Trapping of a Methanesulfonanilide by Closure of the HERG Potassium Channel Activation Gate

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abstract Deactivation of voltage-gated potassium (K\(^+\)) channels can slow or prevent the recovery from block by charged organic compounds, a phenomenon attributed to trapping of the compound within the inner vestibule by closure of the activation gate. Unbinding and exit from the channel vestibule of a positively charged organic compound should be favored by membrane hyperpolarization if not impeded by the closed gate. MK-499, a methanesulfonanilide compound, is a potent blocker ([IC\(_{50}\) = 32 nM] of HERG K\(^+\) channels. This bulky compound (7 × 20 Å) is positively charged at physiological pH. Recovery from block of HERG channels by MK-499 and other methanesulfonanilides is extremely slow (Carmeliet, 1992; Ficker et al., 1998), suggesting a trapping mechanism. We used a mutant HERG (D540K) channel expressed in Xenopus oocytes to test the trapping hypothesis. D540K HERG has the unusual property of opening in response to hyperpolarization, in addition to relatively normal gating and channel opening in response to depolarization (Sanguinetti and Xu, 1999). The hyperpolarization-activated state of HERG was characterized by long bursts of single channel reopening. Channel reopening allowed recovery from block by 2 \(\mu\)M MK-499 to occur with time constants of 10.5 and 52.7 s at −160 mV. In contrast, wild-type HERG channels opened only briefly after membrane hyperpolarization, and thus did not permit recovery from block by MK-499. These findings provide direct evidence that the mechanism of slow recovery from HERG channel block by methanesulfonanilides is due to trapping of the compound in the inner vestibule by closure of the activation gate. The ability of HERG channels to trap MK-499, despite its large size, suggests that the vestibule of this channel is larger than the well studied Shaker K\(^+\) channel.

key words: MK-499 • voltage clamp • Xenopus oocyte

INTRODUCTION

The voltage and channel state-dependent modulation of ionic currents by drugs has provided insights into the structure of voltage-gated ion channels. 30 yr ago, Armstrong (1969, 1971) showed that block of a voltage-gated K\(^+\) channel in squid giant axon by a quaternary ammonium ion (QA)\(^1\) required channel opening, that QA access to the binding site inside the pore occurred from the intracellular side, and that elevation of external [K\(^+\)] hastened dissociation of the QA. Moreover, channel block by small QA compounds (e.g., TEA) was not associated with a change in deactivation rate. These findings suggested that TEA could be trapped inside the channel pore by closure of the activation gate. Drug trapping has also been described for TEA or decytriethylammonium by a mutant Shaker K\(^+\) channel (Holmgren et al., 1997), and small local anesthetics by sodium channels (Strichartz, 1973). These later studies supported Armstrong’s model that the inner pore of a channel was a relatively large water-filled vestibule, situated between the putative selectivity filter and activation gate and lined by hydrophobic residues (Armstrong, 1969, 1971). These predicted features of the vestibule were confirmed when the crystal structure of the KcsA channel was solved (Doyle et al., 1998). The inner pore of KcsA, and by inference other K\(^+\) channels, is lined by the inner helices (homologous to S6 transmembrane domains) from each of four subunits, arranged to form an inverted teepee structure.

