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Long QT 1 Mutation KCNQ1$_{A344V}$ Increases Local Anesthetic Sensitivity of the Slowly Activating Delayed Rectifier Potassium Current

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Background: **Anesthesia in patients with long QT syndrome (LQTS) is a matter of concern. Congenital LQTS is most frequently caused by mutations in KCNQ1 (Kv7.1), whereas drug-induced LQTS is a consequence of HERG (human ether-a-go-go–related gene) channel inhibition. The aim of this study was to investigate whether the LQT1 mutation A344V in the S6 region of KCNQ1, at a position corresponding to the local anesthetic binding site in HERG, may render drug insensitive KCNQ1 channels into a toxicologically relevant target of these pharmacologic agents. This may suggest that LQTS constitutes not only a nonspecific but also a specific pharmacogenetic risk factor for anesthesia.**

Methods: **The authors examined electrophysiologic and pharmacologic properties of wild-type and mutant KCNQ1 channels. The effects of bupivacaine, ropivacaine, and mepivacaine were investigated using two-electrode voltage clamp and whole cell patch clamp recordings.**

Results: **The mutation A344V induced voltage-dependent inactivation in homomeric KCNQ1 channels and shifted the voltage dependence of KCNQ1/KCNE1 channel activation by +30 mV. The mutation furthermore increased the sensitivity of KCNQ1/KCNE1 channels for bupivacaine 22-fold (KCNQ1$_{wt}$/KCNE1: IC$_{50}$ = 2,431 ± 582 μM, n = 20; KCNQ1$_{A344V}$/KCNE1: IC$_{50}$ = 110 ± 9 μM, n = 24). Pharmacologic effects of the mutant channels were dominant when mutant and wild-type channels were coexpressed. Simulation of cardiac action potentials with the Luo-Rudy model yielded a prolongation of the cardiac action potential duration and induction of early afterdepolarizations by the mutation A344V that were aggravated by local anesthetic intoxication.**

Conclusions: **The results indicate that certain forms of the LQTS may constitute a specific pharmacogenetic risk factor for regional anesthesia.**

LONG QT syndrome (LQTS) is a cardiac disease characterized by arrhythmia, ventricular fibrillation of the torsades de pointes type, and sudden death. The perioperative treatment of patients with LQTS is a matter of concern.[1] Cardiac events of LQTS patients are often triggered by adrenergic stimulation caused by physical or emotional stress. Cardiotoxic side effects of local anesthetics may be particularly dangerous, because they alter effective refractory period temporal dispersion[2] and are capable of inducing sudden cardiac arrest.[3,4] Seven genes have been identified to be responsible for congenital LQTS.[5–11] Mutations in these genes may lead to a prolongation of the cardiac action potential detectable as prolonged QT interval in the electrocardiogram.

Apart from the congenital form of LQTS, a variety of drugs can cause acquired LQTS by inhibiting cardiac ion channels.[12] The main molecular sites of interaction for these proarrhythmic pharmacologic agents are human ether-a-go-go–related gene (HERG) potassium channels,[13] which mediate the rapidly activating delayed rectifier current (I_{Kr}) during the repolarization phase of the cardiac action potential. Two aromatic amino acids in the S6 transmembrane domain facing the inner cavity were identified to be important for high-affinity drug binding to HERG channels: tyrosine 652 and phenylalanine 656.[13] In a previous study, we established that these two residues are also involved in interaction of HERG channels with amino-amide local anesthetics.[14] Mutating the aromatic amino acids Y652 and F656 to alanine reduces the inhibition of HERG channels by bupivacaine, ropivacaine, and mepivacaine 4- to 30-fold.[14]

In contrast to HERG channels, KCNQ1/KCNE1 channel complexes, which generate the slowly activating delayed rectifier current (I_{Ks}),[15] are insensitive to the pharmacologic effects of amino-amide local anesthetics such as bupivacaine[16] and also cocaine.[17] Several mutations in KCNQ1 cause LQT1, including more than 10 mutations in the S6 region (LQTS database‖). Some of these are reported to form nonfunctional ion channels.[18–20] A mutation of alanine to valine in the position 344 (A344V) has been reported in a family of LQT1 patients.[21] This position in KCNQ1 corresponds to F656 in HERG channels. Because the mutation of phenylalanine to alanine in HERG channels reduces the affinity for local anesthetics,[14] we hypothesized that a mutation of alanine to the more hydrophobic amino acid valine might render insensitive KCNQ1 channels sensitive to toxicologically relevant concentrations of local anesthetics. An increased sensitivity induced by an LQT1 mutation may be specifically deleterious because I_{Ks} represents an important repolarization reserve in human heart.[22,23] I_{Ks} prevents extensive action potential prolongation in the setting of an elevated sympathetic tone or when action potential duration is prolonged by unintentional I_{Kr} block.[24] Both may be observed during local anesthetic intoxication.

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‖ Available at: http://pc4.fsm.it:81/cardmoc/. Accessed December 20, 2005.

Therefore, the aim of this study was to analyze the pharmacologic interaction of local anesthetics with wild-type and mutant KCNQ1 channels and KCNQ1/KCNE1 channel complexes. Because the mutation A344V has not been characterized before, it was necessary to describe the effects of this mutation on the biophysical properties of KCNQ1 and KCNQ1/KCNE1 channels first.

Materials and Methods

Molecular Biology, cRNA Preparation, and Cell Culture

The mutant KCNQ1 A344V was created by site directed mutagenesis from human KCNQ1. All constructs were cloned in the pcDNA3 expression vector for expression in Chinese hamster ovary (CHO) cells and in the pGEM expression vector for complementary RNA (cRNA) synthesis. The cRNA was synthesized *in vitro* with the T7 mMESSAGE mMACHINE Kit (Ambion, Austin, TX) according to the manufacturer's protocol. The concentration was determined with the RiboGreen method (RiboGreen RNA Quantification Reagent; Molecular Probes, Eugene, OR). The total amount of cRNA was 2 ng per oocyte for injection in *Xenopus laevis* oocytes. The cRNA of KCNQ1 and KCNE1 were injected in a 1:1 ratio. Two-electrode voltage clamp experiments were performed 2–7 days after the cRNA injection. Preparation of *Xenopus* oocytes was performed as described previously.[25] Oocytes were incubated at 17°C in gentamicin containing oocyte Ringer's solution (75 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Na-pyruvate, 5 mM HEPES, pH adjusted to 7.5 with NaOH, 50 μg/ml gentamicin; all from Sigma-Aldrich, Taufkirchen, Germany). Cell culture of CHO cells was performed as described previously.[14]

Electrophysiology

Electrophysiologic experiments were performed as described previously.[14,25] Different pulse protocols were used for characterization of the channels and to establish their pharmacologic sensitivities. The holding potential was −100 mV for *Xenopus* oocytes and −80 mV for CHO cells. To analyze the activation, depolarizing pulses were applied from −80 to +100 mV in 10-mV steps. The duration of the depolarization was 3 s for KCNQ1 alone and 5 s for KCNQ1/KCNE1; tail currents were recorded at −60 mV. For pharmacologic experiments, single square pulses to +60 or +80 mV for 3 and 5 s were used. Repetitive pulses were applied to determine that steady state inhibition was reached. For whole cell recordings in CHO cells, series resistance was 2.5–5.0 MΩ and was actively compensated for by at least 85%. A leak subtraction protocol was used. The recorded signal was filtered at 2 kHz and stored with a sampling rate of 5 kHz for analysis.

