Block of Human Heart hH1 Sodium Channels by Amitriptyline

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ABSTRACT
Amitriptyline is a tricyclic antidepressant used to treat major depression and various neuropathic pain syndromes. This drug also causes cardiac toxicity in patients with overdose. We characterized the tonic and use-dependent amitriptyline block of human cardiac (hH1) Na+ channels expressed in human embryonic kidney cells under voltage-clamp conditions. Our results show that, near the therapeutic plasma concentration of 0.3 to 0.8 μM, amitriptyline is an effective use-dependent blocker of hH1 Na+ channels during repetitive pulses (~55% block at 5 Hz). The tonic block for resting and for inactivated hH1 channels by amitriptyline (0.1–100 μM) yielded IC₅₀ values (50% inhibitory concentration) of 24.8 ± 2.0 (n = 9) and 0.58 ± 0.03 μM (n = 7), respectively. Substitution of phenylalanine with lysine at the hH1-F1760 position, a putative binding site for local anesthetics, eliminates the use-dependent block by amitriptyline at 1 μM. The time constants of recovery from the inactivated-state amitriptyline block in hH1 wild-type and hH1-F1760K mutant channels are 8.0 ± 0.5 (n = 6) and 0.45 ± 0.07 s (n = 6), respectively. A substitution at either hH1-F1760K or hH1-Y1767K significantly increases the IC₅₀ values for resting and inactivated states of amitriptyline, but the increase is much more pronounced with the hH1-F1760K mutation. Because these two residues were proposed to form a part of the local anesthetic binding site, we conclude that amitriptyline and local anesthetics interact with a common binding site. Furthermore, at therapeutic concentrations, the ability of amitriptyline to act as a potent use-dependent blocker of Na+ channels may, in part, explain its analgesic actions.

Amitriptyline is a tricyclic agent used for the treatment of major depression (Baldessarini, 1995). This drug is effective in the treatment of postherpetic neuralgia, diabetic neuropathy, and other neuropathic pain syndromes (Monks and Merskey, 1984). Oral amitriptyline achieves a good or moderate response in about two-thirds of patients with postherpetic neuralgia and three-quarters of patients with painful diabetic neuropathy; such neuropagic pain syndromes are often unresponsive to narcotic analgesics (Bryson and Wilde, 1996). Whether analgesic effects of amitriptyline are linked to its mood-altering activity and/or are attributable to a discrete pharmacological action is unknown. Above the therapeutic plasma concentration of 0.3 to 0.8 μM, the tricyclic antidepressants have significant effects on the cardiovascular system, including direct depression of the myocardium and evidence of prolonged conduction times (Nattel et al., 1984; Nattel, 1985); with an overdose of >3 μM, these effects may be life-threatening (Amsterdam et al., 1980). The known physiological targets of tricyclic antidepressants in the central nervous system are the 5-HT₂ serotonin receptors and the α₁-adrenergic receptors (Baldessarini, 1995).

In addition to these primary targets, tricyclic antidepressants are also effective K+ and Na+ channel blockers. For example, tricyclic imipramine inhibits transient K+ channels in hippocampal neurons with an IC₅₀ of ~6 μM (Kuo, 1998). In adrenal chromaffin cells, amitriptyline blocks peak Na+ currents with an IC₅₀ value of 20.2 μM (Pancrazio et al., 1998). In cardiac myocytes, 0.4 μM amitriptyline elicits a profound use-dependent block of Na+ current during repetitive pulses at a frequency of 5 Hz (Barber et al., 1991). Such a use-dependent phenomenon is qualitatively similar to that found when Na+ channels are exposed to local anesthetics (LAs) (Hille, 1992). Because recovery from the use-dependent block of amitriptyline is slow in cardiac Na+ channels, with a time constant of 13.6 s, Barber et al. (1991) suggested that the block of cardiac Na+ channels by amitriptyline is the probable cause of cardiac toxicity.

The location of the amitriptyline binding site in Na+ channels has not been delimited. Although the blocking effects of amitriptyline are similar to those of LAs, no direct evidence demonstrates that amitriptyline and LAs share a common binding site. Mammalian Na+ channel isoforms consist of a large α-subunit and one or two smaller β-subunits (Catterall, 1995; Fozzard and Hanck, 1996). The α-subunit alone can form functional channels when transiently expressed in human embryonic kidney (HEK) cells. The proposed organization of the α-subunit Na+ channel consists of four homologous domains with six transmembrane segments each. The

ABBREVIATIONS: LA, local anesthetic; HEK, human embryonic kidney; Kᵣ, resting affinity; Kᵢ, inactivated affinity; DMSO, dimethylsulfoxide.

