Consequently, and also for purposes of comparison with ventricular AP clamp data, we studied the effects of the V307L KCNQ1 mutation on the profile of IKCNQ1-KCNE1 during an atrial AP command waveform. The results of atrial AP clamp experiments are shown in Fig. 4. For six WT KCNQ1+KCNE1 expressing cells, a small IKCNQ1-KCNE1 was observed when the atrial AP command was applied (Fig. 4Ai); current increased relatively gradually during the applied AP command, attaining its maximal amplitude relatively late during the AP. This is consistent with a previous simulation of the profile of IC during atrial APs (28). For V307L KCNQ1+KCNE1, IKCNQ1-KCNE1 elicited by the atrial AP waveform was of a similar overall profile to that of WT IKCNQ1-KCNE1 but exhibited a greater magnitude (compare Fig. 4Ai with Fig. 4Aii). Fig. 4B shows instantaneous I-V relations for WT and V307L during atrial AP repolarisation (for the same cells as Fig. 4Ai and Aii); for 6 cells exhibiting WT IKCNQ1-KCNE1 maximal repolarising current occurred at -19.1±1.0 mV, whereas for 8 cells exhibiting V307L IKCNQ1-KCNE1 this value was -20.9±1.0 mV (P<0.01 vs. WT). As shown in Fig. 4C, the mean maximal amplitude of V307L IKCNQ1-KCNE1 during repolarisation was more than three-fold that for WT IKCNQ1-KCNE1. However, for both WT and mutant channels the current was much smaller than the corresponding current seen during ventricular APs (compare Fig. 3B,C and 4B,C). The atrial AP command differed from the ventricular AP used (cf Fig. 4A with Fig. 3A) both in exhibiting an initial rapid repolarisation phase and in possessing a lower and briefer duration plateau phase. Consequently, both the smaller current amplitude and the more negative value of peak repolarising current can be attributed to the relatively lower and shorter plateau phase of the atrial AP command, which would result in less extensive current activation compared to the ventricular AP waveform.

**Fig. 4.** WT and V307L IKCNQ1-KCNE1 during atrial AP clamp. (A) Profile of WT (Ai) and V307L (Aii) IKCNQ1-KCNE1 (the thicker of the pair of overlaid traces) during an applied atrial AP command (the thinner of the pair of overlaid traces; AP command frequency of 1 Hz). Each of (Ai) and (Aii) has its own time axis, whilst the current axis (shown to the left of (Ai)) and voltage axis (shown to the right of (Aii)) apply to both panels. (B) Representative instantaneous current-voltage (I-V) relations for WT (Bi) and V307L (Bii) IKCNQ1-KCNE1 during atrial AP repolarisation (direction of repolarisation denoted by arrows). Instantaneous I-V relations show continuous plots of current recorded during AP repolarisation and the direction of repolarisation on each plot is indicated by the filled ‘arrow’ symbols. (C) Comparison of mean (±S.E.M) maximal repolarising current during AP repolarisation between WT and V307L IKCNQ1-KCNE1 (n=6 and 8 respectively; P<0.05).
A pharmacological inhibitor of V307L IKCNQ1-KCNE1

To date an equi-effective pharmacological inhibitor of WT and V307L IKCNQ1-KCNE1 has not been identified. The archetypal selective 'Ik' inhibitor chromanol 293B (trans-N-[6-Cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2H-1-benzopyran-4-yl]-N-methyl-ethanesulphonamide) has been reported to bind to KCNQ1 at a site comprising residues from the pore-helix and S6 segment, and the V307L mutation has been reported to reduce significantly the sensitivity of KCNQ1 to chromanol 293B, both for KCNQ1 expressed alone and for KCNQ1+KCNE1 (30). In a limited series of experiments with this compound (not shown) we also observed a reduced ability of chromanol 293B to inhibit V307L IKCNQ1-KCNE1. By contrast, the quinoline antimalarial drug mefloquine ([2,8-bis(trifluoromethyl)quinolin-4-yl]-piperidin-2-ylmethanol; which is structurally related to the antiarrhythmic drug quinidine) has been reported to be an effective inhibitor of WT IKCNQ1-KCNE1 that exhibits blocking characteristics suggestive that channel opening may not be obligatory for inhibition to occur (31). We reasoned, therefore, that this drug might be an effective pharmacological inhibitor of V307L IKCNQ1-KCNE1. In order to test this proposition, the protocol shown in the lower panels of Figs. 5Ai and 5Aii was applied repeatedly for both WT and V307L KCNQ1+KCNE1 in control external solution and during the application of a range of mefloquine concentrations. Fractional inhibition of the elicited current typically reached steady-state within 4-6 minutes and WT and V307L IKCNQ1-KCNE1 measurements in control and mefloquine used to construct concentration-response relations. As Figs. 5Ai and Aii show, 30 µM mefloquine produced a marked and similar level of inhibition of WT and V307L IKCNQ1-KCNE1. For a total of six mefloquine concentrations (ranging from 0.1 µM to 100 µM), mean observed fractional block obtained at the end of the 4 s test pulse was plotted against concentration and concentration-response relations were obtained as shown in Fig. 5B. The calculated IC50 value for mefloquine block of WT IKCNQ1-KCNE1 was 3.4 µM (95% CI: 2.4 to 4.8 µM) with a nH of 0.7±.0.1. For the V307L KCNQ1 mutant, the calculated IC50 value was 3.3 µM (95% CI: 2.3 to 4.8 µM) with a nH of 0.7±0.1 (P>0.1). Thus at each of two time-points examined, WT and V307L IKCNQ1-KCNE1 showed a similar sensitivity to inhibition by mefloquine.