Bupivacaine (Sigma-Aldrich, Taufkirchen, Germany), *S*(−)-ropivacaine, (AstraZeneca, Södertalje, Sweden), and mepivacaine (Sigma-Aldrich) were dissolved in the respective extracellular recording solution. Terfenadine (Sigma-Aldrich) was prepared as 5 mM stock in dimethyl sulfoxide and diluted to 1 μM in extracellular recording solution. A hydrostatically driven perfusion system was used to apply the drugs onto the cells and to exchange the extracellular solutions. All experiments were performed at room temperature.

Data Analysis

Data were analyzed with Pulse Fit software (HEKA Elektronik, Lambrecht, Germany) and with KaleidaGraph software (Synergy Software, Reading, PA). The normalized tail currents during the activation protocol were fitted by a Boltzmann equation: $I = I_{max}/[1 + \exp((V_{0.5} - V_m)/k)]$, where $V_{0.5}$ is the voltage of half-maximal activation, V_m is the membrane potential, and k is the slope factor. The inhibition of currents by local anesthetics was quantified by the reduction of the maximal current during the test pulse: $f = 1 - (I_{max,\ drug}/I_{max,\ control})$. Concentration–response curves were fitted by a Hill function: $f = 1/[1 + (IC_{50}/c)^h]$, where IC_{50} is the concentration of half-maximal inhibition, c is the concentration of the local anesthetic, and h is the Hill coefficient. Statistical significance was tested using a two-sided Student *t* test or F test to compare concentration–response curves[26] (Excel; Microsoft, Redmond, WA). *P* values of 0.05 or less were regarded as significantly different. Data are presented as mean ± SD unless stated otherwise; n values indicate the number of experiments.

Computer Simulations of Cardiac Action Potentials

Computer simulations were conducted using a modified version of the Luo-Rudy dynamic (LRd) model (C++ code)[27,28] downloaded from the Internet.# The program was translated into C#, compiled with Visual Studio 2003, Net Framework 1.1 (Microsoft), and executed on personal computers. The program was used to model action potentials for epicardial, endocardial, and midmyocardial layers of the heart.[29] The effect of the mutation $KCNQ1_{A344V}$ on the cardiac action potential was simulated as a 15-mV shift in the voltage dependence of I_{Ks} activation. Parameters for I_{Ks} activation were altered to fit the experimental data. The influence of bupivacaine on the cardiac action potential was simulated by inhibition of sodium channels,[30] L-type calcium channels,[31] and repolarizing potassium channels[14,32] present in human heart by a clinically relevant concentration of 3 μM (inhibition of I_{Na}: 55%, I_{Kr}: 15%; $I_{L\text{-}Ca}$: 5%; I_{to} was not included in the LRd model) and by a toxicologically relevant concentration of 30 μM bupivacaine[33] (inhibi-

Available at: http://rudylab.wustl.edu/research/cell/methodology/cellmodels/LRd/code.htm. Accessed August 10, 2005.

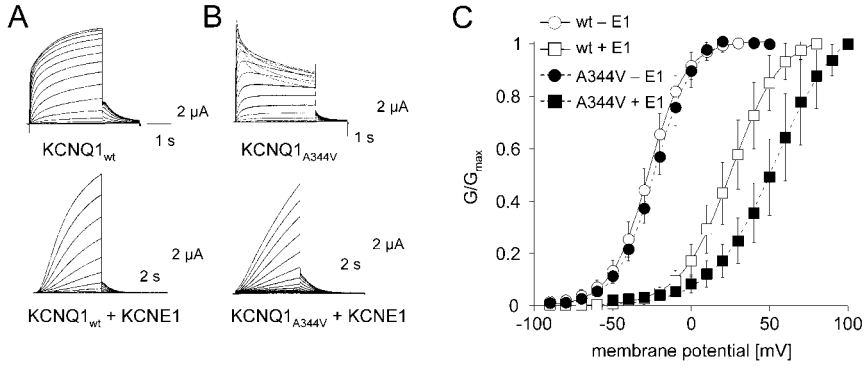

Fig. 1. Biophysical properties of KCNQ1 channels and KCNQ1/KCNE1 channel complexes expressed in *Xenopus laevis* oocytes. (*A* and *B*) Representative currents evoked by the activation protocol through $KCNQ1_{wt}$ (*A*) and $KCNQ1_{A344V}$ (*B*) channels expressed without (*upper panel*) and with the subunit KCNE1 (*lower panel*). (*C*) Conductance–voltage relation of KCNQ1 wild-type (wt) and mutant (A344V) channels expressed with (+ E1) and without KCNE1 (− E1). Curves were fitted with Boltzmann functions (table 1).

tion of I_{Na}: 90%, I_{Kr}: 60%, $I_{L\text{-}Ca}$: 20%). Effects of the mutation A344V were simulated as an additional 15-mV shift in I_{Ks} activation and inhibition of I_{Ks} (5% for 3 μM, 10% for 30 μM). Action potential duration (APD) was automatically calculated as time difference between begin of depolarization and 90% repolarization. Numerical results were written in ASCII formatted text files and visualized with KaleidaGraph software.