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LA receptor has been mapped within the segment D4-S6 of the rat brain type IIA isoform (Ragsdale et al., 1994). The homologous residues of human heart Na+ channels (Gellens et al., 1992) involved in LA binding are hH1-F1760 and hH1-Y1767. However, mutations at these hH1 positions have not been studied to date. In this report, we characterize the amitriptyline block in the hH1 α-subunit channel as a possible target of cardiac toxicity. Because amitriptyline and LAs elicit a similar tonic and use-dependent block, we set out to determine whether amitriptyline and LAs share a common binding site within Na+ channels. For direct comparison we chose a local anesthetic, cocaine, which like amitriptyline is highly cardiotoxic.

Materials and Methods

Mutagenesis and Transfection of HEK293t Cells. hH1 cDNA plasmid was obtained from Dr. Roland Kallen (University of Pennsylvania, Philadelphia, PA). Mutagenesis of hH1 cDNA was performed with the Transformer Site-Directed Mutagenesis Kit (Clontech Laboratories, Palo Alto, CA). The restriction primer has a sequence of 5'-CGAATTCTGCAGAGCTCCATCACACTGG-3' in which the restriction site EcoRV in the polylinker region has been changed to SacI. The mutagenesis primer was synthesized according to the coding sequence of the mutated residue. In vitro synthesis was performed for a total of 4 h, with one addition of dNTPs and T4 DNA polymerase during the reaction. The potential mutants were identified as EcoRV-resistant plasmids. The mutation was confirmed by DNA sequencing with primers near the mutated region.

Stocks of cultured HEK293t cells and CD-8 plasmid were obtained from Dr. Stephen Cannon (Massachusetts General Hospital, Boston, MA). The culture of HEK293t cells and their transient transfection with wild-type and mutant hH1 clones were as described (Cannon and Strittmatter, 1993). Approximately 2 μg of hH1 plasmid, 5 to 10 μg of hH1 mutants, and 1 μg of reporter plasmid CD-8 were found adequate for transfection. At 15 h after transfection, cells were replated in 35-mm tissue culture dishes. Transfection-positive cells were visualized with CD8-Dynabeads (Dynal Inc., Lake Success, NY).

Electrophysiology and Data Acquisition. The whole-cell configuration of a patch-clamp technique (Hamill et al., 1981) was used to study macroscopic hH1 Na+ currents in CD8-coated cells at room temperature (22 ± 2°C). Electrode resistances ranged from 0.5 to 0.8 MΩ. Command voltages were elicited with pCLAMP7 software and delivered by Axopatch 200B (Axon Instruments, Inc., Foster City, CA); data were filtered at 5 kHz and acquired at 10 to 20 kHz unless stated otherwise. Cells were held at −140 mV and dialyzed for at least 20 min before current recording. Most of the capacitative and leak currents were cancelled with an Axopatch 200B device and by P/-4 subtraction. No P/-4 was applied for the use-dependent protocol. Current amplitudes at −50 mV were 3 to 10 nA for wild type and ~1 to 5 nA for mutants. Series resistance compensation of 40 to 75% typically resulted in voltage errors of ±5 mV at ±50 mV. All current measurements were performed at ±30 or ±50 mV for the outward Na+ currents. Such recordings allowed us to avoid complication of series resistance artifact and to minimize inward Na+ ion loading during pulses. Curve fitting was performed by Micrcoral Origin (Microcoral Software Inc., Northampton, MA).

Solutions and Chemicals. Amitriptyline hydrochloride was purchased from Sigma (St. Louis, MO), dissolved in dimethylsulfoxide (DMSO) at 10 and 100 mM, and stored at −20°C. The highest DMSO concentration in solution was 0.1%. DMSO at a final concentration of 1% had no effect on Na+ currents. Cocaine hydrochloride was obtained from Mallinckrodt, Inc. (St. Louis, MO), dissolved in water at 100 mM, and stored at −20°C. Cells were perfused with an extracellular solution containing 65 mM NaCl, 85 mM choline chloride, 2 mM CaCl2, and 10 mM HEPES (titrated with tetramethylammonium hydroxide to pH 7.4). The pipette (intracellular) solution consisted of 100 mM NaF, 30 mM NaCl, 10 mM EGTA, and 10 mM HEPES (titrated with cesium hydroxide to pH 7.2). For each experiment, 100 and 300 μM amitriptyline solutions were first prepared from the stock. Final amitriptyline concentrations were made by serial dilution.

