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Fenamate-induced enhancement of heterologously expressed HERG currents in *Xenopus* oocytes

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Abstract

The human ether-a-go-go related gene (HERG) product encodes for the pore-forming subunit of the rapid component of the delayed rectifier K^+ channel that mediates repolarization of cardiac action potential. HERG channels are also potential targets of a large variety of pharmacological agents most of which tend to block HERG currents. In this study, we examined the effects of the non-steroidal anti-inflammatory agents, flufenamic acid and niflumic acid, on heterologously expressed HERG channels in oocytes. The cRNA of HERG (30 ng) was injected into *Xenopus* oocytes and currents were recorded using two-electrode voltage clamp technique in a low Cl^- solution. Flufenamic and niflumic acids (10^{-4}–5×10^{-4} M) enhanced the amplitude of outward currents evoked by depolarizing pulses. At potentials positive to 0 mV, an initial transient component was also evident in the presence of fenamates. Fenamates accelerated the activation rate of HERG channels and decelerated their deactivation. Flufenamic acid (5×10^{-4} M) shifted the I_{tail}–V relationship from -26.7 ± 0.1 to -31.4 ± 0.2 mV. Neither flufenamic acid or niflumic acid affected the kinetics of HERG channel inactivation. Using a voltage protocol that mimicked the cardiac action potential, both fenamates increased the outward current during the plateau and during the phase 3 repolarization of action potential. The effects of the fenamates were blocked by the HERG channel blocker, E-4031 and were also not observed in water-injected oocytes. Our data suggest that fenamates enhance HERG currents and affect the action potential duration in the heart.

Keywords: Voltage-clamp; Flufenamic acid; Niflumic acid; HERG (ether-a-go-go related gene); Q–T syndrome, long

1. Introduction

The human ether-a-go-go (eag)-related gene (HERG) encodes the pore-forming subunit of the cardiac rapidly activating delayed rectifier K^+ channel ($K_{V(r)}$) and demonstrates inward rectification at positive potentials (Sanguinetti et al., 1995; Trudeau et al., 1995). However, structurally HERG channels differ from classical inward rectifiers in that the molecular structure consists of six putative membrane-spanning domains compared to other inwardly rectifying K^+ channels, which consist of a pore domain with two flanking transmembrane spans (Warmke and Ganetzky, 1994; Smith et al., 1996). The rapid inactivation of HERG current at positive potentials forms the basis for inward rectification and distinguishes $K_{V(r)}$ from other K^+ channels (Spector et al., 1996; Wang et al., 1997). Changes in HERG channels function occurs in both inherited and acquired long QT syndromes, characterized by an unusually slow repolarization of cardiac action potential leading to cardiac arrhythmia, ventricular fibrillation and sudden cardiac death due to torsade de pointes (Curran et al., 1995; Sanguinetti et al., 1996). It has been recently reported, that the loss of channel function is related to multiple mechanisms including abnormal protein trafficking, the generation of nonfunctional channels, and altered channel gating (Zhou et al., 1998a; Furutani et al., 1999).

Long QT syndrome can be inherited as an autosomal dominant trait, but the more common form of this disorder is acquired (Sanguinetti et al., 1995). Studies from multiple laboratories indicate that the vast majority of drugs associated with torsades de pointes are $I_{K(Vr)}$ blockers. HERG channels are molecular targets for widely used pharmacological agents including class III antiarrhythmics (Thomas et al., 2001),

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divalent cations as well as variety of other therapeutic agents (Mohammad et al., 1997; Zhang et al., 1999; Kamiya et al., 2001; Pond and Nerbonne, 2001; Vandenberg et al., 2001). The therapeutic use of many these drugs is limited because of their blocking action on $I_{K(Vr)}$. For example, gastrointestinal prokinetic agent cisapride was recently withdrawn from the U.S. market because of its proarrhythmic effects (Potet et al., 2001). It would thus be of clinical importance to identify compounds that enhance $I_{K(Vr)}$.

Fenamates are nonsteroidal anti-inflammatory drugs belonging to the class of *N*-phenylanthranilic acids that have been reported to affect a variety of ion channels in several tissues (Farrugia et al., 1993a,b; Chouabe et al., 1998; Stumpff et al., 2001). Flufenamic and niflumic acids are the protype of this class of compounds that are generally used in oocyte expression system in order to block endogenous Ca^{2+}-activated Cl^- currents (White and Aylwin, 1990).

In preliminary experiments, we observed that fenamates could increase HERG currents expressed in the *Xenopus* oocytes. We have further evaluated the mechanisms associated with fenamate-induced enhancement of heterologously expressed HERG currents.

2. Materials and methods

2.1. cRNA preparation

The HERG cDNA construct was kindly provided by Dr. P. Spector (University of Oklahoma). The HERG cDNA in Psp64PolyA vector was linearized with *BamH*1. The digested product was ethanol precipitated and the linearized template DNA was resuspended in autoclaved double distilled water at a concentration of 1 μg/μl. MMesseage mMachine kit containing SP6 RNA polymerase enzyme (Ambion) was used to make cRNA from linearized templated DNA, according to the manufacturers instructions.