DISCUSSION

This is only the second study to investigate in detail in vitro the effects of the V307L mutation on IKCNQ1-KCNE1 (15), and we report here additional kinetic modifications (discussed below) to IKCNQ1-KCNE1 with the V307L KCNQ1 mutation to those reported previously (15). In addition, and in contrast with the situation in

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**Fig. 5.** Comparison of sensitivity to inhibition by mefloquine between WT and V307L IKCNQ1-KCNE1. (A) Upper traces show representative current records in normal extracellular solution (Control) and following exposure to 30 µM mefloquine for WT (Ai) and V307L (Aii) IKCNQ1-KCNE1. The lower traces show the voltage protocol used to elicit the observed currents (start-to-start interval between successive applications of the protocol was 10 s). (B) Concentration-response relationships for inhibition by mefloquine of WT and V307L IKCNQ1-KCNE1. End pulse currents were used to assess mefloquine inhibition (n=4-6 cells per concentration; ‘squares’ denote response of WT IKCNQ1-KCNE1, whilst ‘triangles’ denote response of V307L IKCNQ1-KCNE1). IC50 and nH values are given in the Results and Discussion text. The following concentrations of mefloquine were tested: 0.1 µM, 1 µM, 3 µM, 10 µM, 30 µM and 100 µM.
respect of KCNH2 and KCNJ1 mutations involved in the SQT1 and SQT3 variants of the SQT5 (12, 18-21), this is the first investigation to have employed the AP voltage-clamp technique in the study of the effects of the V307L KCNQ1 mutation. Also, it is the first study to report an effective pharmacological inhibitor of V307L IKCNQ1-KCNE1.

**Effects of the V307L mutation under voltage and AP clamp**

Under our conditions, the V307L KCNQ1 mutation led to a marked (-36 mV) leftward shift of half-maximal activation V0.5 for IKCNQ1-KCNE1. This shift is significantly larger than that reported previously for the V307L mutation when I KCNQ1-KCNE1 was studied using COS-7 cells (~18 mV) (15). It is unclear what accounts for the differences in the two studies in this regard. It is possible to speculate that the different expression systems used (simian-derived COS-7 cells in (15) and CHO cells in the present study) may play a role, via differentially affecting the properties of expressed WT and mutant KCNQ1+KCNE1 channels; the two studies are similar in that each employed perforated patch-clamp recording and similar recording temperatures (37°C in the present study; 35°C in that of Bellocq and colleagues (15)). In common with Bellocq et al. (15) we observed a tendency for the time course of activation of IKCNQ1-KCNE1 to accelerate, although in our hands this difference did not attain statistical significance at the majority of voltages - probably because of the considerable heterogeneity in activation time-course exhibited by WT IKCNQ1-KCNE1 in the present study. However, we observed a significant increase of both fast and slow time constants of deactivation. This effect of the V307L KCNQ1 mutation on I KCNQ1-KCNE1 has not been reported previously and may be of functional significance: slowed deactivation would be anticipated to both contribute to membrane conductance immediately following AP peak current during the AP was observed, providing the first evidence for IKCNQ1-KCNE1; this shift is significantly larger than that reported previously for the V307L mutation when I KCNQ1-KCNE1 was studied using COS-7 cells (~18 mV) (15). It is unclear what accounts for the differences in the two studies in this regard. It is possible to speculate that the different expression systems used (simian-derived COS-7 cells in (15) and CHO cells in the present study) may play a role, via differentially affecting the properties of expressed WT and mutant KCNQ1+KCNE1 channels; the two studies are similar in that each employed perforated patch-clamp recording and similar recording temperatures (37°C in the present study; 35°C in that of Bellocq and colleagues (15)). In common with Bellocq et al. (15) we observed a tendency for the time course of activation of IKCNQ1-KCNE1 to accelerate, although in our hands this difference did not attain statistical significance at the majority of voltages - probably because of the considerable heterogeneity in activation time-course exhibited by WT IKCNQ1-KCNE1 in the present study. However, we observed a significant increase of both fast and slow time constants of deactivation. This effect of the V307L KCNQ1 mutation on I KCNQ1-KCNE1 has not been reported previously and may be of functional significance: slowed deactivation would be anticipated to both contribute to membrane conductance immediately following AP peak current during the AP was observed, providing the first direct experimental validation of a prior in silico prediction of little alteration to the voltage of peak I KCNQ1-KCNE1 during ventricular AP repolarisation in the setting of V307L-induced SQT2 (17). The augmented magnitude of V307L I KCNQ1-KCNE1 is indicative of greater I Ks and, thereby, acceleration of ventricular repolarisation and QT interval shortening in SQT2 (15). Prior in silico simulation work based on previously reported effects of the V307L mutation (15) predicts shortening of ventricular APs and effective refractory period (ERP) and increased transmural heterogeneity in these parameters, as well as an increased vulnerable window for unidirectional conduction block - factors that combine to increase the risk of re-entrant arrhythmia (15, 17). Our present data on I KCNQ1-KCNE1 kinetics have value for future improvement of in silico studies of the consequences of the V307L mutation for ventricular electrophysiology. In particular, in order to determine the importance of slowed I KCNQ1-KCNE1 deactivation to ventricular cell and tissue electrophysiology, this effect of the V307L mutation on I KCNQ1-KCNE1 should be incorporated in future in silico investigations of SQT2 as additional parameters rather than mono-exponential activation time-courses for simulated I Ks are necessary to reproduce accurately experimental findings (cf (17)).