Results

Use-Dependent Block of Wild-Type hH1 Na+ Channels by Amitriptyline. To investigate whether the amitriptyline block of hH1 Na+ channels in transfected cells is comparable to that in rabbit cardiac myocytes, we first determined the amitriptyline use-dependent block of hH1 Na+ currents. Near the upper range of the therapeutic plasma concentration (1 μM), amitriptyline produced a significant use-dependent block of Na+ currents when the cell was repetitively depolarized to +50 mV for 21 ms at a frequency of 5 Hz (Fig. 1B). In contrast, without amitriptyline there was no use-dependent block of H1 currents (Fig. 1A). About 55% of peak Na+ currents were blocked by amitriptyline after 60 repetitive pulses (Fig. 1C, closed circles). The time course of this use-dependent block was well fitted by a single exponential with a rate constant of 0.053 per pulse and an estimated steady-state block of ~58%. Such use-dependent block is similar to that found in rabbit cardiac myocytes.

![Fig. 1. Use-dependent block of hH1 by amitriptyline. Repetitive pulses (see inset) elicited no use-dependent block of hH1 Na+ currents (A), but this protocol produced a profound use-dependent block with 1 μM amitriptyline presence (B). Outward Na+ currents were generated by test pulses at +50 mV and sampled at 200 kHz for the first 1.3 ms and at 10 kHz thereafter. Traces correspond to pulse number 1, 10, 20, 30, 40, 50, and 60. The peak amplitude of Na+ currents was measured, normalized by the peak value of the first pulse, and plotted against pulse numbers (C). The data (n = 7) were well fitted by a single exponential function (solid line) with a time constant of 18.7 pulse for the estimated block of 57.8 ± 0.2%. For comparison, cocaine at 10 μM produced an estimated use-dependent block of 43.4 ± 0.2% (n = 6) with a time constant of 15.8 pulse when assayed under identical conditions.](image-url)
(Barber et al., 1991). Thus, amitriptyline near the therapeutic plasma concentration range elicits a potent use-dependent block of hH1 channels.

Like amitriptyline, cocaine overdose (5–90 μM) results in the production of cardiac arrhythmias. At 10 μM, cocaine elicited a use-dependent block of about 40% at 5 Hz in hH1 channels (Fig. 1C, open diamonds), significantly less than that of amitriptyline at 1 μM (P < .05). This cocaine result is comparable with that found in guinea pig cardiac myocytes (Crumb and Clarkson, 1990). These authors also reported that the higher the pulse frequency the greater the steady-state block. At 50 μM cocaine concentration, the block reached 65% at 5 Hz and 43% at 1 Hz. Similar phenomena were found for cocaine in hH1 channels (Wright et al., 1999) and for amitriptyline in native heart Na⁺ channels (Barber et al., 1991).

Steady-State Tonic Block of hH1 Na⁺ Channels by Amitriptyline at Various Voltages. LA binding with hH1 Na⁺ channels is highly voltage-dependent, particularly at a voltage range of −140 to −90 mV (Wright et al., 1997). We examined this voltage-dependent binding of amitriptyline with Na⁺ channels, using the protocol of Wright et al. (1997) (Fig. 2, inset). A prepulse of 10 s at various voltages was applied to allow the drug to bind with Na⁺ channels. A 100-ms interpulse was inserted to allow the drug-free Na⁺ channels to recover from fast-inactivated states. A brief test pulse at +30 mV was then applied to activate drug-free channels. Without drug, the peak Na⁺ current at the brief test pulse was not sensitive to the prepulse at voltages of <−140 mV. A small fraction of Na⁺ channels (<15%) was progressively inactivated by the slow-inactivation process at prepulse voltages >−140 mV (Fig. 2A, top traces; Fig. 2B,

![Diagram](image)

Fig. 2. Steady-state tonic block by amitriptyline at various voltages. A 10-s prepulse to −180 or −70 mV was applied to allow amitriptyline binding (A, inset). Without drug, the outward Na⁺ current at the test pulse showed only a small difference in peak amplitudes at prepulse voltages of −180 and −70 mV (A, top traces). With 1 μM amitriptyline, the difference was much larger (A, bottom traces). The peak amplitudes without drug were measured, normalized with the value at a prepulse voltage of −180 mV, and plotted against the prepulse voltage (B, open circles). The peak amplitudes with 1 μM amitriptyline present were each renormalized with the control data and plotted (B, solid circles). The normal h curve was plotted (C, solid line) along with the simulation (dashed line), which is drawn according to $y = 1/[1 + (C/K_{app})]$, where $C = 1 \mu M$, $1/K_{app} = (h^*/K_R) + [1 - h^*/K_I]$, $h^* = h - 9 \text{ mV}$, $K_R = 24 \mu M$, and $K_I = 0.44 \mu M$. 

