Validation of a [3H]Astemizole Binding Assay in HEK293 Cells Expressing HERG K⁺ Channels

Peter J.S. Chiu1,*, Karen F. Marcoe1, Sidney E. Bounds1, Chun-Hsiung Lin2, Jin-Jye Feng2, Atsui Lin2, Fong-Chi Cheng2, William J. Crumb3, and Richard Mitchell1

1MDS Pharma Services, 22011 30th Drive SE, Bothell, WA 98021-4444, USA
2Pharmacology Laboratories, MDS Pharma Services, Peitou, Taipei 112, Taiwan
3Zenas Technologies and School of Medicine, Tulane University, New Orleans, LA 70112, USA

Received February 5, 2004; Accepted April 21, 2004

Abstract. A radioligand binding assay for the HERG (human ether-a-go-go-related gene) K⁺ channel was developed to identify compounds which may have inhibitory activity and potential cardiotoxicity. Pharmacological characterization of the [3H]astemizole binding assay for HERG K⁺ channels was performed using HERG-expressing HEK293 cells. The assay conditions employed yielded 90% specific binding using 10 µg/well of membrane protein with 1.5 nM of [3H]astemizole at 25°C. The K_d and B_max values were 5.91 ± 0.81 nM and 6.36 ± 0.26 pmol/mg, respectively. The intraassay and interassay variations were 11.4% and 14.9%, respectively. Binding affinities for 32 reference compounds (including dofetilide, cisapride, and terfenadine) with diverse structures demonstrated a similar potency rank order for HERG inhibition to that reported in the literature. Moreover, the [3H]astemizole binding data demonstrated a rank order of affinity that was highly correlated to that of inhibitory potency in the electrophysiological studies for HERG in HEK293 (r_SP = 0.91, P < 0.05). In conclusion, the [3H]astemizole binding assay is rapid and capable of detecting HERG inhibitors.

Keywords: HERG, radioligand binding, astemizole, long QT syndrome, K⁺ channel

Introduction

The rapidly activating delayed rectifier K⁺ currents (I_Kr) critically contribute to cardiac repolarization (1). The human ether-a-go-go-related gene, HERG, which is expressed in the heart of mammalian species including humans, encodes the pore-forming α subunit for I_Kr (2). Heterologously expressed HERG currents in mammalian cells including HEK293 and CHO cells are known to share pharmacological and biophysical properties with I_Kr. Mutations in HERG are characterized by delayed ventricular repolarization, manifested on the electrocardiogram as a prolongation of QT interval (congenital long QT syndrome: LQTS) (3–5). A plethora of drugs including methanesulfonylanilides (e.g., dofetilide, MK-499, and E-4031) are known to cause QT prolongation by blocking I_Kr K⁺ channels (acquired long QT syndrome), which is the underlying cause of life-threatening torsade de pointes, a form of polymorphic ventricular arrhythmia, in susceptible individuals (3, 6). Syncope and sudden death due to torsade de pointes caused by noncardiovascular drugs such as terfenadine (antihistamine), astemizole (antihistamine), and cisapride (gastrokinetic) led to their withdrawal from the market (7, 8). Consequently, cardiac safety relating to I_Kr K⁺ channels has become a major concern of regulatory agencies, as HERG channel inhibition has been identified as the firmest link to QT prolongation. In order to prevent costly attrition, it has become a high priority in drug discovery to screen out inhibitory activity on HERG channels in lead compounds as early as possible. Electrophysiological study using the patch clamp technique in HERG-transfected mammalian cells generates the most definitive data on HERG inhibition. However, the assay is slow and costly. Other functional assays using Rb flux or voltage-sensitive dyes have relatively low sensitivity, limiting their usefulness as efficient drug screens (9, 10).
Given the fact that drug-induced blockade of $I_{Kr}$ is the primary cause of life-threatening arrhythmias, a $[^3]$Hastemizole binding assay in the HERG transfected embryonic kidney cell line HEK293 (11) was developed in order to provide a rapid and low-cost screen for HERG-binding activity. Astemizole is known to be a potent blocker of HERG channels (7, 12). The assay was validated with 32 known HERG channel blockers with diverse structures. Furthermore, the radioligand binding data were compared with results from patch clamp studies.