Results

Electrophysiologic Properties of $KCNQ1_{A344V}$ and $KCNQ1_{A344V}$/KCNE1

KCNQ1 wild-type and mutant channels were heterologously expressed in *Xenopus laevis* oocytes. Expression of $KCNQ1_{wt}$ resulted in a slowly activating, noninactivating voltage-dependent current. Expression of $KCNQ1_{A344V}$ yielded functional channels displaying a voltage-dependent inactivation of the macroscopic current not seen in wild-type channels (figs. 1A and B, upper panel). Coexpression of the pore-forming subunit together with the accessory subunit KCNE1 slowed activation of the currents and increased current amplitudes for both wild-type and mutant. Notably, the macroscopic voltage-dependent inactivation of $KCNQ1_{A344V}$ was abolished by coexpression with KCNE1 (figs. 1A and B, lower panel). The voltage dependence of activation was marginally changed by the mutation when KCNQ1 was expressed alone (fig. 1C and table 1). Coexpression with KCNE1 caused a shift in the voltage dependence of activation compared with KCNQ1 without KCNE1 by +53 mV for $KCNQ1_{wt}$/KCNE1 and by +80 mV for $KCNQ1_{A344V}$/KCNE1, resulting in a 30-mV difference between wild-type and mutant channel complexes (fig. 1C and table 1). However, the maximal current amplitudes were not significantly different between $KCNQ1_{wt}$ and $KCNQ1_{A344V}$ or between $KCNQ1_{wt}$/KCNE1 and $KCNQ1_{A344V}$/KCNE1 ($P > 0.05$; table 1).

Inhibition of KCNQ1 and KCNQ1/KCNE1 by Local Anesthetics

The pharmacologic effects of different amino-amide local anesthetics on $KCNQ1_{wt}$ and $KCNQ1_{A344V}$ alone as well as on complexes formed by $KCNQ1_{wt}$ or $KCNQ1_{A344V}$ and KCNE1 were analyzed in *Xenopus* oocytes. Because of the differences in activation behavior, different protocols were used for the pharmacologic experiments. Channels were activated by 3-s pulses to +60 mV for $KCNQ1_{wt}$ and $KCNQ1_{A344V}$, 5-s pulses to +60 mV for $KCNQ1_{wt}$/KCNE1, and 5-s pulses to +80 mV for $KCNQ1_{A344V}$/KCNE1. Both wild-type and mutant channels were inhibited by bupivacaine in a concentration-dependent manner (figs. 2A and B). The concentration–response data were mathematically described by Hill functions (fig. 2C and table 2). The mutant channel $KCNQ1_{A344V}$ was 17-fold more sensitive to the inhibition by bupivacaine than $KCNQ1_{wt}$. Coexpression with KCNE1 reduced the sensitivity by a factor of 0.4 for

Table 1. Gating Parameters of KCNQ1 and KCNQ1/KCNE1 Channels

	Xenopus Oocytes				CHO Cells	
	wt		A344V		wt	A344V
	−KCNE1	+KCNE1	−KCNE1	+KCNE1	+KCNE1	+KCNE1
$V_{0.5}$, mV	−25.6 ± 3.6	27.1 ± 9.0	−23.2 ± 2.9	57.3 ± 14.8	−2.2 ± 9.0	37.2 ± 8.8
P $V_{0.5}$ (wt-A344V)	—	—	< 0.05	< 0.001	—	< 0.001
Slope factor, mV	11.2 ± 1.3	15.1 ± 2.1	13.2 ± 2.0	19.6 ± 3.6	11.7 ± 3.2	16.3 ± 3.0
G_{max}	2.8 ± 1.7 μA	3.3 ± 1.7 μA	2.2 ± 1.3 μA	2.4 ± 1.4 μA	2.7 ± 0.81 nA	2.6 ± 0.45 nA
n	14	16	27	22	24	20

Parameters of Boltzmann fits for activation of KCNQ1 and KCNQ1/KCNE1 channels expressed in *Xenopus laevis* oocytes and Chinese hamster ovary (CHO) cells. Data are presented as mean ± SD. *P* values indicate the significance of the difference between values for half-maximal activation of wild-type (wt) and mutant (A344V) channels.

G_{max} = maximal conductance during the tail pulse; n = number of experiments; $V_{0.5}$ = voltage of half-maximal activation.