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open circles). With 1 µM amitriptyline, there was little difference in peak amplitude with prepulse voltages of <−140 mV except for a small tonic block of Na⁺ current by amitriptyline. However, when prepulse voltages were >−140 mV, a strong block of peak Na⁺ currents was evident. The amplitude of this voltage-dependent block reached a steady-state level of ~70% between −90 and −60 mV (Fig. 2A, bottom traces; Fig. 2B, closed circles). The data could be well fitted by a Boltzmann equation with midpoint and slope of −113.0 ± 0.3 mV and 6.2 ± 0.2 mV (n = 6), respectively (Fig. 2B, fitted line). Figure 2C shows a simulation using an equation described in the figure legend. Details of this simulation will be discussed later. Our result demonstrates that the binding of amitriptyline with hH1 Na⁺ channels is strongly voltage-dependent. As shown for LAs (Wright et al., 1997), low-affinity binding at prepulse voltages from −150 to −190 mV may correspond to amitriptyline binding with the resting state of hH1 Na⁺ channels, whereas the high-affinity binding from −90 to −60 mV corresponds to binding with the inactivated state.

**Low and High Affinities of Amitriptyline Binding with hH1 Na⁺ Channels.** To assess directly the amitriptyline affinities, we measured the tonic block at −180 mV for resting-state affinity and at −70 mV for inactivated-state affinity at various drug concentrations. These two voltages were chosen on the basis of voltage-dependent block by 1 µM amitriptyline (Fig. 2), the voltage at which the block reaches asymptote. Figure 3A shows that the IC₅₀ of amitriptyline at −180 mV is 24.8 ± 2.0 µM (open circles, n = 9; defined as resting affinity, Kᵣ), whereas the inactivated affinity (Kᵢ) at −70 mV is 0.58 ± 0.03 µM (closed circles, n = 7). The concentration-response curves yielded Hill coefficients of 1.29 and 1.23 (Fig. 3A, solid lines for Kᵣ and Kᵢ, respectively), which suggests that one amitriptyline molecule blocks one Na⁺ channel. The ratio of Kᵣ to Kᵢ is 42.8. By definition, a low dissociation constant designates high affinity. Thus, like

![Fig. 3](https://example.com/fig3.png)

Fig. 3. Concentration-response curve for resting and inactivated hH1 channels. The resting affinity for amitriptyline in wild type (A), hH1-F1760K (B), and hH1-Y1767K (C) was measured with a prepulse of −180 mV for 10 s (see Fig. 2, inset) at various drug concentrations (open circles), and the inactivated affinity was measured at −70 mV for 10 s (solid circles). The data were fitted by the Hill equation (solid lines). Hill coefficients are given in brackets. The IC₅₀ values for resting and for inactivated channels were defined as Kᵣ (open circles) and Kᵢ (closed circles), respectively, and plotted in (D) as the hH1 wild type along with hH1-F1760K and hH1-Y1767K mutants measured under the same conditions. * and † indicate P < .05 using an unpaired Student’s t test with its wild-type counterpart. For comparison, the fitted dashed lines in (A) represent the cocaine block of hH1 channels under identical conditions. The cocaine data were omitted here for clarity.
inactivated block at 1 μM amitriptyline can be determined by varying the duration of the prepulse at −70 mV (Fig. 4A, inset). A 100-ms interpulse at −140 mV was inserted before the test pulse to allow the recovery from fast inactivation. At 1 μM amitriptyline, the block at −70 mV developed with a time constant of 2.75 s and nearly reached a steady-state block at approximately 10 s (Fig. 4A).

The recovery time course from the inactivated drug-bound state of the hH1 Na⁺ channel was measured by a two-pulse protocol (Fig. 4, inset). Without drug, the recovery time course after a 10-s prepulse at −70 mV was biphasic. A large portion of the current (82%) recovered with a fast time constant of 9.0 ± 0.5 ms (n = 6), and a smaller portion (17%) recovered with a slower time constant of 0.18 ± 0.04 s (n = 6). The fast time constant was due to recovery from fast inactivation at −70 mV, and the slower time constant was due to recovery from residual slow inactivation. However, with 1 μM amitriptyline, the recovery time course was drastically changed; a small portion of the current (30%) recovered with a time constant of 18.7 ± 2.3 ms (n = 6), and a large portion (67%) recovered with a time constant of 8.02 ± 0.50 s (n = 6). The latter slow recovery time constant was due to recovery from drug-bound inactivated channels because the amount of inactivated block at 1 μM amitriptyline was estimated to be about 63% (Kᵢ = 0.58 μM). This result also validates the pulse protocol used in the studies depicted in Figs. 2 and 3, where a 100-ms interpulse at −140 mV was used to recruit the drug-free resting channel. This interpulse duration of 100 ms is too short to allow recovery of drug-bound inactivated channels with a time constant of 8 s.