**Material and Methods**

**Reagents**

Astemizole, (±)sotalol, quinidine, amiodarone HCl, disopyramide, flecainide acetyl, procainamide, terfenadine, diphenhydramine, tamoxifen, bepridil HCl, verapamil, risperidone, haloperidol, thioridazine, pimozide, erythromycin, ketoconazole, amitriptyline HCl, fluoxetine HCl, imipramine HCl, KCl, MgCl$_2$, HEPES, EGTA, ATP-Na$_2$, NaCl, CaCl$_2$, dextrose, and DMSO were purchased from Sigma-Aldrich Company (St. Louis, MO, USA); minimum essential medium with Earle’s salts supplemented with nonessential amino acids, sodium pyruvate, penicillin, streptomycin, Geneticin®, and fetal bovine serum were purchased from Mediatech (Herndon, VA, USA); E-4031 (4’-[1-[2-(6-methyl-2-pyridyl)ethyl]-4-piperidyl]carbonyl)methanesulfonanilide) was purchased from Wako Chemicals USA, (Richmond, VA, USA). Dofetilide was a generous gift of Pfizer, Inc. (Sandwich, UK). Loratadine, fexofenadine, ciaspride, olanzapine, ziprasidone, glibenclamide, sumatriptan succinate, and sildenafil citrate were purchased from Sequoia Research Products, Ltd. (Oxford, UK). [O-Methyl-$[^3]$H(N)]astemizole (represented as $[^3]$Hastemizole, 85 Ci/mmol) was a custom synthesized preparation from PerkinElmer Life Sciences (Wellesley, MA, USA). Human embryonic kidney (HEK293) cells stably expressing the HERG K+ channel were licensed from the University of Wisconsin Alumni Research Foundation (Madison, WI, USA) (11).

**Membrane preparation**

Cell cultures of HERG transfected HEK293 cells were maintained in 90% Minimum Essential Medium, supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and non-essential amino acids in a humidified incubator, 37°C, 5% CO$_2$. Membranes were prepared from cell cultures following the method of Finlayson et al. (13) for small scale preparations or by modified methods for large scale production. Small scale preparations employed cell monolayers, which were washed with Hank’s Balanced Salt Solution (HBSS), then harvested by detachment with trypsin /EDTA, EDTA alone, or scraping into 0.32 M sucrose containing protease inhibitors (4 µg/mL aprtinin, 1 µg/mL leupetin, 100 µM 4-(2-amino-ethyl)benzenesulfonyl fluoride HCl [Pefabloc® SC, AEBSF], and 1 µg/mL pepstatin A). Scraped cells remained in sucrose, while the others were centrifuged, resuspended in HBSS to wash them, centrifuged, and resuspended in 0.32 M sucrose and protease inhibitors. Cells were homogenized using a glass/Teflon tissue grinder. The suspension was centrifuged 10 min 650 x g, 4°C and the supernatant was retained on ice during pellet resuspension in high purity H$_2$O, 0°C. Following 10 min incubation, the pellet suspension was homogenized and centrifuged as above. The two supernatants were combined and centrifuged for 15 min at 48,000 x g, 4°C. Pelleted membranes were resuspended and homogenized as before in membrane buffer (10 mM HEPES, pH 7.4, 5 mM KCl, 130 mM NaCl). Alternatively, large scale preparations employed cells grown in Cell Factories (Nunc, Rochester, NY, USA). Cells were rinsed with HBSS, detached with EDTA, washed, and resuspended in membrane buffer containing protease inhibitors. The cell suspension was disrupted using a Polytron tissue grinder (Kinematica, Lucerne, Switzerland) and centrifuged for 10 min at 700 x g. The supernatant was centrifuged for 30 min at 48,000 x g, 4°C. Pelleted membranes were resuspended and homogenized using a glass/Teflon tissue grinder in membrane buffer. Membrane protein was assessed by the BCA method (Pierce Biotechnology, Inc., Rockford, IL, USA), and aliquots were frozen in liquid nitrogen and stored at −80°C. Freshly thawed membranes were employed in all assays.