Most previous investigations of open channel-dependent block and trapping by drugs have used low affinity, charged molecules such as QA compounds for K\(^+\) channels, or local anesthetic agents for Na\(^+\) channels. High micromolar to millimolar concentrations of local anesthetics or QA compounds are required to block these channels. In contrast, methanesulfonanilide compounds such as dofetilide and MK-499, antiarrhythmic agents that also appear to block by a trapping mechanism (Carmeliet, 1992), reduce the rapid delayed rectifier K\(^+\) current (I\(_{\text{Kr}}\)) of cardiac myocytes at nanomolar concentrations. I\(_{\text{Kr}}\) channels are formed by coassembly of HERG subunits (Sanguinetti et al., 1995; Trudeau et al., 1995). Recent studies have confirmed that methanesulfonanilides are potent blockers of heterologously expressed HERG channels (Kiehn et al., 1996;
Dependent opening of D540K is half-maximal at -171 mV, whereas depolarization-dependent opening of these channels is half-maximal at -9 mV. The aim of the present study was to exploit the unique gating properties of D540K HERG and determine whether the very slow recovery from block of HERG channels by methanesulfonanilide compounds results from a trapping mechanism. We first characterized the properties of hyperpolarization-activated channel opening of D540K HERG and showed that these openings were associated with long periods of single channel bursting activity. We then demonstrated that strong hyperpolarization of D540K HERG, but not wild-type (WT) HERG channels, promotes recovery from block by MK-499. These studies provide a direct demonstration that, if not for closure of the activation gate, membrane hyperpolarization favors unbinding of a large positively charged compound. More specifically, these studies provide a molecular mechanism for the slow recovery from HERG channel block by methanesulfonanilide compounds, a potent and clinically important class of antiarrhythmic agents.

Materials and Methods

Isolation and maintenance of Xenopus oocytes, site-directed mutagenesis, and cRNA injection were performed as described previously (Goldin, 1991; Goldin and Sumikawa, 1992; Sanguinetti and Xu, 1999). Whole cell currents were recorded 2–4 d after cRNA injection using a GeneClamp 500 amplifier, a Pentium computer with a Digidata 1200 computer interface (Axon Instruments), and standard two-electrode voltage clamp techniques (Stühmer, 1992). To attenuate endogenous chloride currents, Cl⁻ was replaced with Mes in the external solution that contained (mM): 96 NaMES, 2 KMES, 2 CaMES, 5 HEPES, 1 MgCl₂, adjusted to pH 7.6 with methane sulfonic acid. Some oocytes had large endogenous inward currents when the membrane was pulsed to very negative potentials. The presence of these currents varied between batches of oocytes and was easily discernible from the D540K HERG inward currents based on their negative activation threshold and very slow activation kinetics. Batches of oocytes with large endogenous currents were discarded.

Bath solutions were applied with a switching device that directed flow through a 1-ml Gilson pipette tip and could be placed within 0.5 mm of the oocyte. Flow rates of 3–4 ml min⁻¹ were thus restricted to a small volume around the cell, ensuring complete and rapid exchange of solutions and preventing external K⁺ accumulation/depletion when current amplitudes were limited to ±4 μA. Whole-cell current records were on-line filtered and digitized at 1 kHz, unless otherwise stated. Currents were not leak subtracted.

Single channel recording from Xenopus oocytes was performed as described previously (Zou et al., 1997) using an Axopatch 200 patch clamp amplifier (Axon Instruments). The extracellular solution contained (mM): 120 KCl, 10 HEPES, 5 EGTA; adjusted to pH 7.2 with KOH. Current signals were on-line filtered at 1 kHz with a low-pass Bessel filter and digitized at 2.5 kHz with a Pentium computer and Digidata 1200 interface. Single channel data were analyzed using Fetchan and Pstat software (Pclamp 6; Axon Instruments). Single channel amplitudes were generated from all point histograms of binned single channel data and fit with Gaussian distributions using a Levenberg-Marquardt least-squares fit routine. Amplitude threshold analysis was used to generate idealized single channel traces for calculating dwell time and time-dependent open probability histograms.

MK-499 (Claremon et al., 1993), supplied by Merck and Co., Inc., was prepared daily by dilution to the required concentration from a 5-mM stock solution that was dissolved in dimethylsulphonic acid and kept at -20°C.

Data are expressed as mean ± SEM. Statistical analysis was performed using paired and unpaired t tests (Excel; Microsoft Corp.). Curve fitting was performed with Origin software (Microcal).