Development of and Recovery from the High-Affinity Block of hH1 Na⁺ Channels by Amitriptyline. The development of the high-affinity block of hH1 Na⁺ channels by amitriptyline can be determined by varying the duration of the prepulse at −70 mV (Fig. 4A, inset). A 100-ms interpulse at −140 mV was inserted before the test pulse to allow the recovery from fast inactivation. At 1 μM amitriptyline, the block at −70 mV developed with a time constant of 2.75 s and nearly reached a steady-state block at approximately 10 s (Fig. 4A).

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Amitriptyline Block of hH1-F1760K Mutant Channels. As for the F1764 position in rat brain type IIA Na⁺ channels, the homologous hH1-F1760 position at the D4-S6 segment has been proposed to be involved in binding with the tertiary amine group of LAs. To determine whether this residue is involved in amitriptyline binding, we chose the mutant hH1-F1760K (phenylalanine—lysine) and measured the voltage dependence of amitriptyline binding from −190 to −60 mV. Figure 5A shows that there is little block of peak Na⁺ currents at any of the voltages that were tested, with or without 1 μM amitriptyline. Even at −70 mV, 1 μM amitriptyline inhibits only about 5% of hH1-F1760K channels. This result is in sharp contrast to that for wild-type current, which is blocked ~70% by the same concentration of amitriptyline at voltages from −90 to −60 mV (Fig. 5A, dotted line). Clearly, the inactivated hH1-F1760K channels have a drastically reduced affinity for amitriptyline. It is noteworthy that the steady-state inactivation of hH1-F1760K measured as hᵥ curve (Hodgkin and Huxley, 1952) reaches its completion like wild type with h₀.₅ = −100.8 ± 1.3 mV and kᵥ = 5.4 ± 0.2 mV (n = 7). For comparison, the parameters for wild type are h₀.₅ = −100.1 ± 2.2 mV and kᵥ = 7.8 ± 0.1 mV (n = 6). The activation kinetics of hH1-F1760K measured as conduction-voltage curve (Hodgkin and Huxley, 1952) are also comparable with wild type with Eᵥ₀.₅ = −42.6 ± 3.4 mV and kₑ = 8.9 ± 0.9 mV (n = 6). Activation parameters for wild type are Eᵥ₀.₅ = −52.0 ± 2.5 mV and Kₑ = 8.3 ± 0.4 mV

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Fig. 4. Development of and recovery from amitriptyline block of inactivated hH1 channels. The pulse protocols are shown in the inset. For the development of inactivated-state block, the prepulse duration at −70 mV was varied, and the peak current at the test pulse was measured, normalized to the initial peak amplitude (t = 0), and plotted against the prepulse duration (A). With 1 μM amitriptyline, the data were well fitted by a single exponential function (solid line). For recovery from the inactivated-state block, the interpulse duration at −140 mV was varied, and the peak current at the test pulse was measured, normalized with respect to the peak amplitude without the prepulse, and plotted against the interpulse duration (B). The data were well fitted by a double-exponential function (solid line).
respectively (Fig. 3B). The ratio of $K_m$ for wild-type current at 1 mM amitriptyline (Fig. 2) was comparable with hH1-F1760K. However, the use-dependent block of wild-type hH1 Na$^+$ currents by 1 μM amitriptyline during repetitive pulses (Fig. 1C) was completely missing in hH1-F1760K (data not shown). The lack of use-dependent block persisted at 30 μM amitriptyline. The $K_t$ value at −70 mV and the $K_R$ value at −180 mV for hH1-F1760K mutant channels were estimated to be 12.4 ± 0.8 μM (n = 5) and 42.8 ± 3.1 μM (n = 5), respectively (Fig. 3B). The ratio of $K_R$ to $K_t$ affinity is 3.5 in hH1-F1760K, significantly less than the 42.8 ratio for the wild type. The point mutation at hH1-F1760K reduces resting affinity by 1.7-fold but reduces inactivated affinity by 21.4-fold.