$[^3]$Hastemizole binding assay

$[^3]$H Astemizole binding in HERG transfected HEK293 cell membranes was assessed by modification of previous methods developed for HERG K+ channel binding (13 – 15). All binding assays were performed in a total volume of 100 µL: 60 µL of buffer (10 mM HEPES, pH 7.4, 130 mM NaCl, 5 mM KCl, 0.8 mM MgCl$_2$, 1 mM NaEGTA, 10 mM glucose, 0.1% BSA) and membrane suspension, 20 µL of test drug or vehicle, and 20 µL of $[^3]$Hastemizole. Non-specific binding (NSB) was defined by 10 µM astemizole. Incubation was conducted in 96-well polypropylene plates (Costar) at 25°C with equilibrium attainment experimentally verified at 60 min. Binding was terminated by rapid filtration onto GF/B glass fiber filters, presoaked in 0.3% polyethyleneimine, followed by rapid washing 6 times (2 mL) with ice-cold solution containing 25 mM
Tris-HCl, pH 7.4, 130 mM NaCl, 5 mM KCl, 0.8 mM MgCl₂, 0.05 mM CaCl₂, and 0.1% BSA using a Tomtek Mach 2 Harvester. Captured radiolabel was detected using a Wallac LKB 1205 Betaplate liquid scintillation counter.

For optimization of HERG protein concentration in the assay, tissue linearity experiments with 0 to 40 µg/well of membrane protein and [³H]astemizole in the final concentration of 1.5 nM were conducted to demonstrate that under the conditions employed, radioligand was present in excess of the receptors. Tissue linearity experiments also were performed under the assay conditions described above with wild type HEK293 cell membranes (PerkinElmer Life Sciences).

For saturation binding studies, 10 µg/well of HERG membrane protein was incubated with [³H]astemizole diluted in assay buffer to concentrations of 0 to 30 nM, thus extending the concentration range 10-fold above the expected Kᵦ. All final [³H]astemizole concentrations were calculated from total ligand cpm. For DMSO tolerance studies, the binding activity of 10 nM [³H]astemizole was evaluated in the presence of 0%, 0.5%, 1%, 2%, 5%, and 10% DMSO. For competitive binding studies, assays were performed in the volumes mentioned above with [³H]astemizole in final concentrations of 1.4 to 18 nM and 10 µg/well of HERG membrane protein. All test compounds were solubilized and serially diluted in 100% DMSO prior to dilution in assay buffer. Seven to 12 concentrations in the appropriate range for each competitor were tested. Radioligand concentration was approximately one fourth the experimental Kᵦ, with at least a 10-fold excess over bound ligand with 10 µg membrane protein. Mean data and associated standard errors of the mean were calculated using Microsoft Excel.