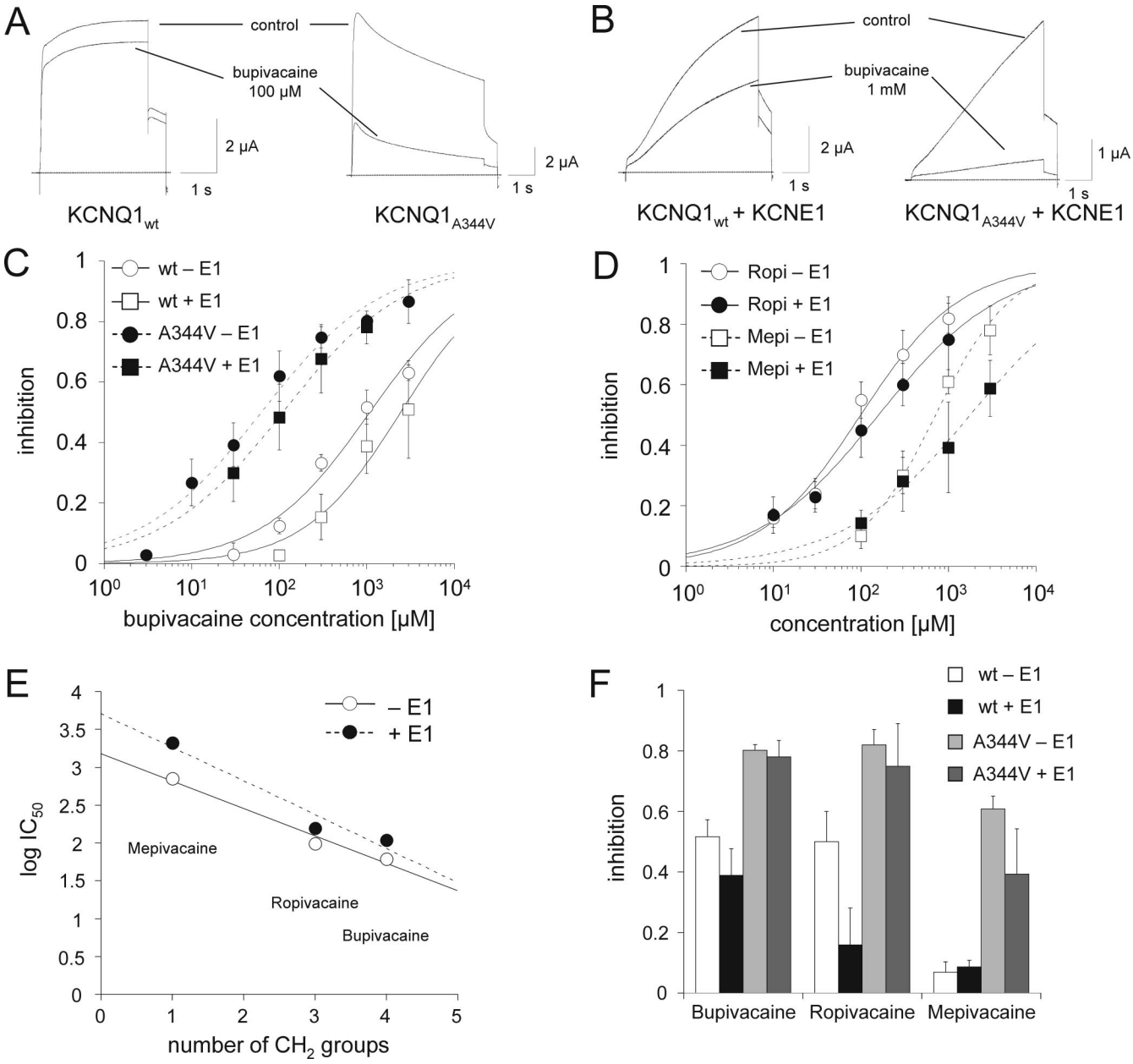

Fig. 2. Pharmacologic properties of KCNQ1 wild-type (wt) and mutant (A344V) channels expressed in *Xenopus laevis* oocytes in the presence (+ E1) and absence (− E1) of the β subunit KCNE1. (*A*) Inhibition of $KCNQ1_{wt}$ and $KCNQ1_{A344V}$ channels by 100 μM bupivacaine. (*B*) Inhibition of $KCNQ1_{wt}$/KCNE1 and $KCNQ1_{A344V}$/KCNE1 channel complexes by 1 mM bupivacaine. (*C*) Concentration–response curves for inhibition by bupivacaine were fitted with Hill functions (table 2). (*D*) Concentration–response curves of $KCNQ1_{A344V}$ and $KCNQ1_{A344V}$/KCNE1 channel block by *S*(−)-ropivacaine (Ropi) and mepivacaine (Mepi). Parameters of Hill fits are given in table 3. (*E*) Correlation between the length of the N-substituent of the homologue series of local anesthetics used in our experiments and the $logIC_{50}$ for $KCNQ1_{A344V}$ and $KCNQ1_{A344V}$/KCNE1 (linear fit for $KCNQ1_{A344V}$: $r = 0.99$; for $KCNQ1_{A344V}$/KCNE1: $r = 0.98$). (*F*) Comparison of the inhibition of $KCNQ1_{wt}$ and $KCNQ1_{A344V}$ channels expressed alone or with KCNE1 by 1 mM bupivacaine, *S*(−)-ropivacaine, and mepivacaine.

wild-type and by a factor of 0.6 for mutant channels. The ratio of sensitivity between wild-type and mutant channels (22-fold increase by the mutation A344V) was only slightly changed. Bupivacaine (100 μM) did not significantly change the voltage dependence of activation either for $KCNQ1_{A344V}$ ($V_{0.5} = -26.4 \pm 3.2$ mV, n = 7; $P > 0.05$) or for $KCNQ1_{A344V}$/KCNE1 ($V_{0.5} = +54.8 \pm 5.0$ mV, n = 5; $P > 0.05$). The inhibition of $KCNQ1_{A344V}$ channels or of $KCNQ1_{A344V}$/KCNE1 channel complexes was not voltage dependent for depolarizations to potentials between −40 and +100 mV.

To establish whether the lipophilicity of amino-amide local anesthetics influences the pharmacologic potency on KCNQ1 inhibition, the effects of ropivacaine and mepivacaine were analyzed next. Complete concentration–response curves were determined for inhibition of $KCNQ1_{A344V}$ and for inhibition of $KCNQ1_{A344V}$/KCNE1 (fig. 2D and table 3). The rank order of inhibitory po-

Table 2. Inhibition of KCNQ1 and KCNQ1/KCNE1 Channels by Bupivacaine

	Xenopus Oocytes				CHO Cells	
	wt		A344V		wt	A344V
	−KCNE1	+KCNE1	−KCNE1	+KCNE1	+KCNE1	+KCNE1
IC_{50}, μm	1,078 ± 194	2,431 ± 582	62.2 ± 12.8	110 ± 9.3	667 ± 30	37.6 ± 2.4
P IC_{50} (wt-A344V)	—	—	< 0.001	< 0.001	—	< 0.001
Hill	0.70 ± 0.10	0.80 ± 0.17	0.63 ± 0.08	0.63 ± 0.05	1.01 ± 0.05	0.91 ± 0.05
n	23	20	32	24	17	26

Parameters of Hill fits for inhibition of KCNQ1 and KCNQ1/KCNE1 channels expressed in *Xenopus laevis* oocytes and Chinese hamster ovary (CHO) cells by bupivacaine (mean ± SEM). *P* values indicate the significance of the difference between concentration of half-maximal inhibition (IC_{50}) values for wild-type (wt) and mutant (A344V) channels.

Hill = Hill coefficient; n = number of experiments.

tency was bupivacaine > ropivacaine > mepivacaine and thus correlated with the length of the N-substituent (fig. 2E). Inhibition of $KCNQ1_{wt}$ and $KCNQ1_{wt}$/KCNE1 by ropivacaine and mepivacaine was analyzed at a concentration of 1 mM (fig. 2F). The rank order of inhibitory potency was the same for $KCNQ1_{wt}$ and $KCNQ1_{wt}$/KCNE1 and $KCNQ1_{A344V}$ and $KCNQ1_{A344V}$/KCNE1, whereas the sensitivity was significantly increased by the mutation A344V for all three local anesthetics.

The effect of the mutation A344V on the biophysical and pharmacologic properties of KCNQ1/KCNE1 channel complexes was independent of the expression system because we obtained the same results in mammalian CHO cells (tables 1 and 2 and fig. 3). Although the absolute values for the half-maximal activation differ between CHO cells and *Xenopus* oocytes, the relative difference between $KCNQ1_{wt}$/KCNE1 and $KCNQ1_{A344V}$/KCNE1 is similar. Because of the small currents of KCNQ1 channels without KCNE1 in CHO cells, pharmacologic experiments in CHO cells were only performed with KCNQ1/KCNE1 channel complexes. The sensitivity of KCNQ1/KCNE1 channels in CHO cells was increased by a factor of 3.6 for wild-type and by a factor of 2.9 for mutant channel complexes when compared with *Xenopus* oocytes. However, the increase in bupivacaine sensitivity of $KCNQ1_{A344V}$/KCNE1 compared with $KCNQ1_{wt}$/KCNE1 was similar (18-fold in CHO cells, 22-fold in *Xenopus* oocytes).