Recovery Time Course of hH1-F1760K Channels by Amitriptyline. Because the binding affinity of the inactivated hH1-F1760K channels was reduced by 21.4-fold (12.43 μM versus 0.58 μM for wild type), we sought to determine whether this affinity reduction is associated with the fast dissociation of drug-bound hH1-F1760K channels. If this were the case, a rapid dissociation of amitriptyline-bound inactivated channels would result in a rapid recovery time course. Figure 5B shows the recovery time course of hH1-F1760K mutant channels with and without 30 μM amitriptyline. Without drug, most hH1-F1760K current recovered more rapidly than wild-type current with a fast time constant of 2.0 ± 0.2 ms (n = 6) (versus 9.0 ms for wild type; Fig. 5B, dotted line). With 30 μM amitriptyline, the time course yielded a second time constant of 0.45 ± 0.07 s (n = 6) that corresponded to the recovery of amitriptyline-bound channels. The high drug concentration was needed for this mutant because of its low affinity. For comparison, the recovery time constant for wild-type current was 8.0 s at 1 μM amitriptyline, a difference of 17.8-fold (Fig. 5B, dotted line).

Amitriptyline Block of hH1-Y1767K Mutant Channels. The homologous hH1-Y1767 position has been proposed to interact with the phenyl group of LAs (Ragsdale et al., 1994). We found that this hH1-Y1767K channel still displayed a relatively high inactivated-state affinity for amitriptyline when the prepulse voltage was >−90 versus <−160 mV (Fig. 6A). Without drug, however, the peak Na$^+$ currents were highly sensitive to the prepulse voltage; at >−140 mV, the peak currents decreased progressively to a new steady state of about 45 to 50% at voltages between −90 and −60 mV. The underlying mechanism of this decrease is unknown. Whatever the cause of this current decrease, it is not isoform-specific because rat muscle μ1-F1579K channels display a similar phenotype (Wright et al., 1998). With 1 μM amitriptyline present, a voltage-dependent block of hH1-Y1767K similar to that of the wild type was observed (Fig. 6A versus Fig. 2). After normalization and curve fitting with a Boltzmann equation, the block reached steady state at a voltage of >−80 mV to a fitted value of 67.0 ± 0.3% (n = 6), which was not significantly different from 69.6 ± 0.4% (n = 6) of the wild type (P > 0.05). The slope (7.0 mV) and the midpoint potential (−114 mV) were comparable with those of the wild type (6.2 and −113 mV, respectively). These results indicate that the binding of amitriptyline to the inactivated state is reduced minimally, to a degree far less than that found in hH1-F1760K. As in the wild type, repetitive pulses at 5 Hz elicited a sizable use-dependent block of hH1-Y1767K currents (Fig. 6B; bottom traces), although the degree of this additional block was less than that of the wild type.

To determine the $K_R$ and $K_t$ in hH1-Y1767K channels directly, we constructed the concentration-response curve at various amitriptyline concentrations (Fig. 3C). The ratio of $K_R$ (35.3 ± 1.1 μM, n = 9) to $K_t$ (0.73 ± 0.04 μM, n = 6) was 48.3 in the hH1-Y1767K mutant, comparable with the ratio of 41.7 in the wild type. $K_R$ and $K_t$ values increased in hH1-Y1767K by 1.4- and 1.3-fold, respectively. Thus, the reduction of amitriptyline affinity in the hH1-Y1767K mutant was minimal compared with the hH1-F1760K mutant (Fig. 3B). The gating parameters for hH1-Y1767K (h0.5 = −102.2 ± 1.0 mV, $k_h = 8.1 ± 0.4$ mV, n = 5; and $E_{0.5} = −43.8 ± 1.7$ mV, $k_h = 10.4 ± 0.8$ mV, n = 4), however, are comparable with hH1-F1760K.
and plotted against prepulse voltages (open diamonds). Significant use-dependent block by 1 mM Na structure (Fig. 7) are probably important for amitriptyline's phenomenon. Further investigation of this complicated use-dependent block channel blocker (see later), may prove to be a useful probe for necessary to generate those effects. The involvement of a fast gate potentiates the use-dependent effects of LAs but is not use-dependent block (Wang et al., 1987; Vedantham and Hz). Channel activation clearly plays a significant role in the 50, and 60.

were well fitted by a single exponential function with a time constant of 12.5 ± 0.1 pulse (n = 6). Traces correspond to pulse number 1, 10, 20, 30, 40, 50, and 60.