**Electrophysiological studies**

HEK293 cells (11) were maintained in minimum essential medium with Earle’s salts supplemented with nonessential amino acids, sodium pyruvate, penicillin, streptomycin, Geneticin® and fetal bovine serum. The internal (composition: 130 mM KCl, 5 mM MgCl₂, 10 mM HEPES, 5 mM EGTA, 5 mM ATP-Na₃) and external (137 mM NaCl, 4 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂, 10 mM HEPES, 11 mM dextrose) recording solutions were adjusted to pH 7.2 using KOH and to pH 7.4 with NaOH, respectively. The vehicle was either de-ionized H₂O or DMSO. Historical data from this lab indicate that over the course of approximately 10 min, 1% DMSO produced a 1.4 ± 1.5% (n = 11) reduction in HERG current amplitude. The lowest dilution used in the present study was 0.025% DMSO. Experiments were performed at 37 ± 1°C. Currents were measured using the whole-cell variant of the patch clamp method. Glass pipettes were pulled from borosilicate glass by a horizontal puller (Sutter Instruments, Novato, CA, USA) and then fire polished to produce tip openings of 1 to 2 µm for K⁺ current recordings. Pipette tip resistance was approximately 1.0 to 2.0 MΩ when filled with K⁺ internal solutions. Bath temperature was measured by a thermistor placed near the cell under study and was maintained by a thermosteal device (model No. 806-7243-01, Cambion/Midland Ross, Cambridge, MA, USA). An Axopatch 1-B amplifier (Axon Instruments, Foster City, CA, USA) was used for whole-cell voltage clamping. Creation of voltage clamp pulses and data acquisition were controlled by an IBM PC running pClamp software (Axon Instruments). After rupture of the cell membrane (entering whole-cell mode), current kinetics and amplitudes were allowed to stabilize as the cell was dialyzed with internal solution and paced at 0.1 Hz using protocol A (typically 5 – 7 min). If the HERG current did not stabilize over this time period, the cell was discarded. Currents were considered stable if currents elicited by a series of voltage pulses given at 0.1 Hz were superimposed. Peak HERG current was measured as the maximum outward deflection of the tail current elicited upon return to −40 mV. Experiments were performed in the following order: establish whole-cell configuration, dialyze cell and allow to reach steady-state condition, wash in the first concentration of the test drug, holding at −75 mV pulse at 0.1 Hz until steady state block is observed, wash in subsequent concentrations of drug (Fig. 6).

**Data analyses**

For binding studies, the final concentration of [³H]astemizole was calculated using the following equation: 

\[
[M] = \frac{(X \text{ cpm} / (D \times V \text{ mL})) / (1/E)(1 \mu Ci / 2.22E6 dpm)(1/SA)}{V \text{ mL} / 2.22E6 dpm}\]

where [M] is the molar concentration, X is the measured cpm for the volume of [³H] per assay well (20 µL) spotted on the filtermat, D is the decay factor (Note: the fractional decay was considered negligible as all experiments were performed within four months of synthesis of the [³H]astemizole), V is the assay volume (mL), E is the counter efficiency for [³H] (approximately 0.5 cpm/dpm), SA is the specific activity of [³H]astemizole (85 Ci/mmol). Assay results were evaluated using GraphPad Prism software (San Diego, CA, USA). IC₅₀ values were derived from a 4 parameter logistics fit using Prism. Each inhibitor was tested in duplicate in 5 separate experiments. Kᵦ values were calculated using the Cheng-Prusoff equation (16): 

\[
Kᵦ = IC₅₀ / [1 + ([L]/Kᵦ)]
\]

where IC₅₀ is the half maximal inhibition concentration, [L] is the ligand.
concentration, $K_d$ is the equilibrium dissociation constant. The experimentally-derived saturation $K_d$ was used for $K_i$ calculations. Total bound cpm was used to calculate the interassay and intraassay variations. Intraassay variation was calculated as the average of daily %CV, where %CV = (standard deviation / mean value) $\times$ 100. Interassay variation was calculated employing the mean and standard deviation of individual experimental means. Percent CV for 7 separate experiments was determined by dividing standard deviation with mean value. The electrophysiological data are presented as percent reduction of current amplitude. This was measured as current reduction after a steady-state effect had been reached in the presence of drug relative to current amplitude before drug was introduced (control). Each cell served as its own control. Potency ranking is expressed in terms of the concentration that causes 50% reduction of current amplitude (IC$_{50}$) in nM.