Results

Properties of Whole Cell D540K HERG Channel Current Activated by Hyperpolarization

We previously described the biophysical properties of D540K HERG (Sanguinetti and Xu, 1999). Before studying the block of D540K HERG by MK-499, we fur-
ther characterized the unique gating properties of this mutant channel using whole-cell and single-channel recording techniques. D540K HERG current elicited by depolarization from a holding potential of −90 mV appeared to activate instantaneously, followed by rapid inactivation. In the example shown in Fig. 1A, currents were elicited by 2-s depolarizations to potentials ranging from −60 to +20 mV. Deactivation elicited by repolarization to −70 mV was relatively fast. In the same cell, hyperpolarization from the holding potential of −90 mV activated a small instantaneous current followed by a much slower inward current (Fig. 1B). The instantaneous component represents channels that were open at the holding potential of −90 mV. In contrast to deactivation of depolarization-activated current, the deactivation of hyperpolarization-activated channels was quite slow at −70 mV (compare tail currents in Fig. 1A and B). Thus, channels opened by depolarization activate and deactivate rapidly, whereas channels opened by hyperpolarization activate and deactivate slowly. The time course of current activation in response to depolarizing pulses is obscured by the rapid onset of inactivation. However, when other mutations (G628C, S631C) that remove inactivation (Smith et al., 1996) were introduced into the D540K HERG background, the rate of activation was easily discernible (Fig. 1C), with time constants of 15 ± 1 and 92 ± 8 ms at 0 mV (n = 9). Inactivation-removed D540K HERG channels also reopened upon hyperpolarization (Fig. 1C, bottom). The I-V relationship for steady state D540K HERG channel currents activated by either depolarizing or hyperpolarizing pulses showed inward rectification, with a peak outward current near −20 mV (Fig. 1D). The small slope conductance between −60 and −90 mV indicates the voltage range where D540K HERG channels are primarily in a closed state.

The contrast between the properties of depolarization and hyperpolarization-activated open states of D540K HERG channels are most obvious using a two-pulse protocol. Channels were opened and/or inactivated by a 1-s prepulse to +40 mV, followed by a pulse to −160 mV (Fig. 2A). Hyperpolarization elicited a large transient inward current followed by a second, slowly activating inward current (Fig. 2B). The fast component resulted from rapid recovery of depolarization-activated channels from inactivation and their subsequent rapid deactivation (Schonherr and Heinemann, 1996; Smith et al., 1996; Spector et al., 1996b). The slow component of inward current resembles the hyperpolarization-activated current (Fig. 1B), and presumably is caused by channels reopening from the deactivated, closed state. After extrapolating back to time zero to correct for deactivation (Fig. 2C), the amplitude of the depolarization-activated current component was clearly larger than the second component, even though the driving force was the same. The disparity in amplitude could result from either a difference in single channel current amplitude or open probability between the two open states. To investigate this further, we compared the single channel properties of the depolarization-activated
Drug Trapping in HERG Pore

Single Channel Properties of Hyperpolarization-activated D540K HERG

WT and D540K HERG single channel currents were recorded using the cell-attached configuration of the patch-clamp technique. Channel openings were elicited by stepping to −80, −140, or −160 mV for 5 s, after a 500-ms prepulse to +40 mV. At −80 mV, inward single channel events were observed throughout the 5-s step for both WT and D540K HERG currents. However, at −140 and −160 mV, single channel WT HERG currents were only observed at the beginning of the pulse (Fig. 3 B). In contrast, D540K HERG single channels opened repetitively throughout the pulse (Fig. 3 C). There was no significant difference between WT and D540K HERG single channel amplitudes at any potential (P > 0.05, Fig. 3 D). The I-V relationship for both channels displayed weak inward rectification, as previously reported for WT HERG (Zou et al., 1997) and cardiac I_{Kr} (Ito and Ono, 1995) channels. This may result from voltage-dependent transitions to a substrate or flicker block by an unidentified intracellular molecule (Zou et al., 1997). The single channel conductance, determined by linear regression analysis of mean data was 22.0 pS between −160 and −120 mV and 12.4 pS between −120 and −60 mV. The ensemble-averaged current at −160 mV for a macropatch containing at least six channels is shown in Fig. 4. The number of open channels increased progressively during the 5-s pulse and the ensemble average closely resembled the whole-cell current at this test potential (Fig. 1 B).