Fig. 6. Characteristics of mutant hH1-Y1767K channels. The tonic inhibition of hH1-Y1767K channels at various voltages is shown in (A) with 1 μM amitriptyline. The pulse protocol was the same as in Fig. 2. The data (closed circles, n = 6) were renormalized with control data (open circles, n = 6) and plotted against prepulse voltages (open diamonds). Significant use-dependent block by 1 μM amitriptyline was observed at a frequency of 5 Hz (B). The degree of use-dependent block in hH1-Y1767K channels (42.5 ± 0.2%, n = 6) was less than in the wild type (Fig. 1). The data (not shown) were well fitted by a single exponential function with a time constant of 12.5 ± 0.1 pulse (n = 6). Traces correspond to pulse number 1, 10, 20, 30, 40, 50, and 60.

Discussion

The major findings of this study are 3-fold. (1) Amitriptyline at 1 μM elicits a high degree of use-dependent block in human heart hH1 Na+ channels. (2) Amitriptyline displays a high affinity to the inactivated hH1 Na+ channels at the submicromolar range. (3) Affinities for amitriptyline in hH1 Na+ channels were affected by mutations at the putative LA receptor site. Details and significance of these findings are discussed below.

Amitriptyline Is a Potent Use-Dependent Blocker of hH1 Channels. Amitriptyline elicits a profound use-dependent block of hH1 Na+ currents at 1 μM. Approximately 58% of Na+ currents are blocked after 60 pulses (Fig. 1). At higher concentrations, tonic inhibition of hH1 Na+ currents becomes evident (Fig. 3). Therefore, tonic block and use-dependent block are the common features of LAs and amitriptyline. However, amitriptyline appears more potent than most LAs in producing use-dependent block. For example, bupivacaine and cocaine at 1 μM produce the use-dependent block of Na+ channels (Fig. 1C, at 5 mV at various drug concentrations. The rationale in choosing these specific voltages is based on the voltage dependence of amitriptyline binding (Figs. 2, 5, and 6). There are two different binding affinities for amitriptyline with hH1 Na+ channels. The resting affinity for amitriptyline can be determined at voltages between −150 and −190 mV and the inactivated affinity at voltages between −80 and −60 mV. The term "inactivated affinity" is used here because at −70 mV the inactivated state is the absorbing state (Aldrich et al., 1983).

The resting affinity for amitriptyline in hH1 Na+ channels is 24.8 ± 2.0 μM, whereas the inactivated affinity is 0.58 ± 0.03 μM (Fig. 3), a difference of 42.7-fold. To date, there is no report on the amitriptyline affinity in native inactivated cardiac Na+ channels for comparison with our measurements. This ratio of $K_R$ to $K_I$ for amitriptyline block in hH1 channels is higher than that for cocaine under identical conditions (16.2-fold; $K_R = 343 ± 27 μM, n = 5; K_I = 27.2 ± 0.8 μM, n = 9$; Fig. 3A, dashed lines). It is noteworthy that the voltage dependence of LA block can be simulated using the values of $K_R$, $K_I$, along with an imposed equilibrium shift (h*) in h by higher use-dependent block of hH1 channels because this drug is more potent than lidocaine, bupivacaine, and cocaine in blocking Na+ currents during repetitive pulses. The facts that (1) amitriptyline is a potent use-dependent blocker near the therapeutic plasma concentration, and that (2) it has an unusually high affinity toward the inactivated state of Na+ channels could, in part, explain its analgesic actions. In particular, high-frequency abnormal impulses of sensory afferents (~20 Hz; Devor, 1984) may facilitate the use-dependent block, as well as the inactivated-state block, of amitriptyline in these nerve fibers.

Amitriptyline Binding with hH1 Channels Is State-Dependent. We determined directly the resting tonic block of amitriptyline at −180 mV and the inactivated block at −70 mV at various drug concentrations. The rationale in choosing these specific voltages is based on the voltage dependence of amitriptyline binding (Figs. 2, 5, and 6). There are two different binding affinities for amitriptyline with hH1 Na+ channels. The resting affinity for amitriptyline can be determined at voltages between −150 and −190 mV and the inactivated affinity at voltages between −80 and −60 mV. The term "inactivated affinity" is used here because at −70 mV the inactivated state is the absorbing state (Aldrich et al., 1983).

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The resting potential of myocardial cells is approximately -90 mV, and the inactivated-state affinity should dominate the binding of amitriptyline in vivo. Under these “resting potential” conditions, more than 70% of hH1 channels are blocked by 1 μM amitriptyline via the inactivated state. Furthermore, the drug-bound inactivated channels recover extremely slowly to their resting state; the time constant for recovery is 8.0 s, slightly slower than that of cocaine (6.8 s) in hH1 channels.