**Results**

**Optimization of assay conditions for $[^3H]$astemizole binding using HERG transfected membranes**

Astemizole binding to HERG increased with protein concentration with 91% specific binding observed at 100 µg/mL and 95% at 400 µg/mL (Fig. 1). The concentration of $[^3H]$astemizole employed equated to approximately 14,000 to 18,000 cpm per well. The final assay radioligand concentration consistently was found to be in the range of 1.4 to 1.8 nM. In subsequent assays, a protein concentration of 10 µg (100 µg/mL) per well was selected to avoid radioligand depletion. At this protein concentration, the obtained 8.5% bound radioligand allowed testing under acceptable zone A conditions (GraphPad Prism software, San Diego, CA, USA) and ensured a reliable window between total and non-specific binding. A comparison of specifically bound $[^3H]$astemizole to the HERG transfected HEK293 cell membrane preparation and wild-type HEK293 cell membrane preparation is shown in Fig. 1. $[^3H]$Astemizole binding above background was observed in the wild-type HEK293 cell membrane preparation at detectable levels. However, specifically bound signals were at least 8-fold higher in the transfected membrane preparations.

**Time course of $[^3H]$astemizole binding in HERG transfected membranes**

Binding equilibrium was attained by 60 min and binding levels remained constant for at least 180 min at 25°C (Fig. 2). A 60-min standard incubation time was subsequently selected for all assays.

**Concentration dependence of $[^3H]$astemizole binding in HERG transfected membranes**

$[^3H]$Astemizole binding to HERG transfected HEK293 cell membranes determined in the presence or absence of 10 µM astemizole demonstrated that binding was high-affinity, specific, and saturable (Fig. 3). Membranes prepared in small and large-scale formats were essen-

![Fig. 1. Comparison of tissue linearity between 0 – 40 µg of HERG transfected HEK293 cell membrane preparation (solid bar) and wild-type HEK293 cell membrane preparation (open bar) for specific binding using 1.5 nM $[^3H]$astemizole. The ordinate represents specific binding of $[^3H]$astemizole in cpm. Non-specific binding was determined in the presence of 10 µM unlabeled astemizole (cf., Fig 3). Data shown are representative of results from individual tests that were performed in duplicate. Data represent the mean ± S.E.M. SB, specific binding.](image1)

![Fig. 2. Time course of $[^3H]$astemizole binding in HERG transfected HEK293 cell membranes. Assay conditions: 25°C, 1.5 nM $[^3H]$astemizole and 10 µg of HERG transfected HEK293 cell membrane preparation. The ordinate represents specific binding of $[^3H]$astemizole in cpm. Data shown are representative of results from individual tests that were performed in duplicate. Data represent the mean ± S.E.M.](image2)
Astemizole Binding for HERG

Initially indistinguishable in these studies, yielding an overall estimated equilibrium dissociation constant (K_d) of 5.93 ± 0.81 nM (n = 4) and receptor expression level (B_max) of 6.36 ± 0.24 pmol/mg membrane protein (n = 4). Figure 3 depicts a representative saturation binding experiment. The K_d is an order of magnitude lower than literature reports for [3H]dofetilide binding to HERG membranes (13). A Rosenthal transformation of some binding data (not shown) suggested the possibility of more than one binding site class. However, fitting such data to a two-site hyperbolic model demonstrated no improvement in correlation coefficient over a single-site model. Furthermore, astemizole competitive binding data highly correlated with a one-site competition model (cf., Fig. 5).

**Intraassay and interassay variations**

Intraassay variation for the [3H]astemizole binding assay, based on results from seven separate assays (n = 8–32 each), was 11.4%, whereas interassay variation was 14.9% (2,557 ± 380 cpm, n = 7). The results speak for a robust [3H]astemizole binding assay for HERG.

**Effect of DMSO on [3H]astemizole binding in HERG transfected membranes**

The concentration of DMSO in the assay buffer of up to 2% had little effect on [3H]astemizole binding (Fig. 4). The final concentration of DMSO used in competition assays was 1%. Test compounds could be dissolved in 100% DMSO in mM concentrations which allowed top test concentrations of 10 μM or greater.