The single channel behavior of D540K HERG at −80 and −160 mV was most clearly observed during long gap-free recordings. At −80 mV, the open probability was low and open channel bursting was rare and of short duration (Fig. 5 A). In contrast, the open probability at −160 mV was much greater and was associated with long bursts of channel openings (Fig. 5 B). Fig. 5, C and D, shows plots of open probability during 4–5 min of continuous recording. At −80 mV, single channel openings were rare and appeared random. In contrast, single channel open probability appeared modal at −160 mV, with long periods of bursting activity inter-
rupted by prolonged pauses. These data suggest that destabilization of the closed state of D540K is voltage dependent. This was confirmed by estimating the open probability of channels as a function of membrane potential. Single channel open probability ($P_o$) was strongly voltage dependent (Fig. 5 E), increasing from $0.01 \pm 0.003$ ($n = 6$) at $-80$ mV to $0.37 \pm 0.027$ ($n = 3$) at $-180$ mV. The voltage dependence of $P_o$ determined from single channel analysis was shifted in the negative direction compared with whole cell recordings, where $P_o$ was half-maximal at $-117$ mV (Sanguinetti and Xu, 1999). This shift was largely due to the different ionic conditions used for whole-cell and single-channel recordings. When we recorded whole-cell currents in ionic conditions more similar to the single-channel recording conditions (96 mM K$^+$, nominally Ca$^{2+}$-free solution), the voltage dependence of D540K HERG activation was shifted by at least $-40$ mV (not shown).

In summary, the single channel conductance of D540K HERG activated by hyperpolarization was identical to WT HERG activated by depolarization. Progressive hyperpolarization increased single channel burst duration and open probability. These results suggest that the D540K mutation destabilizes the closed state of HERG, but does not affect the conductance or selectivity properties of the channel.

Hyperpolarization-induced Channel Opening of D540K HERG Facilitates Recovery from Block by MK-499

The properties of HERG channel block by the methanesulfonanilide MK-499 has many of the hallmarks typical of drug trapping. Block occurs from the intracellular side of the channel (Zou et al., 1997) and requires prior channel opening (Spector et al., 1996a). Moreover, block is essentially irreversible and the rate of HERG channel deactivation is not altered by MK-499 (Spector et al., 1996a). These findings suggest that block results from trapping, and not a "foot in the door" mechanism. MK-499 is a large molecule (Fig. 6 A) that is nearly all charged at physiological pH. At the pH 7.6 of the extracellular solution, $\approx 99\%$ of the drug has a single positive charge and $\approx 65\%$ of the drug has two positive charges ($pK_a = 7.86$ and 9.52). The drug trapping hypothesis predicts that if HERG channels could reopen from the closed state in response to hyperpolarization, then the channels should recover from block by a positively charged drug like MK-499. We exploited the unique gating properties of D540K HERG to determine whether block of HERG channels by MK-499 results from a trapping mechanism.