2.2. Oocyte isolation and cRNA injection

Female *Xenopus laevis* (Nasco, Fort Atkinson, WI, USA) were anaesthetized by a 20- to 30-min exposure to 3-aminobenzoic acid ethylester (tricaine, 1.5 g/l in water). All protocols were approved by University of Oklahoma IACUC Committee. Ovarian lobes were removed through a small incision in the abdominal wall and washed in Ca^{2+}-free OR-2 solution containing in mM: 82.5 NaCl; 2 KCl; 1 $MgCl_2$; 5 HEPES (pH adjusted to 7.6 with NaOH). Stage IV and V *Xenopus* oocytes were defolliculated by treatment with 1 mg/ml collagenase (Clostridiopeptidase A; Type IA, Sigma) in Ca^{2+}-free OR-2 solution for 1.5 h. Oocytes were incubated at 18 °C in modified Barth's solution with antibiotics, containing 96 mM NaCl; 2 mM KCl; 1 mM $MgCl_2$; 1.8 $CaCl_2$, 2.5 mM HEPES (free acid), 2.5 mM HEPES (sodium salt); 0.1 g/l Streptomycin sulfate; 0.27 g/l Pyruvic acid (sodium salt) and 0.5 g/l Gentamicin sulfate. Twenty-four hours after the isolation procedure, oocytes were injected with 23 nl of HERG cRNA (1.1–1.6 μg/μl) using a Drummond "Nanoject II" automatic nanoliter injector (Drummond Scientific, Broomall, PA).

2.3. Solutions and drugs

Currents were expressed in *Xenopus* oocytes and recorded using two-electrode voltage clamp technique 2–4 days after injection of cRNA. Oocytes were perfused at room temperature (22–24 °C). To attenuate endogenous chloride currents, Cl^- was replaced with D-gluconic acid in the external solution (in mM): 30 NaCl; 66 D-gluconic acid (sodium salt); 2 KCl; 1 $MgCl_2$; 1.8 $CaCl_2$, 2.5 HEPES (free acid) and 2.5 HEPES (sodium salt). Glass microelectrodes were filled by 3 M KCl with tip resistances of 0.7–3 MΩ. Oocytes were clamped with a Geneclamp 500 amplifier and voltage-clamp protocols were generated with pClamp software (Axon Instruments). Unless noted otherwise, the oocyte membrane potential was held at −70 mV between test pulses.

Flufenamic and niflumic acid solutions at concentrations 10^{-4} and 5×10^{-4} M each were bath applied at a rate 3 ml/min. Final concentration of the fenamates were prepared by adding appropriate volume of a 0.1 M stock prepared in DMSO (Dimethyl sulfoxide). Current recordings were measured after 3 min of perfusion at a given concentration to ensure steady-state conditions.

2.4. Current recording and data analysis

HERG currents were evoked by 2 s depolarizing voltage steps ranging from −70 to +50 mV in 10-mV increments. The current–voltage relationships were obtained by measuring the current amplitude at the end of the depolarizing steps and at the peak of tail currents. The activation curves were determined by fitting peak values of tail currents (I) versus test potential (V_t) to a Boltzmann function: $I = I_{max}/(1 + \exp[(V_{0.5} - V_t)/k])$, where I_{max} is the maximum tail current.

To assess kinetics of deactivation, a double-pulse protocol was used. Test potentials from −70 to +50 mV were applied followed by repolarization to −140 mV. The deactivation process (at −140 mV) was fit to double-exponential functions to obtain time constants of deactivation.

The kinetics of HERG inactivation were determined using voltage protocols similar to that previously published (Zou et al., 1998; Thomas et al., 2001). The ramp-clamp protocol is similar to that of Chen et al. (1999) and the action potential clamp protocol is similar to that of Zhou et al. (1998b). To simulate the voltage transition that occurs during action potential, we applied a voltage step from −80 to +40 mV (2 ms) followed by a slow (1.7 s) voltage ramp back to −80 mV. The simulated action potential clamp duration was 250 ms. Potential was stepped from −80 to

+40 mV, followed by a fast ramp to 0 mV (10 ms) The potential was then slowly ramped to −5 mV (120 ms) to mimic the plateau phase, followed by a ramp to −80 mV (120 ms) (Lees-Miller et al., 2000).

pClamp software (Axon Instruments) was used for the generation of voltage-clamp protocols and data acquisition. Fitting of currents was performed on Origin software. Results are expressed as mean ± standard error. Statistical significance of the observed effects was assessed by mean of a Student's *t*-test. A value of $P \leq 0.05$ was considered statistically significant.

3. Results

Depolarizing pulses from −70 to +50 mV in 10-mV steps (Fig. 1A, upper panel) evoked outward K^+ currents in *Xenopus* oocytes expressing HERG currents. All experiments were carried out in low Cl^- solutions in order to abolish activation of endogenous Cl^- currents. Outward currents activated slowly upon depolarization up to 0 mV, thereafter peak current amplitude decreased. This reflects the inwardly rectifying property of HERG currents that is due to fast inactivation at potentials positive to 0 mV. Upon repolarization to −70 mV, hook like tail currents were evoked due to the process of recovery from inactivation and whereby the channels pass through an open state before closing (Fig. 1A, middle panel). The current–voltage relationship for currents measured at the end of the step potentials, showed the typical bell-shaped response of HERG channels (Fig. 1B). Perfusion of the oocytes with flufenamic acid (10^{-4} M) showed two significant effects: (1) an increase in the amplitude of outward currents measured at the end of pulse and (2) an increase in the transient component of the current at potentials positive to 0 mV (Fig. 1A, bottom panel). In order to take into account the variation in current amplitudes between oocytes, currents were normalized to peak amplitude under control