Table 3. Inhibition of $KCNQ1_{A344V}$ and $KCNQ1_{A344V}$/KCNE1 channels by *S*(−)-Ropivacaine and Mepivacaine

	S-Ropivacaine		Mepivacaine	
	A344V		A344V	
	−KCNE1	+KCNE1	−KCNE1	+KCNE1
IC_{50}, μm	99 ± 14	158 ± 14	711 ± 61	1764 ± 213
Hill	0.76 ± 0.08	0.62 ± 0.04	1.00 ± 0.09	0.60 ± 0.06
n	33	24	23	22

Parameters of Hill fits for inhibition of $KCNQ1_{A344V}$ and $KCNQ1_{A344V}$/KCNE1 channels expressed in *Xenopus laevis* oocytes by *S*(−)-ropivacaine and mepivacaine (mean ± SEM).

Hill = Hill coefficient; IC_{50} = concentration of half-maximal inhibition; n = number of experiments.

Dominant Effect of $KCNQ1_{A344V}$

Simulating the heterozygous state in a patient, $KCNQ1_{wt}$ and $KCNQ1_{A344V}$ were coexpressed in *Xenopus* oocytes in a 1:1 ratio. Figures 4A and B show the current response of $KCNQ1_{wt}$/$KCNQ1_{A344V}$ expressed without and with KCNE1. In the absence of KCNE1, the channels displayed voltage-dependent inactivation, similar to $KCNQ1_{A344V}$ when expressed alone. The voltage of half maximal activation of $KCNQ1_{wt}$/$KCNQ1_{A344V}$ was not significantly different from the half-maximal activation of $KCNQ1_{wt}$ (fig. 4C; $V_{0.5}$ = −26.8 ± 4.0 mV, slope = 10.9 ± 1.2 mV, n = 5; $P > 0.05$). Coexpression of $KCNQ1_{wt}$/$KCNQ1_{A344V}$ with KCNE1 caused a shift of the voltage dependence of activation to more depolarizing potentials (fig. 4C). The $V_{0.5}$ value was 38.6 ± 3.9 mV (slope = 15.2 ± 1.7 mV, n = 5) which was between the $V_{0.5}$ values of $KCNQ1_{wt}$/KCNE1 ($P < 0.05$) and $KCNQ1_{A344V}$/KCNE1 ($P < 0.05$).

The pharmacologic properties of heteromeric $KCNQ1_{wt}$/$KCNQ1_{A344V}$ channels were analyzed at a concentration of 100 μM bupivacaine (fig. 4D). The sensitivity of the channel to the inhibitory action of bupivacaine was also increased in the heteromeric channel compared with the wild-type. The action of 100 μM bupivacaine on the different channel complexes was compared (fig. 4E). The heteromeric channel was less sensitive than the mutant channel $KCNQ1_{A344V}$ for bupivacaine ($P < 0.05$) but significantly more sensitive than the wild-type channel, regardless of coexpression with KCNE1. The difference between expression without and with KCNE1 was not significant ($P > 0.05$). Similar to inhibition of $KCNQ1_{A344V}$, the block of $KCNQ1_{wt}$/$KCNQ1_{A344V}$ was not voltage dependent, and the half-maximal activation was not changed by application of 100 μM bupivacaine.

Inhibition of KCNQ1 by Terfenadine

To establish whether the increased sensitivity of $KCNQ1_{A344V}$ was specific for amino-amide local anes-

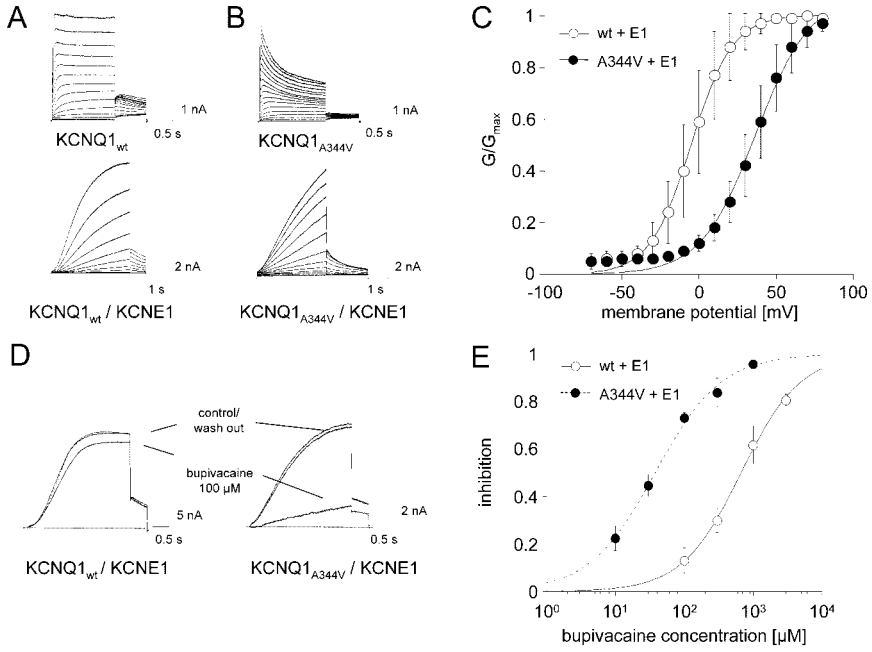

Fig. 3. Characterization of biophysical and pharmacologic properties of $KCNQ1_{wt}$ and $KCNQ1_{A344V}$ channels and $KCNQ1_{wt}$/KCNE1 (wt + E1) and $KCNQ1_{A344V}$/KCNE1 (A344V + E1) channel complexes in Chinese hamster ovary cells. (*A* and *B*) Original currents through $KCNQ1_{wt}$ (*A*) and $KCNQ1_{A344V}$ channels (*B*) expressed without (*upper panel*) and with the subunit KCNE1 (*lower panel*) elicited by the activation protocol. (*C*) Voltage dependence of activation of $KCNQ1_{wt}$/KCNE1 and $KCNQ1_{A344V}$/KCNE1 channels was fitted by a Boltzmann equation; parameters are given in table 1. (*D*) Original currents through $KCNQ1_{wt}$/KCNE1 and $KCNQ1_{A344V}$/KCNE1 channels under control conditions, under application of 100 μM bupivacaine, and after washout. (*E*) Concentration–response curves were fitted by Hill functions (table 2).