We first determined the concentration-response relationship for MK-499 block of HERG channels. Since HERG activation is slow, we used long test pulses with a short inter-pulse duration so that channels spent the majority of time in an activated or inactivated state. From a holding potential of $-90$ mV, 5-s pulses to 0 mV were followed by repolarization to $-70$ mV for 400 ms. The membrane was held at $-90$ mV for only 600-ms between successive pulses. Oocytes that had a steadily incremented current magnitude during the control period, indicative of a shift in the voltage dependence of inactivation due to extracellular accumulation of K$^+$, were discarded. WT and D540K HERG channel currents were blocked by MK-499 in a concentration-dependent manner (Fig. 6). Concentration-response curves were constructed by measuring steady state current amplitudes at each MK-499 concentration and normalizing to control, and then fitting with a Hill equation. The concentration at which 50% inhibition occurred ($IC_{50}$) was $32 \pm 4$ nM ($n = 4$) and $104 \pm 8$ nM ($n = 5-7$) for WT and D540K HERG channel currents, respectively. Steady
state (end pulse) current reflects off and on rates for MK-499 binding at −90 mV (holding potential) and 0 mV (test potential). At −90 mV, a small fraction of D540K, but not WT HERG channels, are in the open state. The reduced potency of MK-499 for block of D540K HERG channels could be caused by unblock of open channels that occurred at the holding potential of −90 mV. For the remaining experiments, we used 2 μM MK-499 to investigate the mechanisms of recovery from HERG channel block by this drug. Under steady state conditions, this concentration completely blocked D540K HERG channels.

To investigate WT HERG recovery from MK-499 block, we used the voltage protocol illustrated in Fig. 7 A (top). Voltage pulses to 0 mV were applied repetitively at 10-s intervals. Once current amplitudes had stabilized in control conditions (Fig. 7 A, a), 2 μM MK-499 was applied. When block reached >85% (Fig. 7 A, b), 5-s hyperpolarizing pulses to −160 mV were applied in the continued presence of MK-499 (Fig. 7 A, c). These repetitive hyperpolarizing pulses were applied at 20-s intervals for 15 min. To assess recovery from block, a depolarizing pulse to 0 mV was applied (Fig. 7 A, e). The amplitude of peak current as a function of time for the whole experiment is illustrated in Fig. 7 B. The percent recovery from block was calculated from the difference in peak current amplitude between e and b, divided by peak current in a. After 40–50 hyperpolarizing pulses (~13–17 min), the mean recovery from block of WT HERG was 5.0 ± 0.9% (n = 5).

The same protocol described for WT HERG was used to examine the kinetics of recovery from block by MK-499 of D540K HERG current. Whole cell “control” current (Fig. 8 A, a) was blocked >85% by repetitive pulsing to 0 mV in the presence of 2 μM MK-499 (b). The first (Fig. 8 B, c) and last (d) of 38 hyperpolarizing pulses to −160 mV are shown. In response to the first hyperpolarizing pulse (c), the current was initially near 0, but became progressively larger during the 5-s pulse. Currents at the end of the pulse and peak tail currents were substantially larger for the last (d) compared with the first (c) hyperpolarizing pulse, indicating recovery from block. Recovery was assessed with a depolarizing pulse to 0 mV (e). Comparing a and e, it is apparent that repetitive pulsing to −160 mV resulted in considerable recovery (in this example, 85%) from MK-499 block. The time course of MK-499 block and unblock is illustrated in Fig. 8 B. Both onset and recovery from block had an exponential time course. D540K HERG recovery from MK-499 block was calculated in the same way as described above for WT HERG. The mean recovery with 35–50 hyperpolarizing pulses was 95.3 ± 2.8% (n = 6).

It could be argued that the greater recovery from block of D540K HERG than WT HERG channels was caused by an allosteric affect on the drug binding site caused by mutation of D540 and not by untrapping when channels reopened in response to hyperpolarization. To test for this possibility, we performed the same recovery from block experiment on D540A HERG channels. These channels activate and deactivate rapidly and have identical voltage dependence for depolarization-activated current as D540K HERG, but do
not open with hyperpolarization (Sanguinetti and Xu, 1999). Representative D540A HERG current traces are shown in Fig. 9 A and current amplitudes are plotted against time in B. Greater than 90% block was obtained with 2 μM MK-499 (b), but little recovery (e) was observed after 50 pulses to −160 mV (c and d). The mean recovery from block was 12.4 ± 1.7% (n = 6). Thus, recovery from block was minimal for WT and D540A HERG channels that do not reopen with hyperpolarization. The small, but significant recovery from block of these channels was likely due to rare channel openings at the holding potential of −90 mV. Overall, our data suggest that unblock of the channel by MK-499 can only occur when the activation gate is open.