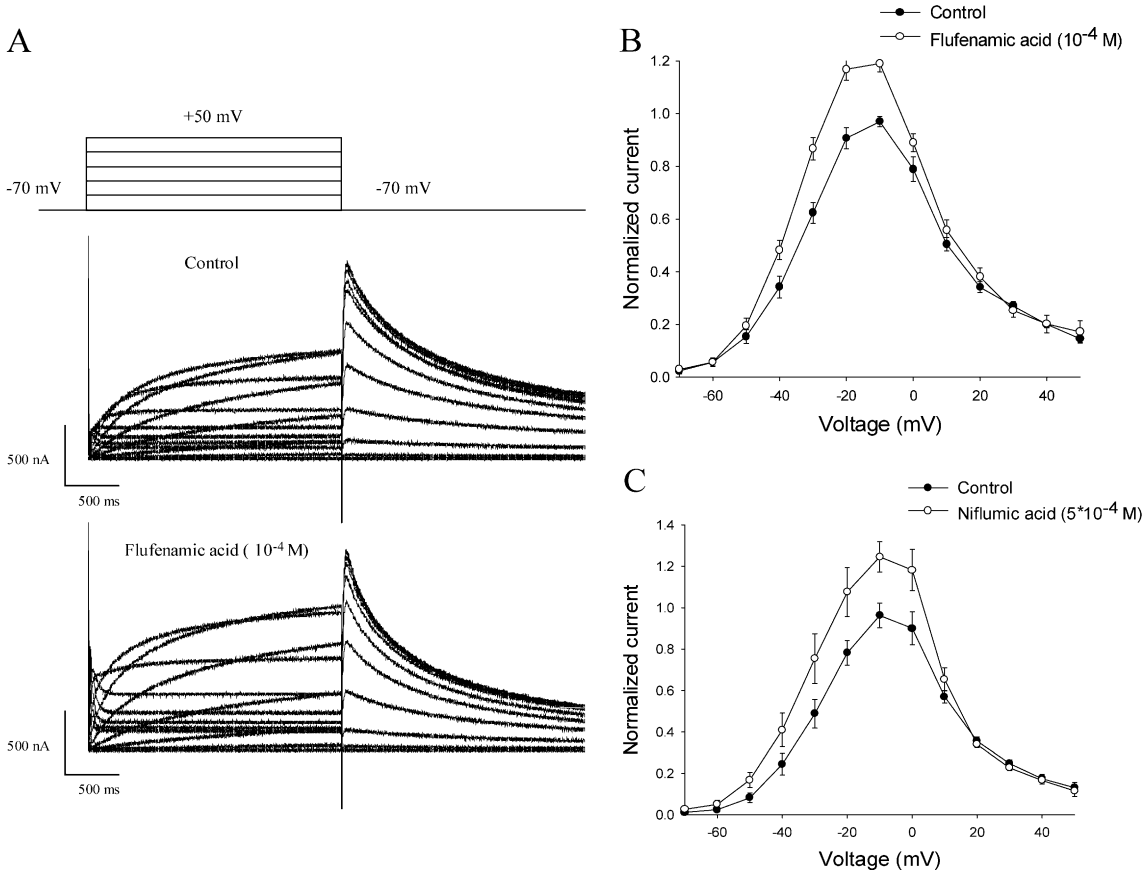

Fig. 1. Effects of fenamates on HERG currents elicited by depolarizing voltage pulses in *Xenopus* oocytes. (A) Superimposed current traces elicited by depolarizing voltage pulses (2 s, upper panel) from −70 to +50 mV in 10-mV increments from holding potential of −70 mV in control conditions (middle panel) and after application of 10^{-4} M flufenamic acid (lower panel) obtained from the same cell. (B and C) *I–V* relationship for HERG current, obtained from the protocol described in panel (A), measured at the end of depolarizing pulses against the pulse potential before and after application of flufenamic (10^{-4} M, B) and niflumic acids (5×10^{-4} M, C). Currents were normalized to the peak amplitude obtained in control conditions. Closed circles with error bars represent mean ± S.D. for control and open circles show data for drug application.

conditions. Between −40 and 0 mV, flufenamic acid increased the average current by 20–22% (Fig. 1B, $P \leq 0.05$; $n = 9$). At more positive potentials, between +10 and +50 mV, the current amplitude at the end of the pulse was not significantly different between control and flufenamic acid. Increasing the concentration of flufenamic acid to 5×10^{-4} M resulted in a larger enhancement of the current both at the end of pulse and the transient component. The enhancement was also observed with niflumic acid (Fig. 1C). At 5×10^{-4} M niflumic acid, the amplitude of the K^+ current represented an increase of 25.1 ± 0.1% at −10 mV ($P \leq 0.05$, $n = 8$). Neither of the fenamates demonstrated a shift in the current–voltage relationship.

In order to establish that these effects were not related to endogenous channels of the oocytes, the effects of flufenamic and niflumic acid were examined in water-injected oocytes. No appreciable current was evoked in the low Cl^- solutions and flufenamic acid had no effect on the currents (data not shown). Additionally, expressed HERG currents were blocked by the HERG channel blocker E-4031 (10 μM) and the effects of flufenamic acid were abolished in the presence of E-4031 (data not shown).

The increase in current amplitude and the enhancement of the transient current indicated either an increase in the activation or a decrease in inactivation of the HERG channels by fenamates. Fig. 2 shows the effects of fenamates on the time course of HERG current activation. HERG currents activate more slowly between −30 and 0 mV, with some inactivation appearing at 0 mV (Fig. 2A, middle panel). The rate of HERG channel activation was best fit by two exponentials. The rate of activation was markedly increased by flufenamic acid at all potentials between −30 and 0 mV for both the fast and slow components (Fig. 2A, bottom panel). The effect was more pronounced for the fast time constant (Fig. 2B) than the slow component. Flufenamic acid (5×10^{-4} M) decreased the fast time constant of activation at −30 mV from 322.8 ± 24.9 to 207.7 ± 28.9 ms (Fig. 2C, $P \leq 0.05$, $n = 9$).