thetics, we investigated the effect of terfenadine on wild-type and mutant KCNQ1 channels. Similar to local anesthetics, terfenadine preferentially blocks HERG channels.[34] The effect of 1 μM terfenadine, a value close to the IC_{50} for the inhibition of HERG potassium channels,[34] was examined on HERG channels and on KCNQ1 wild-type and mutant channels expressed with and without KCNE1 (fig. 5). In agreement with literature,[34] terfenadine (1 μM) inhibited HERG channels by 54 ± 11% (n = 6) and only marginally affected $KCNQ1_{wt}$ channels (inhibition: 4.5 ± 1.7%, n = 5). The sensitivity of KCNQ1 channels for terfenadine was significantly increased by the mutation A344V in both the homomeric and the heteromeric situations (inhibition of $KCNQ1_{A344V}$: 17 ± 6.7%, n = 5, $P < 0.05$; of $KCNQ1_{wt}$/$KCNQ1_{A344V}$: 7.0 ± 1.5%, n = 5, $P < 0.05$). However, the inhibition of KCNQ1 wild-type and mutant channels by 1 μM terfenadine was significantly reduced when they were coexpressed with KCNE1. As a result, there was no significant difference between inhibition of wild-type and mutant or heteromeric channels when they were coexpressed with KCNE1.

Effect of KCNQ1 A344V on a Cardiac Action Potential Model

We used the LRd model for the guinea pig cardiac action potential[27,28] to simulate the effect of the mutation on cardiac action potential morphology (fig. 6). The simulation was performed with a basic cycle length of 1,000 ms for 500 cycles for epicardial, endocardial, and

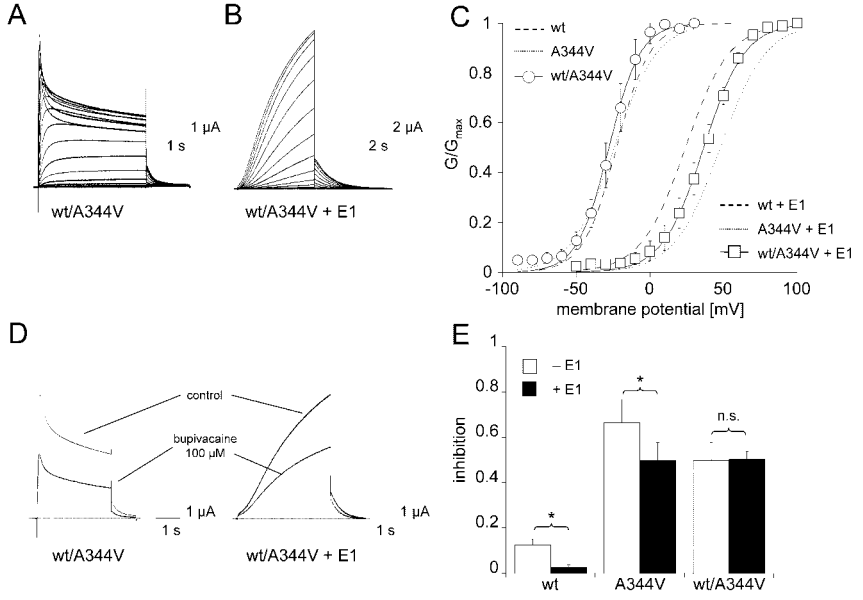

Fig. 4. Coexpression of $KCNQ1_{wt}$ (wt) and $KCNQ1_{A344V}$ (A344V) channels in the presence (+ E1) and absence (− E1) of KCNE1 in *Xenopus laevis* oocytes. Equal amounts of $KCNQ1_{wt}$ and $KCNQ1_{A344V}$ complementary RNA (cRNA) were injected without or with KCNE1 cRNA. Family of current traces evoked by the activation protocol through $KCNQ1_{wt}$/$KCNQ1_{A344V}$ channels (*A*) and $KCNQ1_{wt}$/$KCNQ1_{A344V}$/KCNE1 channel complexes (*B*). (*C*) Voltage dependence of activation of heteromeric $KCNQ1_{wt}$/$KCNQ1_{A344V}$ channels. Boltzmann fits of the homomeric channels were added for comparison. (*D*) Inhibition of $KCNQ1_{wt}$/$KCNQ1_{A344V}$ and $KCNQ1_{wt}$/$KCNQ1_{A344V}$/KCNE1 channels by 100 μM bupivacaine. (*E*) Comparison of inhibition of homomeric and heteromeric channels by 100 μM bupivacaine. * Significant difference between channels without and with KCNE1. n.s. = not significant.

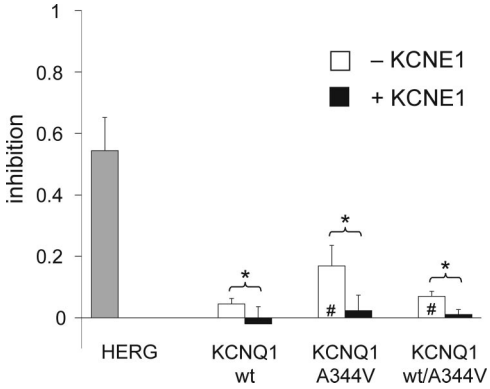

Fig. 5. Inhibition of human ether-a-go-go–related gene (HERG) and different homomeric and heteromeric KCNQ1 channel complexes expressed in *Xenopus laevis* oocytes by 1 μM terfenadine. * Significant difference between channels without and with KCNE1. # Significant differences compared with $KCNQ1_{wt}$.

midmyocardial layers of the heart. According to the LRd model, the depolarizing shift of 15 mV in the voltage dependence of I_{Ks} activation as present in the heterozygous situation resulted in a prolongation of the action potential (fig. 6A). This prolongation was larger in the endocardial layer (41 ms or 19%) than in the epicardial layer (0.2 ms or 0.1%) and largest in the midmyocardial layer. Furthermore, in midmyocardial myocytes, repolarization did not occur within one stimulation interval, and early afterdepolarizations (EADs) developed.