To determine whether recovery of D540K HERG channels from MK-499 block was sensitive to membrane potential, we determined rates for recovery at two different potentials. A single 2-min test pulse was applied to −120 mV (Fig. 10 A) or −160 mV (B) before (Control) and after repetitive pulsing to 0 mV to achieve steady state block with 2 μM MK-499. At either test potential, control currents activated within 4 s to a steady state level that was maintained throughout the 2-min pulse. After block and in the continued presence of MK-499, the holding and initial inward currents were attenuated. However, with continued hyperpolarization, the inward current slowly increased as channels...
became unblocked. The rate and extent of recovery from block (MK-499 current as a proportion of control current) was greater at $-160$ than at $-120$ mV. Time constants for recovery from block were determined by curve-fitting the ratio of drug-sensitive current to control current ($I_{d-s}/I_c$) as a function of time (Fig. 10 C). Recovery was best described with a biexponential function at $-160$ mV and a single exponential function at $-120$ mV. In five cells, the $\tau_f$ and $\tau_s$ for recovery at $-160$ mV was, respectively, $5.1 \pm 1.6$ and $52.6 \pm 9.3$ s. At $-120$ mV, $\tau_s$ was $116.5 \pm 30.9$ s ($n = 5$; significantly different from $\tau_s$ at $-160$ mV, $P < 0.05$). Thus, as expected for a positively charged compound, the recovery from block of D540K HERG channels was accelerated by greater hyperpolarization.

**DISCUSSION**

**Mechanism of Hyperpolarization-dependent Channel Opening of D540K HERG Channels**

As we previously reported, replacement of Asp with Lys at position 540 in LS4-S5 of HERG destabilized the closed state, permitting the channel to reopen in response to membrane hyperpolarization (Sanguinetti and Xu, 1999). This destabilization results in a biphasic voltage dependence of channel opening, a unique behavior for a voltage-gated ion channel. Hyperpolarization-activated current was associated with an increased burst duration and probability of channel opening, but no change in single channel conductance. The $P_o$-$V$ relationship for D540K HERG current is shaped like an inverted bell. Depolarization-dependent opening of D540K HERG channels is half-maximal at $-9$ mV, whereas hyperpolarization-dependent opening is half-maximal at $-117$ mV (Sanguinetti and Xu, 1999). The $P_o$-$V$ relationship is steeper for hyperpolarizing than depolarizing pulses, but both types of channel openings are likely to occur in response to movement of the S4 domain. However, it is unclear whether the D540K mutation alters the movement of the S4 domain in response to hyperpolarization, the interaction of LS4-S5 with another portion of the channel (S6 domain?), or in some other way destabilizes the closed state at negative transmembrane potentials. Mutation of residues other than D540 located in the LS4-S5 also affect activation and deactivation of HERG (Wang et al., 1998; Sanguinetti and Xu, 1999). The structure of the activation gate is not known, but it has been suggested that twist-
ing of the S6 domains in response to a change in trans-
membrane voltage could narrow or widen the access to
the pore, analogous to opening and shutting of a “trap
door.” Based on mutational analysis (McCormack et al.,
1993; Sanguinetti and Xu, 1999; Shieh et al., 1997), it is
conceivable that LS4-S5 is an important component of
the K1 channel activation gate, perhaps by acting as a
transducer between movement of the S4 voltage sensor
and the narrow region of the S6 domains. Further stud-
ies are needed to define the molecular basis of the al-
tered channel gating induced by the D540K mutation.

Regardless of the exact gating mechanism, D540K
HERG channel behavior is useful for testing the drug-
trapping hypothesis that predicts that if channels could
reopen from the closed state in response to hyperpolar-
ization, then positively charged compounds would dis-
sociate from their receptor site and exit the channel.