The amplitude of HERG currents at potentials positive to 0 mV is markedly reduced due to the inactivation of the channels. The fact that inactivation is faster than activation results in a small transient component. As shown in Fig. 3, fenamates markedly enhanced the transient component

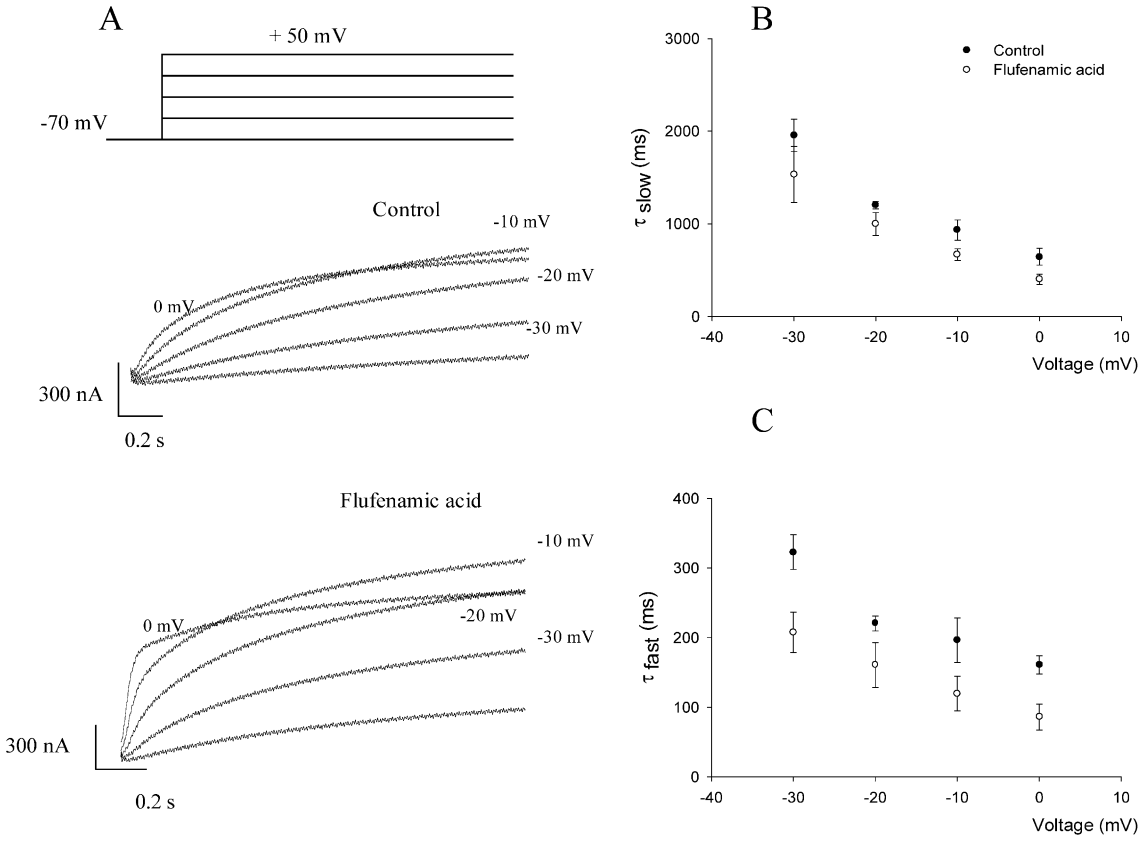

Fig. 2. Effects of flufenamic acid on HERG activation rate. (A) Slow activation of HERG currents at potentials from −30 to 0 mV. Middle panel shows currents obtained in control and bottom panel shows currents obtained in the presence of flufenamic acid (5×10^{-4} M). Each current trace was fit by two exponentials. (B and C) Voltage-dependent time constants for fast (B) and slow (C) components of activation. Flufenamic acid increases the fast component of activation rate of HERG channels.