The effects of 3 μM and 30 μM bupivacaine on the cardiac action potential simulated for the homozygous and heterozygous conditions are demonstrated in figures 6B and C (see Materials and Methods for values of inhibition). In wild-type conditions (fig. 6B), 3 μM bupivacaine induced a prolongation of the cardiac action potential only in the midmyocardial layer of the heart by 16%. At 30 μM, bupivacaine prolonged the action potential in the epicardial and endocardial layers by 8% and 12%, respectively. In addition, the higher concentration of bupivacaine caused the development of EADs in the midmyocardial layer. Simulation of the effect of 3 μM bupivacaine for the heterozygous condition (fig. 6C) demonstrated that the local anesthetic prolonged APD in the epicardial layer by 23% and further prolonged APD in the endocardial layer by 10%. The low concentration of bupivacaine furthermore induced EADs and a loss of repolarization in the midmyocardial layer of the myocardium. At 30 μM, bupivacaine prolonged the APD in the epicardial layer by 56% and caused EADs in the midmyocardial and endocardial layers with a loss of repolarization in the midmyocardial layer.

Discussion

In this work, we describe biophysical and pharmacologic properties of the LQT1 mutant $KCNQ1_{A344V}$.[21] The electrophysiologic features of this mutation in the lower part of the S6 helix are a depolarizing shift of the voltage dependence of activation and induction of voltage-dependent inactivation.

A positive shift in the voltage dependence of activation has been reported for LQT1 mutations in the S4 domain as well as for LQT1 mutations in the C-terminal region of KCNQ1.[19,35] Similar to our findings, the shift is only observed when the KCNQ1 mutants are coexpressed with KCNE1. It is reasonable to assume that mutations in the voltage sensor containing S4 domain may change the voltage dependence of activation by directly affecting the positive charge of the voltage sensor. However, there are several possible explanations for the effect of a

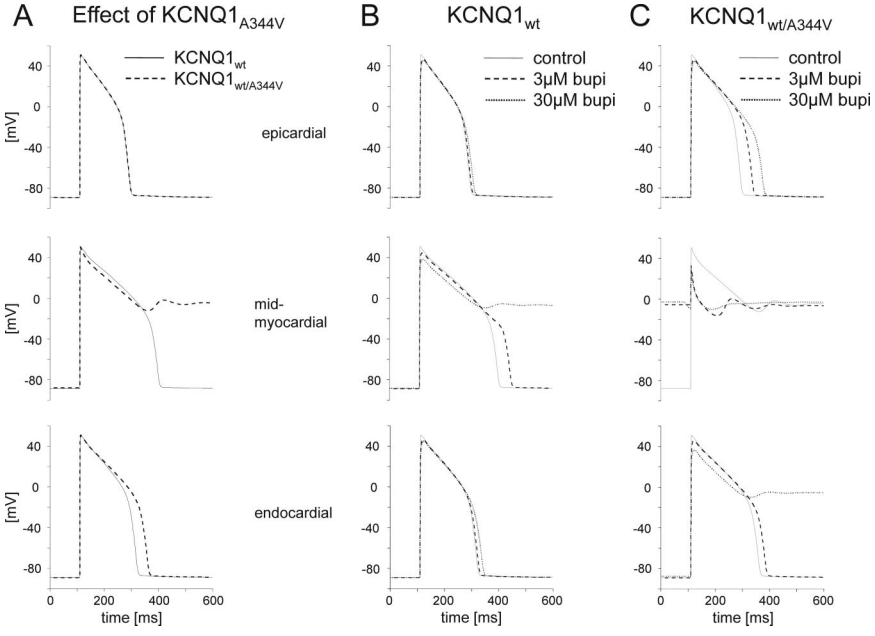

Fig. 6. Simulation of cardiac action potentials with the Luo-Rudy dynamic model. Simulation was performed for epicardial (*upper panel*), midmyocardial (*middle panel*), and endocardial (*lower panel*) layers of the heart. (*A*) Simulation of ventricular action potential with standard settings (wt) and with the assumption of a shift in activation of I_{Ks} by 15 mV for the heterozygous mutant channel (wt/A344V). (*B* and *C*) Effects of 3 and 30 μM bupivacaine (bupi) are simulated by inhibition of sodium, L-type calcium and potassium channels (3 μM: inhibition of I_{Na}: 55%, $I_{L\text{-}Ca}$: 5%, I_{Kr}: 15%; 30 μM: inhibition of I_{Na}: 90%, $I_{L\text{-}Ca}$: 20%, I_{Kr}: 60%) for wild-type (*B*) and heterozygous (additional 15-mV shift in I_{Ks} activation; additional inhibition of I_{Ks}: by 5% [3 μM] and 10% [30 μM]) conditions (*C*).

mutation in the S6 region on activation gating. First, the coupling between the activation gate and the voltage sensor may be impaired. Mutagenesis studies in voltage-gated potassium channels demonstrate that the lower part of the S6 region couples with the S4–S5 linker and that mutations in this region affect the voltage dependence of gating.[36,37] These results were substantiated by the recently resolved crystal structure of Kv1.2 revealing a close vicinity of the S4–S5 linker and the S6 helix.[38] Second, the shift in the voltage dependence of activation may also be compatible with a destabilization of the open state relative to the closed state due to profound structural changes in the pore region. This destabilized channel would require higher activation energy and therefore a more positive membrane potential to open.[39]

The mutant channels $KCNQ1_{A344V}$ exhibit macroscopic inactivation that is abolished by coexpression with KCNE1. A similar voltage-dependent inactivation in the absence of KCNE1 is caused by other mutations in both the S6 and S5 regions of KCNQ1.[40,41] The voltage-dependent inactivation of KCNQ1 channels has been suggested to result from a constriction of the pore, mediated by interaction of the residues F340 and L273 in the S6 and S5 helix with V310 in the pore helix of the channels.[42] A change in the size of these residues leads to an enhanced inactivation behavior due to a destabilization of the open state of the pore.[42] The residue A344 is a single turn below F340 in the S6 helix, and an increased side chain at position 344 may disrupt the interactions of the S6 helix with the pore as well. Because KCNE1 eliminates delayed inactivation of $KCNQ1_{wt}$ channels by stabilizing the pore by an indirect mechanism,[43] KCNE1 may prevent inactivation in $KCNQ1_{A344V}$ channels by the same mechanism. Our results indicate that the interaction of KCNE1 with KCNQ1 is not disturbed by the LQT1 mutation A344V because the mutant channels display the biophysical effects attributed to coassembly of KCNQ1 with KCNE1, such as a slower activation, a positive shift in the activation midpoint, and abolishing of inactivation.[15,43] Taken together, our results allow us to hypothesize that the effects of the mutation A344V on channel gating may result from a destabilization of the open state of the pore.