I Ks is known to be present in human atrial myocytes (34) and gain-of-function mutations in KCNQ1 have been associated with cases of atrial fibrillation (14, 35-38). Whilst few, if any, prior AP clamp data have been obtained regarding the profile of WT I KCNQ1-KCNE1 during human AP waveforms, prior simulations of atrial I Ks are suggestive of a modest contribution of native I Ks to atrial repolarisation under normal conditions (e.g. 28, 39, 40). Our data are in agreement with this in that, by comparison to its response to ventricular AP clamp, WT I KCNQ1-KCNE1 under atrial AP clamp was of modest amplitude. However, our experiments show a potential for an increased role for I Ks when channels incorporate V307L KCNQ1 subunits. Future simulation work is warranted to determine whether an increase to I Ks with V307L mutant KCNQ1 could shorten atrial APs sufficiently to abbreviate atrial ERP and increase the risk of atrial re-entrant arrhythmia.

**Mefloquine inhibition of I KCNQ1-KCNE1**

The pore-helical location of the V307 residue in KCNQ1 channels means that mutations at this position could have the potential to affect drug binding within the channel pore. Indeed, the V307L mutation has been shown previously to increase the IC50 for inhibition of I KCNQ1-KCNE1 by the comparatively selective Ic, chromanol 293B inhibition by -7-fold (30). This suggests that pharmacological strategies employing chromanol-related compounds would be less potent against V307L-linked SQT2. By contrast, our study identifies for the first time an effective pharmacological inhibitor of recombinant I Ks channels incorporating the V307L KCNQ1 mutation: similar IC50 values were observed for mefloquine inhibition of I KCNQ1-KCNE1 for WT and V307L KCNQ1. A prior detailed characterisation of the effects of mefloquine on WT I KCNQ1-KCNE1 showed that the compound's inhibitory action exhibited modest inverse voltage and time dependence (31). These features are consistent with at least part of mefloquine's action being attributable to one or both of involvement of closed-channel block and stabilization of KCNQ1+KCNE1 channels in pre-open close state(s) (31). Either eventuality would be anticipated to involve drug-channel interactions at site(s) that differ from that which mediates chromanol block of the channel. Our data are consistent with this in the observation of: (i) a tendency towards a lower IC50 at 1 second than at 4 seconds into an applied depolarisation (consistent with a modest inverse time-dependence (31)) and (ii) the similarity at each time-point in blocking potency of mefloquine for WT and V307L-KCNQ1 I KCNQ1-KCNE1.

The use of implantable cardioverter defibrillators (ICDs) is the first choice treatment to protect SQTS patients from ventricular fibrillation (10, 11). However, an increased risk of inappropriate shock delivery is inherent in ICD use for the SQTS (41) and for some patients, it is possible that this strategy may not be appropriate or even feasible (42). Pharmacological approaches that help offset the functional consequences of SQTS K+ channel mutations therefore offer useful adjunct treatments for (42-43). The present study provides 'proof of concept' data demonstrating that it is possible to identify effective pharmacological inhibitor(s) of SQTS2 mutant I KCNQ1-KCNE1. In terms of the potential clinical application of our observations with respect to mefloquine, it is perhaps interesting to note that a previous study of maintained chronic prophylactic mefloquine administration to 73 military personnel reported modest QTc interval prolongation over a 3 month period of drug administration (44). Excessive QTc prolongation beyond the normal range was not observed (44). This may correlate with the fact that in a direct in vitro comparison (31), mefloquine has been observed to be more potent against recombinant I KCNQ1-KCNE1 than against hERG-the main mediator of drug-induced QTc prolongation and torsades de pointes arrhythmia (45). It might be anticipated that, because in the setting of SQT2 the contribution of I Ks to ventricular repolarisation is increased, the
net effect of mefloquine administration on ventricular repolarisation might also be anticipated to be greater, although this proposition requires experimental validation. It should be noted, however, that in its current clinical use as an antimarial treatment, mefloquine is known to be associated with some incidence of CNS side effects (46, 47). Such effects might be problematic for chronic use of this agent to offset increased risk of adverse drug events. Mefloquine is therefore only recommended for short-term use, particularly if associated with risk of heart problems.

Conflict of interests: None declared.

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