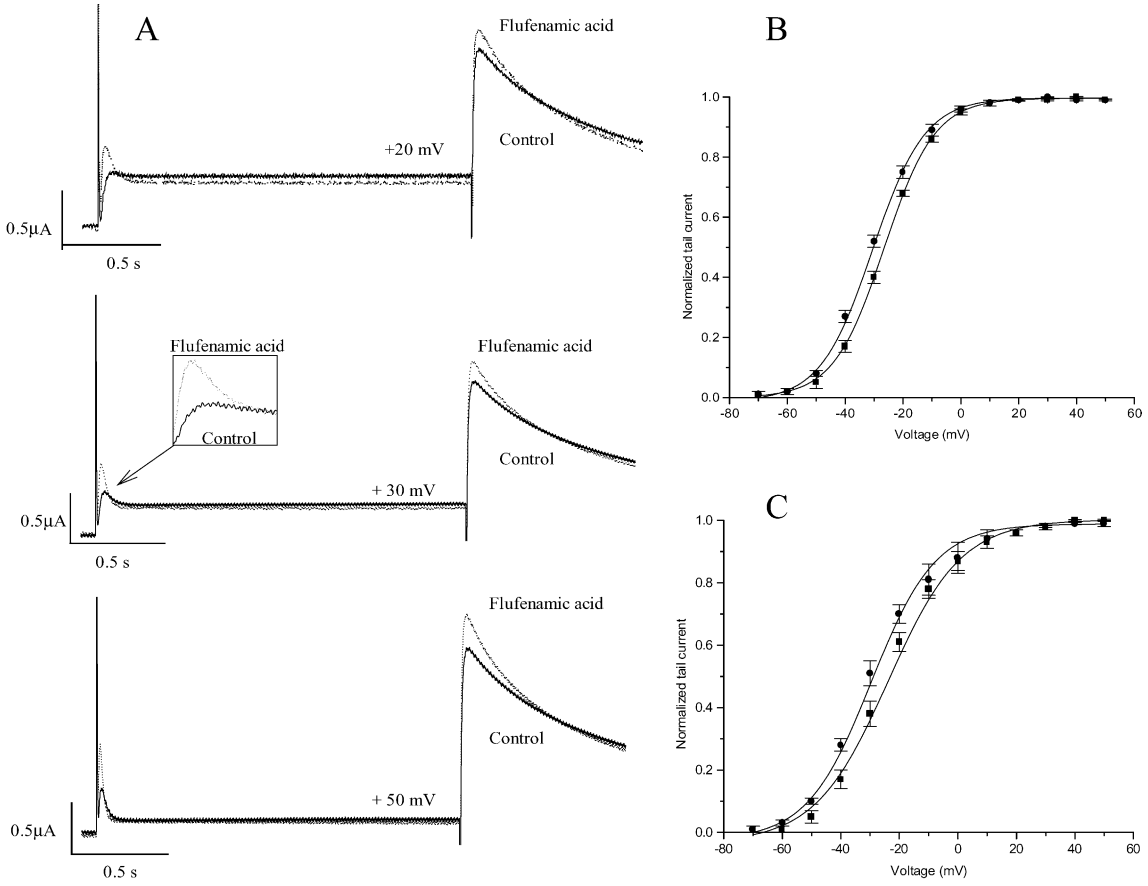

Fig. 3. Characterization of transient peak and tail currents after application of flufenamic and niflumic acids. Panel (A) shows the enhancement of HERG currents transient peak at voltages +20 mV (upper panel), +30 mV (middle panel) and +50 mV (lower panel) recorded before and after application of 10^{-4} M flufenamic acid. (B and C) Plot of normalized tail current measured in the absence (squares) and presence (circles) of flufenamic acid 10^{-4} M (B) and niflumic acid 5×10^{-4} M (C). $I_{tail}-V$ curves were fit by Boltzmann relationship.

followed by a sharp decline in the current amplitude to levels seen in control conditions. Upon repolarization, the tail current amplitude was also enhanced. Fig. 3A shows a typical example at potentials of +20, +30 and +50 mV. The effect of the fenamates on the transient component increased with increasing depolarization. The effect on the transient component was also seen with niflumic acid. The tail current voltage relationship was well fit by the Boltzmann function with $V_{1/2} = -26.7 \pm 0.1$ mV and slope factor $k = 8.6 \pm 0.1$ ($n = 9$). Flufenamic acid (5×10^{-4} M) shifted the $I_{tail}-V$ relationship to the left with a $V_{1/2}$ of -31.4 ± 0.2 mV (Fig. 3B). Similar shift in the $I_{tail}-V$ relationship was observed with niflumic acid (5×10^{-4} M) from -23.4 ± 0.9 to -29.4 ± 0.7 mV (Fig. 3C, $P \leq 0.05$, $n = 8$). These data indicate that although the channels inactivated to the same extent upon depolarization, there were a larger population of channels available for opening in the presence of fenamates upon repolarization.

We next examined whether flufenamic acid may alter the deactivation kinetics of the HERG channel. To analyze the deactivation time constants, a two-pulse protocol was used (Fig. 4A, inset). The cells were stepped to various test potentials from a holding potential of −70 mV, followed by hyperpolarization to −140 mV. At −140 mV, channels recover from inactivation followed by deactivation. The deactivating inward tail currents were fit by a double exponential. Both flufenamic acid (Fig. 4A) and niflumic acid (Fig. 4B), markedly slowed the rate of deactivation. The fast component which most likely represents inactivation and some deactivation was only slightly affected by the fenamates (Fig. 4D and F), while the time constants for the slow component was significantly increased. For flufenamic acid (10^{-4} M), τ_{slow} increased from 313.0 ± 9.1 to 679.9 ± 21.4 ms following test potential of 0 mV, and from 322.2 ± 7.9 to 695.3 ± 27.8 ms at +40 mV (Fig. 4C, $P \leq 0.01$, $n = 9$). There was no voltage-dependence to the rate of deactivation for either of the fenamates. At 0 mV niflumic acid (5×10^{-4} M) decreased τ_{slow} by 63% and at +50 mV it was reduced by 76% (Fig. 4E, $P \leq 0.01$, $n = 8$). These results suggest that in addition to increasing the rate of activation, fenamates additionally decrease the rate of deactivation.