Block of HERG Channels by MK-499: A Clear Example of
Drug Trapping

According to the modulated receptor model for chan-
nel block (Hille, 1977; Hondeghem and Katzung,
1977), a charged compound gains access to its binding
site from the cytoplasm only after the channel has
opened in response to membrane depolarization. Once
bound to its receptor site within the inner vestibule of
the pore (behind the activation gate), a charged com-
pound can become “trapped” when the channel deacti-
vates in response to membrane repolarization. A posi-
tively charged compound would not be capable of exit-
ing the inner vestibule through the highly hydrophobic
environment that typifies the wall of the pore or the
surrounding membrane. Trapping can only occur if the
drug is small enough to fit into the restricted space of
the inner vestibule of the pore region (Hille, 1977;
Hondeghem and Katzung, 1977). If the drug is charged
and appropriately sized, then block is nearly irrevers-
ible as long as the channels are not reopened. High af-
finity block of HERG channels by methanesulfonani-
lide compounds is characterized by most of these prop-
erties. HERG channels are not blocked when oocytes
are exposed to a high concentration (1 µM) of MK-499
if the membrane is held at −80 mV without pulsing.
However, applying a train of repetitive depolarizing
pulses causes block that is essentially irreversible. Thus,
HERG block only occurs after the channels have
opened. The pKas of MK-499 are 7.86 for the piperidine
group and 9.52 for the methanesulfonamide group.
Thus, the drug is 99% charged in the bathing solution
(pH 7.6) used in our experiments. At a concentration
of 2 µM, 20 nM of the drug would be in the un-
charged form. Neutral MK-499 might not require chan-
nel opening to access the vestibule of the pore, but
would then instantly reequilibrate in the aqueous envi-
riment of the vestibule, such that only 0.2 nM would
be stable in the charged state. Because the IC50 of MK-
499 is 32 nM, it is likely that only the charged form of
the drug binds with high affinity to the HERG channel.

One property of MK-499 is not consistent with it being
trapped by channel deactivation. MK-499 is a large mole-
cule (20 × 7 Å) that would probably not fit into the in-
ner vestibule of many K+ channels, including Shaker and
KcsA. Although there is little sequence homology be-
tween the inner helices of KcsA and S6 of Shaker or
HERG, the basic pore structure is likely to be similar
(Doyle et al., 1998; MacKinnon et al., 1998; reviewed in

Figure 10. Rate of D540K HERG recovery from block is voltage
dependent. Representative current traces elicited by 2-min hyper-
polarizing voltage steps to −120 mV (A) or −160 mV (B). Control
currents were elicited once outward currents stimulated with 5-s
pulses to 0 mV had reached steady state. After the addition of 2
µM MK-499, the oocyte was pulsed repetitively to 0 mV to achieve
>85% block. Then the test pulse to −120 was repeated. Block was
reestablished before recording current at −160 mV. (C) Drug sen-
sitive current, Igs, was calculated by subtracting the currents in the
presence of MK-499 from the control currents, Is. Igs was normalized
to control current (Igs/Is, dot plot) for each potential and fitted with exponential functions (solid lines)
to obtain time constants for recovery from MK-499 block. Igs/Is at
−120 mV was fitted with a single exponential function (τ = 109 s).
Igs/Is at −160 mV was fitted with a biexponential function (τ1 =
10.5 s, τ2 = 52.7 s).
behind the activation gate, then the inner vestibule of a normal channel deactivation. If MK-499 binds to a site MK-499 is bound to its receptor it does not interfere with the activation gate. In contrast, channel block caused by trapping is not associated with an altered rate of current deactivation because channels are unable to close when occupied by drug, presumably because of steric interference with the activation gate. In contrast, channel block caused by trapping is not associated with an altered rate of current deactivation because only drug-free channels are capable of conducting current. MK-499 has no effect on the rate of channel deactivation (Spector et al., 1996a), consistent with our conclusion that when MK-499 is bound to its receptor it does not interfere with normal channel deactivation. If MK-499 binds to a site behind the activation gate, then the inner vestibule of a HERG channel must be $>20 \times 7 \, \text{Å}$, the size of a single MK-499 molecule. Our study provides no direct evidence that the site of MK-499 block of HERG channels is the vestibule. This would require knowing the crystal structure of HERG and mapping of residues important for high affinity drug binding. It is conceivable that the binding site is located outside the vestibule in a position that is allosterically affected by the D540K mutation in such a way that unbinding can occur in response to hyperpolarization in D540K, but not D540A or wild type HERG channels.