KCNQ1 channels as well as KCNQ1/KCNE1 channel complexes are insensitive to many pharmacologic agents that block HERG channels, such as local anesthetics.[16] The LQT1 mutation A344V not only alters the gating behavior of KCNQ1 channels and KCNQ1/KCNE1 channel complexes, but it also changes their pharmacologic sensitivity to local anesthetics. The single amino acid exchange in the S6 region of KCNQ1 leads to a 22-fold increase in the sensitivity of KCNQ1/KCNE1 channel complexes to bupivacaine, rendering $KCNQ1_{A344V}$/KCNE1 channel complexes nearly as sensitive as HERG channels.[14,32] This effect did not depend on the expression system because similar results were obtained in CHO cells and in *Xenopus* oocytes. Both wild-type and mutant channels were approximately three times less sensitive to bupivacaine when expressed in *Xenopus* oocytes than in CHO cells. A similar reduction in sensitivity caused by the expression system has previously been described for inhibition of HERG channels by bupivacaine as well.[14,16,32] Although we cannot exclude that the increased sensitivity of $KCNQ1_{A344V}$ is related to the changes in the gating behavior, this seems less likely for several reasons. First, local anesthetic sensitivity of KCNQ1 and of KCNQ1/KCNE1 channels is increased to a similar extend by the mutation, although the effects of the mutation A344V on the gating are qualitatively different in KCNQ1 channels and in KCNQ1/KCNE1 channel complexes. Furthermore, the residue A344 mediates binding of benzodiazepines to KCNQ1 channels,[40] and it has been described as a potential site of interaction in a pharmacophore model of KCNQ1 blockers.[44] Finally, a direct structural effect of the mutation A344V is strongly supported by the experiments with heterologous expression of $KCNQ1_{wt}$ and $KCNQ1_{A344V}$ channels in a 1:1 ratio. The increased sensitivity of the mutant channels seems to be a dominant effect, because there was no significant difference between the inhibition of $KCNQ1_{A344V}$/KCNE1 and $KCNQ1_{wt}$/$KCNQ1_{A344V}$/KCNE1 complexes by 100 μM bupivacaine (fig. 4E). In contrast, the changes in the gating seem not to be dominant. The $V_{0.5}$ value of the $KCNQ1_{wt}$/$KCNQ1_{A344V}$/KCNE1 complex is between the value for $KCNQ1_{wt}$/KCNE1 and $KCNQ1_{A344V}$/KCNE1 (fig. 4C). The increased sensitivity is thus rather caused by structural changes in the pore than by changes in channel gating.

Hydrophobic interactions between the drug molecule and the channel pore seem to be important for inhibition of $KCNQ1_{A344V}$ and $KCNQ1_{A344V}$/KCNE1 by local anesthetics because plotting the log IC_{50} against the lipophilicity of the local anesthetics yields a linear correlation with a correlation coefficient close to unity (fig. 2E). Hence, it may be hypothesized that the mutation A344V generates a hydrophobic interaction site. In addition or as an alternative, the mutation A344V might change the pore structure in such a way that the hydrophobic residues F340 and I337[44] become better accessible for local anesthetics. The observation that the mutation did not alter the sensitivity of KCNQ1/KCNE1 channel complexes to terfenadine suggests that not only the lipophilicity of the drug (terfenadine[45]: $logD_{oct} = 4.4$; bupivacaine[46]: $logD_{oct} = 3.4$) but also the size of the drug molecule (terfenadine: 471.7 g/mol; bupivacaine: 324.9 g/mol) influences the effect of the mutation A344V. In addition, the three-dimensional structure and orientation of the drug molecule may also be a contributing factor. The pharmacogenetic results may thus be specific for hydrophobic pharmacologic agents of a critical size such as local anesthetics.

The depolarizing shift in the voltage dependence of activation of KCNQ1/KCNE1 channel complexes caused by the mutation A344V may explain the pathophysiologic mechanism of this LQT1 mutation. We applied the LRd model for the guinea pig cardiac action potential[27,28] to simulate the effect of the mutation on cardiac action potential morphology (fig. 6). This model has been used before to simulate the effect of LQT mutations on cardiac action potentials.[47,48] These simulations demonstrate an increased dispersion and a repolarization deficiency for the mutant resulting in a more heterogeneous excitation pattern compared with the wild type. Because differences in APD and an increased dispersion of the repolarization can increase the risk for severe cardiac arrhythmia,[49] these simulations confirm the arrhythmogenic potential of the LQT1 mutation A344V.

Simulating the effects of bupivacaine on the cardiac action potential under wild-type conditions showed a prolongation of the cardiac action potential in the epicardial and endocardial layer and development of EADs in the midmyocardial layer. The results of the modeling are therefore consistent with the observation that bupivacaine intoxication causes QT prolongation and an increase in QT dispersion.[2] Under heterozygous conditions, the local anesthetic prolonged APD in epicardial layer and induced EADs in endocardial and midmyocardial layers of the myocardium. Because of different ion channel densities across the ventricular wall that are incorporated in the LRd model as different I_{Kr} to I_{Ks} ratios,[29] effects of the mutation and of bupivacaine were more pronounced in endocardial and midmyocardial layers of the heart.

The results of these simulations are in agreement with experimental data on human as well as on canine cardiomyocytes demonstrating that I_{Ks} blockade causes a more pronounced prolongation of the action potential in the presence of I_{Kr} blockade.[24,50] Mutations in LQT related genes that *per se* do not cause severe clinical effects or show a low penetrance may thus only become apparent when additional factors, such as drug-induced I_{Kr} block, increase the susceptibility to develop severe cardiac arrhythmia.[22,51,52] Because mutation carriers of A344V present with a mild phenotype of LQT1[21] that may not become clinically apparent, our results confirm and extend the concept of I_{Ks} serving as a repolarization reserve.[22,23] LQT1 patients may be exposed to an increased risk of experiencing severe cardiac arrhythmia during local anesthetic intoxication. This study's results allow us to hypothesize that genotype-directed perioperative treatment of patients with congenital LQT syndrome is warranted.

In summary, the results of our study demonstrate that the LQT1 mutation A344V profoundly changes the gating of KCNQ1 channels and of KCNQ1/KCNE1 channel complexes. The mutation induces a voltage-dependent inactivation in KCNQ1 channels and a depolarizing shift in activation of KCNQ1/KCNE1 channel complexes. Furthermore, the mutation leads to a 22-fold increase in local anesthetic sensitivity. The increased sensitivity results from structural changes in the three-dimensional structure induced by the mutation A344V or by the *de novo* formation of an interaction site. The results of our study suggest that certain forms of the LQT syndrome may constitute a specific rather than a nonspecific risk factor for the development of severe cardiac arrhythmia during anesthesia.

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