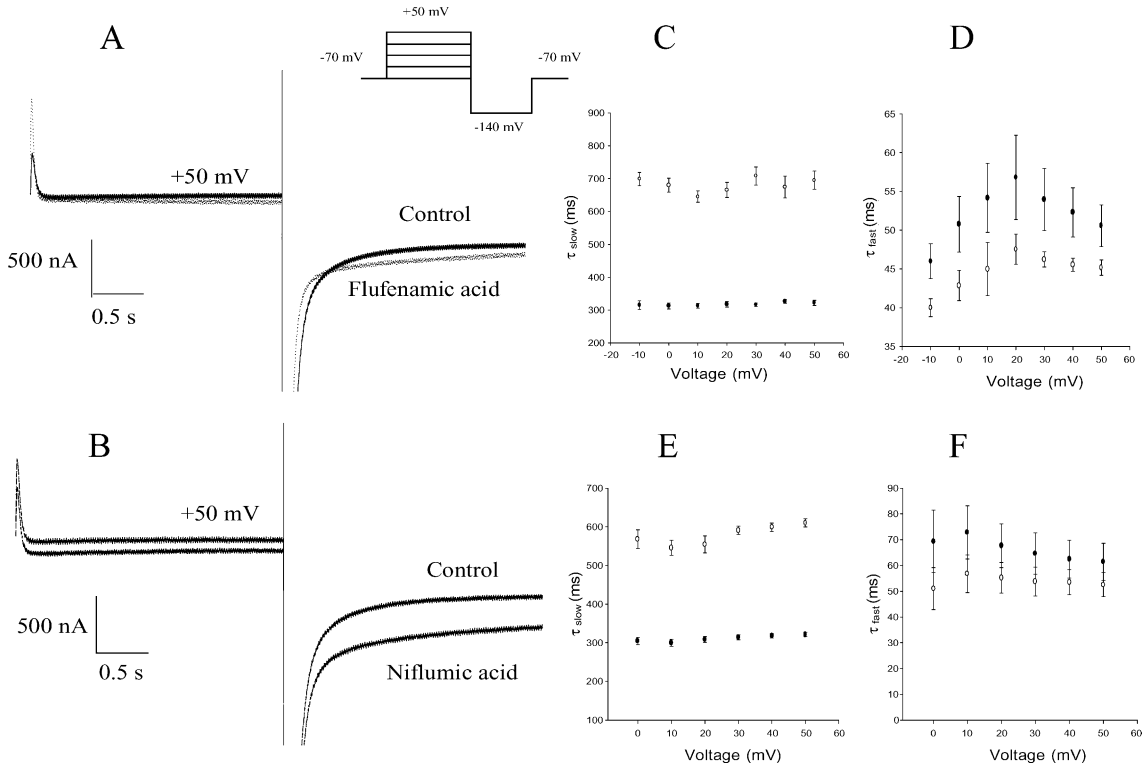

Fig. 4. Flufenamic and niflumic acids decelerate deactivation of HERG currents. (A and B) Currents were recorded during test pulses to potentials ranging from −70 to +50 mV followed by hyperpolarizing step to −140 mV to rapidly remove inactivation. The time constants of deactivation were determined from tail currents measured at −140 mV and fit with double exponential function. (C and D) Voltage-dependent time constants for slow (C) and fast (D) components of deactivation after application of flufenamic acid (10^{-4} M). (E and F) show the slow and fast time constants of deactivation in the presence of niflumic acid (5×10^{-4} M). Close circles represent control data, open circles show drug application.

In order to examine the effects of fenamates on the voltage-dependence of channel inactivation, a three-pulse protocol was applied. The cell was depolarized to +20 mV for 2 s to establish inactivation, followed by a 30-ms prepulse to potentials between −140 and +40 mV to allow recovery from inactivation but little deactivation. The cell was then depolarized to a test potential of +20 mV to assess the relative number of channels that recovered from inactivation during the prepulse (Zou et al., 1998). The voltage protocol is shown in inset of Fig. 5A. The amplitude of the current obtained during the test potential was plotted as a function of the prepulse potential and fit with a Boltzmann function. As shown in Fig. 5A, flufenamic acid (5×10^{-4} M) did not affect the kinetics of channel inactivation. Fig. 5B shows the voltage-dependence of the peak current versus the prepulse potential.

The rate of channel inactivation was measured by using a three-pulse protocol as illustrated in Fig. 5C (inset). The cell was held at +40 mV for 2-s duration followed by a brief repolarization to −140 mV for 15 ms to allow recovery from inactivation. Variable test potentials were then applied between −60 and +40 mV. As shown in Fig. 5C, flufenamic acid (5×10^{-4} M) did not significantly alter the rate of inactivation. The time constant for a single exponential fit was measured and plotted against the test potential (Fig. 5D). There was no significant difference in the time constants between control and fenamates.

In a final series of experiments, we examined the effects of the fenamates on the repolarizing currents using ramp protocols. In the ramp protocol (Fig. 6A, upper panel) that involves an initial step depolarization from −80 to +40 mV, followed by a slow ramp (1.7 s) to −80 mV, an initial transient peak was obtained in the presence of niflumic acid (10^{-4} M) followed by a slight enhancement of the outward current upon slow repolarization.

The effects of the fenamates were further examined using a ramp protocol mimicking a cardiac action potential. Potential was stepped from −80 to +40 mV, followed by a fast ramp to 0 mV (10 ms) The potential was then slowly ramped to −5 mV (120 ms) to mimic the plateau phase, followed by a ramp to −80 mV (120 ms). Application of this protocol resulted in small outward current during the plateau of action potential, with a significant activation of the current during phase 3 repolarization of the action potential. Flufenamic acid at 10^{-4} M (Fig. 6B, middle panel) and niflumic acid at 5×10^{-4} M (Fig. 6B, lower panel) significantly increased the amplitude of outward current both during the plateau of action potential