Other methanesulfonanilide compounds are also likely to block HERG channels by a trapping mechanism. Carmeliet (1992) studied the kinetics of $I_{K1}$, block by dofetilide in cardiac myocytes. Dofetilide is a methanesulfonanilide with similar potency and kinetics of HERG block as MK-499. Unblock of $I_{K1}$ by dofetilide was very slow at $-50 \, \text{mV}$, but was virtually irreversible at $-75 \, \text{mV}$. This pattern of unblock is consistent with drug trapping because some channels are open at $-50 \, \text{mV}$ (but not at $-75 \, \text{mV}$) and would therefore allow drug to escape.

Channel State-dependent Binding of Methanesulfonanilides

It is clear that HERG channels must open before being blocked by MK-499 or other methanesulfonanilide compounds. However, it is not clear if drug then binds preferentially to the open or inactivated state of the channel. Some studies have suggested that inactivated HERG channels have a lower affinity for dofetilide than the open state (Kiehn et al., 1996; Snyders and Chaudhary, 1996). More recent studies using HERG mutants in which inactivation has been modified have reached the opposite conclusion. Wang et al. (1997) reported that block of wild-type HERG by E-4031 required 10-fold less compound than block of a mutant HERG (G628C:S631C) that removes inactivation. HERG/eag chimera channels that did not inactivate were also less sensitive to block by dofetilide (Ficker et al., 1998), eag channels do not inactivate and are relatively insensitive to methanesulfonanilides. These studies suggest that methanesulfonanilides preferentially bind to the inactivated state but do not rule out the possibility that reduced binding affinity to inactivation-deficient mutant channels results from an allosteric effect unrelated to channel state. Thus, it remains unclear whether channel inactivation alters the affinity of the methanesulfonanilide binding site.

Heterologous expression of HERG in Xenopus oocytes induces a $K^+$ current with properties very similar to the rapidly activating delayed rectifier $K^+$ current ($I_{Kr}$) that mediates repolarization of cardiac myocytes (Sanguinetti et al., 1995; Trudeau et al., 1995). However, it was recently reported that coassembly of HERG and MiRP1 (minK-related peptide) subunits induces a current with properties almost exactly like $I_{K1}$, recorded in cardiac myocytes (Abbott et al., 1999). Association of MiRP1 with HERG also alters the potency and kinetics of block by the methanesulfonanilide E-4031. Thus, association of MiRP1 with HERG may alter the state dependency or accessibility of drug to its receptor site.

In summary, our findings provide the most direct evidence to date that slow recovery from channel block by a charged drug is due to trapping of the compound in the inner vestibule by deactivation. The ability of HERG channels to trap MK-499, despite its large size, indicates that the vestibule of this channel is larger than the more well studied Shaker $K^+$ channel.

We thank Monica Lin and Mike Martines for valuable technical assistance. MK-499 was kindly provided by Merck and Company, Inc. This work was supported by a Wellcome Prize Traveling Research Fellowship (J.S. Mitcheson), and a grant from the National Heart, Lung, and Blood Institute of the National Institutes of Health (HL55236).

Submitted: 2 December 1999
Revised: 18 January 2000
Accepted: 18 January 2000
Released online 14 February 2000

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