Our results are thus consistent with the suggestion that tricyclic antidepressants cause cardiac toxicity in overdose patients through the Na⁺ channel route (Ogata and Narahashi, 1989; Barber et al., 1991). In fact, the cardiotoxic drug cocaine is one order less potent than amitriptyline in blocking heart hH1 Na⁺ channels (Figs. 1C and 3A). The rapid development of amitriptyline block during repetitive pulses, the slow recovery from amitriptyline-induced inactivated-state block, and the high potency of amitriptyline as a blocker can readily explain the clinical findings during tricyclic antidepressant overdose, such as increased atrioventricular conduction time, decreased intraventricular conduction, and increased propensity for arrhythmias.

Amitriptyline and LAs Interact with Common Residues in hH1 Channels. The blocking phenomena of amitriptyline are similar to those of LAs (Pancrazio et al., 1998). All these drugs elicit tonic and use-dependent block; they all bind preferentially to the inactivated state with a high affinity. Nonetheless, there is no direct evidence to support that amitriptyline and LAs bind to common residues in Na⁺ channels. Our results demonstrate that hH1-F1760 and hH1-Y1767 can affect the amitriptyline binding significantly. Both of these homologous residues in brain (Ragsdale et al., 1994) and skeletal muscle (Wright et al., 1997) channels are known to affect the LA binding. The mutant hH1-F1760K exhibits a reduced resting affinity (by 1.7-fold) as well as a reduced inactivated-affinity for amitriptyline (by 21.4-fold). In rat skeletal muscle Na⁺ channels, resting affinity for cocaine in the mutant channel μ1-F1579K is reduced by 2.1-fold (Wright et al., 1998), and inactivated affinity is reduced by 21.3-fold. To explain such differential reduction in cocaine block, Wright et al. (1998) invoked conformational changes at the LA receptor during state transition. In wild-type channels, the transition from the resting state to the inactivated state may increase the affinity of the LA receptor by shifting the orientation of the residue within D4-S6. The homologous residue at the hH1-F1760 position has been suggested to interact with the tertiary-amine moiety of LAs (Ragsdale et al., 1994; Qu et al., 1995). Although an allosteric effect by hH1-F1760K mutation cannot be dismissed at this time, a positive charged residue at the hH1-F1760 position may indeed destabilize the amitriptyline binding through charge-charge repulsion, particularly during the inactivated-state transition.

Mutation at the hH1-Y1767K position affects amitriptyline block but much less than mutation at the hH1-F1760K position. For example, the Kᵣ for amitriptyline in hH1-Y1767K channels is reduced by 1.4-fold, as is the Kᵣ (by 1.3-fold). Lesser effects of cocaine block are also found in homologous μ1-Y1586K. The Kᵣ for cocaine in μ1-Y1586K is not reduced, and the Kᵣ is reduced by 6.1-fold. Wright et al. (1998) suggested that a π-cation interaction between the aromatic ring of LAs and the amine moiety of lysine on Y1586K provides some of the binding energy needed. Such an explanation is appealing for amitriptyline because it contains a tricyclic ring with two aromatic groups. Figure 7 illustrates the amitriptyline molecule along with the putative structure of the D4-S6 α-helix. This figure also includes the structure and the orientation of individual amino acid residues within the D4-S6 α-helix. At the atomic scale, the residues at hH1-F1760 and hH1-Y1767 can indeed accommodate the amitriptyline molecule. Alternatively, the hH1-Y1767 residue may be involved allosterically in amitriptyline binding. Li et al. (1999) recently suggested an indirect involvement of this tyrosine residue in the LA binding interaction. They systematically mutated the homologous tyrosine residue Y1717 of the rat brain RB-III clone, and found no correlation between LA binding and the size, hydrophobicity, or aromaticity of the mutated RBIII-Y1717 residue. Therefore, the precise role of the hH1-Y1767 residue in LA binding remains unclear.

Finally, Barber et al. (1991) provided evidence that amitriptyline and diphenylhydantoin interact with two separate...
binding sites. Because anticonvulsant diphenylhydantoin has been shown to bind to the LA receptor (Ragsdale et al., 1996), this inconsistency will require further attention. Regardless, our results demonstrate that amitriptyline and LAs share a common receptor in hH1 Na+ channels. The list of agents that bind to this receptor now includes LAs, antiarrhythmic drugs, anticonvulsants, and the antidepressant amitriptyline.

References