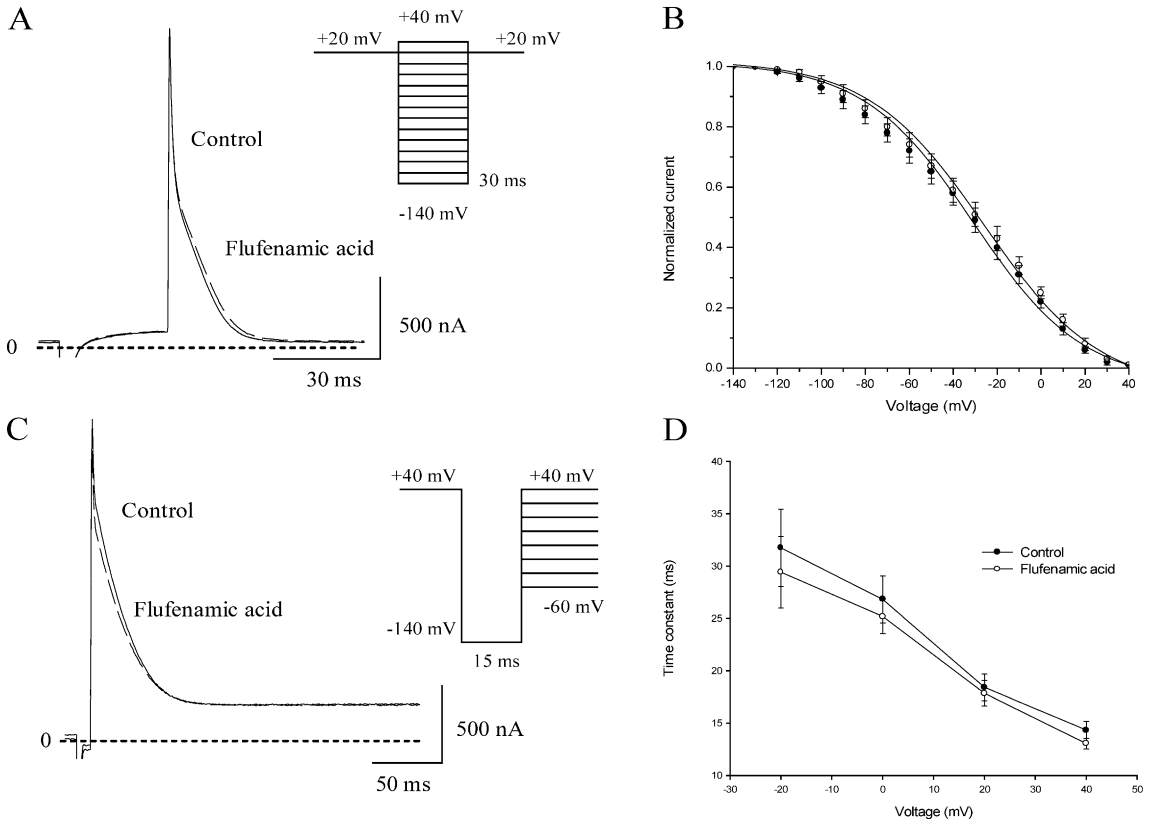

Fig. 5. Fenamates do not markedly affect HERG current inactivation. Panel (A) shows measurements of the steady-state inactivation at test potential +20 mV after prepulses between −140 and +40 mV in 10-mV increments for 30 ms. The voltage protocol is illustrated in the inset. Superimposed traces after hyperpolarization pulse of −50 mV are displayed before and after application of flufenamic acid (5×10^{-4} M). The normalized current amplitude at the beginning of the tail current at 20 mV was plotted against prepulse potential (panel B), resulting in a steady-state inactivation curve under control conditions (closed circles) and after application of flufenamic acid (5×10^{-4} M, open circles). The difference between control and experimental data was not significant. (C) To examine the inactivation time course, a conditioning pulse to +40 mV for 2 s from holding potential of −70 mV was followed by a hyperpolarizing pulse to −140 mV for 15 ms, and subsequent depolarizing test pulses between −60 and +40 mV in 20-mV steps were applied. The inset illustrates the voltage protocol. Superimposed traces at +20 mV are shown before and after application of flufenamic acid (5×10^{-4} M). Inactivation time constants (τ) were measured by fitting with a single-exponential function. (D) τ values representing inactivation time constants plotted as a function of test potential.

(69.9 ± 7.8%, $n = 8$) and phase 3 of the simulated action potential (52.8 ± 9.6%, Fig. 6C).

4. Discussion

There has been considerable interest in HERG since the discovery that it is mutated in chromosome 7 form of long QT syndrome (Curran et al., 1995). HERG channels expressed in *Xenopus* oocytes display inward rectification resulting from a rapid inactivation mechanism at potentials positive to 0 mV, followed by large transient currents that occur upon repolarization. This effect is due to inverse gating kinetics compared to classical Shaker-like K^+ channels, i.e. inactivation is faster than activation and recovery from inactivation is considerable faster than deactivation. In the present study, we found that fenamates at potentials up to 0 mV enhanced the amplitude of the outward currents and at more positive potentials, application of fenamates resulted in an enhanced transient component. Between −80 and 0 mV, HERG currents are slowly activating whereas inactivation predominates at more positive potentials resulting in inward rectification. The increase in current amplitude by fenamates could result from an increase in activation or decrease in the rate of inactivation. Both the fast and slow components of activation were significantly enhanced by fenamates between −30 and 0 mV. In the presence of fenamates, the HERG deactivation rates were also slowed, particularly the slow component. However, neither of the fenamates affected the voltage-dependence or the rate of inactivation.

The net result of these is an enhancement in the amplitude of the HERG currents and the presence of a transient component at potentials positive to 0 mV, where normally inactivation is faster than activation resulting in reduction in the outward current. The most plausible explanation for the presence of a transient peak at potentials positive to 0 mV is that activation becomes faster than inactivation resulting in an enhanced amplitude of current. This effect is similar to the effects of fenamates on IsK where there is an increase in the instantaneous current (Busch et al., 1994a). However, unlike IsK, HERG currents inactivate resulting in a transient peak. The physiological importance of an accelerated HERG chan-

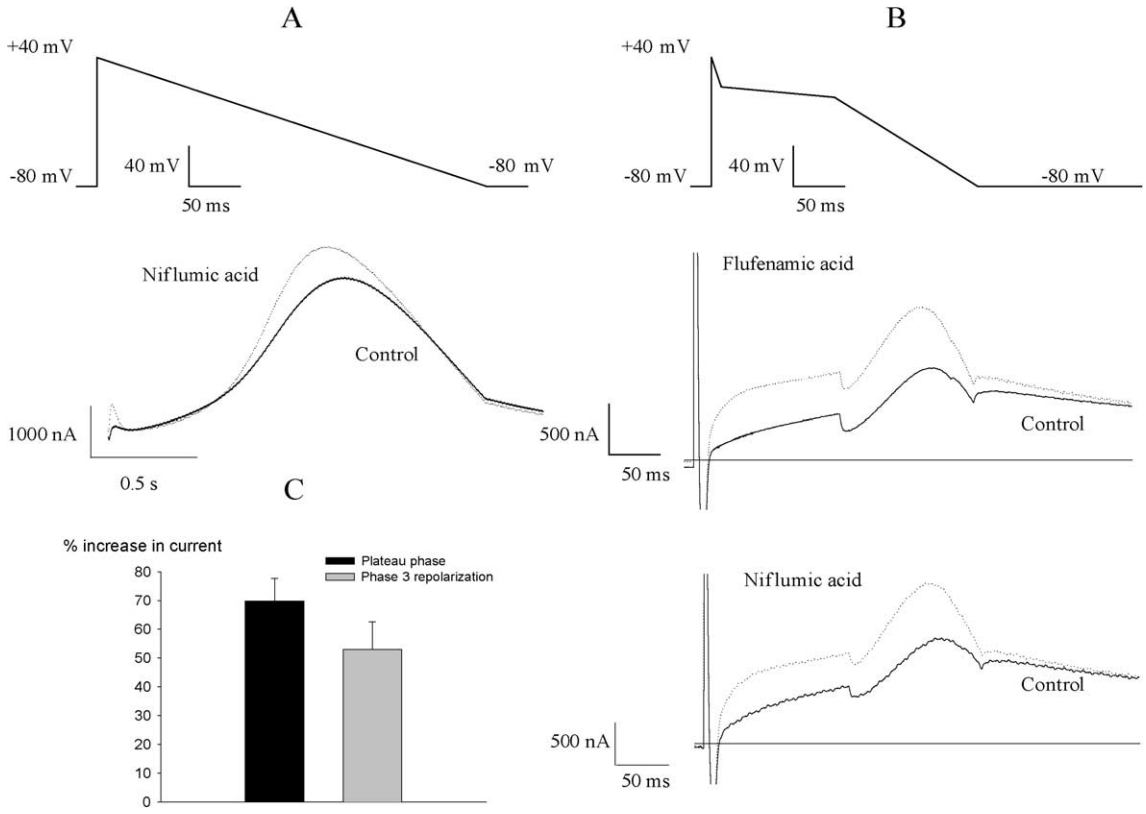

Fig. 6. Physiological significance of the fenamates using ramp and action potential voltage protocols. (A) Examples of fenamates-sensitive currents elicited by ramp clamp. Protocol is shown in the inset. During repolarizing, ramp niflumic acid (5×10^{-4} M) increased the transient peak and the amplitude of repolarizing current. Similar increase in transient current and repolarizing current was obtained with flufenamic acid (5×10^{-4} M). (B) Flufenamic and niflumic acids (5×10^{-4} M) increased the amplitude of HERG current during phases 2 and 3 of simulated action potential. Dotted lines represent current obtained in the presence of the fenamates. (C) Percentage increase by flufenamic acid of current during plateau phase and during repolarization (phase 3).

nel activation and decelerated deactivation was evident using the ramp clamp and simulated action potential protocols (Fig. 6). Ramp clamp data suggest that an outward HERG current is produced during repolarization between 0 and -50 mV reaching maximum amplitude at -40 mV. Additionally, flufenamic and niflumic acids increased outward voltage-dependent HERG current during plateau phase and phase 3 repolarization of simulated action potential clamp that would result in shortening of the action potential duration. The amplitude of current during the declining voltage ramp is determined by the declining driving force for outward flux of K^+, the number of channels that recover from inactivation and the rate of channel deactivation (Chen et al., 1999).

Due to the leftward shift in the $I_{tail}-V$ relationship by fenamates and since neither the voltage-dependence of inactivation nor the time constants for inactivation were significantly affected, it is suggested that these compounds may affect the activation gate of the HERG channel. A possible interpretation is that the fenamates accelerate channel opening and slow channel closure.

Fenamates are known to affect several types of ion channels. Of particular interest has been the recent findings of Abitbol et al. (1999) describing the rescue of IsK mutation by fenamates. Mutations of IsK which forms the β-subunit of the delayed rectifier K^+ channel ($K_{V(s)}$) are present in long Q−T syndrome. Co-expression of KvLQT1 and IsK produces the current resembling the cardiac delayed rectifier current, $I_{K(Vs)}$. Abitbol et al. (1999) demonstrated that fenamates could rescue the dominant negative effect of IsK on outward K^+ currents. Our finding that expressed HERG channels may be similarly modified in their gating kinetics indicate that fenamates may modify both $I_{K(Vs)}$ and $I_{K(Vr)}$.

Many structurally diverse group of drugs block the HERG channel, which has led to untoward side effects of these therapeutically used agents on cardiac function. By using alanine-scanning mutagenesis, Mitcheson et al. (2000) have recently identified the importance of aromatic residues within the S6 domain of erg/eag channels in the interaction with these agents. These residues appear to face the pore of the HERG channel. It remains to be determined whether these sites are involved in the interaction with the fenamates. It is noteworthy, that in the case of the interaction of fenamates with IsK channel, the interaction appears to be at the extracellular N-terminal segment which allosterically interacts with KvLQT1 to affect channel gating (Abitbol et al., 1999).

The concentrations of the flufenamic and niflumic acids we used in our experiments were within the range used to

block endogenous Ca^{2+}-activated Cl^- currents of oocytes (White and Aylwin, 1990; Busch et al., 1994a,b). Therefore, in order to avoid any effects on the endogenous currents, experiments were carried out using low Cl^- solutions. The lack of enhancement or inhibition by the fenamates in water injected oocytes confirm that these effects did not arise from inhibition of endogenous Cl^- currents. Moreover, the effects of fenamates were abolished in the presence of the HERG channel blocker, E-4031.

Synthetic molecules such as fenamates and other openers of K^+ channel offer novel therapeutical approach of stabilizing and controlling cellular function (Lawson, 2000). Our results, to our knowledge, demonstrate for the first time a link between the fenamate class of non-steroidal anti-inflammatory agents and the electrophysiological properties of the HERG K^+ channel. The positive regulation by fenamates on HERG and previously described effects on IsK and K_VLQT1 channels (Busch et al., 1994a; Chouabe et al., 1998; Abitbol et al., 1999) suggest a potential role of fenamates in prevention or treatment of long QT syndrome.

Acknowledgements

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