**Competition binding of [3H]astemizole in HERG transfected membranes**

A summary of affinity values for 32 compounds tested for HERG binding is presented in Table 1. The inhibition curves of specific [3H]astemizole binding by four representative compounds from three different therapeutic classes is shown in Fig. 5. Astemizole demonstrated greatest potency among the 32 test compounds against [3H]astemizole binding, showing K_i = 3.3 ± 0.70 nM, in accord with the observed K_d = 5.91 ± 0.81 nM and IC_{50} of 4.2 ± 1.0 nM. These closely similar values indicate that the [3H]astemizole binding assay is internally consistent and can provide highly predictive data regarding binding affinity to HERG. In contrast, aspirin and sildenafil both showed K_i values exceeding 1 mM. Furthermore, the majority of compounds produced Hill coefficients close to 1.0. Compounds that deviated significantly from 1.0 included E-4031 (0.81 ± 0.03) and loratadine (2.05 ± 0.19). Several compounds including ziprasidone and diphenhydramine consistently displayed elevated [3H]astemizole binding at very high concentrations, while binding was reduced at lower competitor concentrations. The observations are illustrated in Fig. 6 with ziprasidone. Data trended upward rising above lower plateaus defined by lower compound concentrations. Such elevated values may have been due in part to poor solubility at high concentrations, although other compounds (e.g., tamoxifen) did not show this behavior.
despite evident poor solubility. Data corresponding to such high concentrations were excluded from curve fitting for $K_i$ estimations.

**Comparison of binding data with electrophysiology data**

The $IC_{50}$ values ($n = 5 - 7$) for inhibition of HERG currents in HEK293 cells by 14 compounds were as follows: cisapride, 6.9 nM; E-4031, 18.1 nM; amiodarone, 25.9 nM; astemizole, 26 nM; fluoxetine, 197 nM; tamoxifen, 198 nM; imipramine, 616 nM; flecainide, 810 nM; ketoconazole, 1,920 nM; procainamide, 7,450 nM; disopyramide, 9,640 nM; and erythromycin, 62,600 nM. The effect of $I_{Kr}$ blockers on HERG currents is depicted in Fig. 7, using astemizole as an example.

In comparison with $[^3H]$astemizole binding data generated with the same compounds in HEK 293, it was demonstrated that the rank order of affinity ($K_i$) was highly correlative to that of inhibitory potency in the electrophysiology studies for HERG in HEK 293 ($r_{SP} = 0.91, P<0.005$) (Fig. 8). Furthermore, the 32 reference compounds including the 14 compounds from diverse structural classes demonstrated binding affinity values for HERG with similar rank order of inhibition of HERG currents as reported in the literature (6). However, the potency for HERG inhibition in the binding assay is in general about one order of magnitude less than that in the electrophysiology assays (Fig. 8).

### Table 1.

<table>
<thead>
<tr>
<th>Class</th>
<th>Compound</th>
<th>$K_i$, nM</th>
<th>$n_H$</th>
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<tr>
<td>Antiarrhythmic</td>
<td>Dofetilide</td>
<td>28 ± 6</td>
<td>0.90 ± 0.09</td>
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<tr>
<td></td>
<td>E-4031</td>
<td>58 ± 5</td>
<td>0.81 ± 0.03</td>
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<td></td>
<td>Amiodarone</td>
<td>591 ± 173</td>
<td>1.22 ± 0.12</td>
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<tr>
<td></td>
<td>Quinidine</td>
<td>10,697 ± 1,547</td>
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<tr>
<td></td>
<td>Flecainide</td>
<td>28,256 ± 9,365</td>
<td>0.97 ± 0.06</td>
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<td>Disopyramide</td>
<td>164,434 ± 25,188</td>
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<td>(+/-)Sotalol</td>
<td>147,079 ± 22,112</td>
<td>0.91 ± 0.08</td>
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<td>Procainamide</td>
<td>613,782 ± 58,950</td>
<td>1.09 ± 0.11</td>
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<td>Antihistamine</td>
<td>Astemizole</td>
<td>3.3 ± 0.7</td>
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<td>Terfenadine</td>
<td>101 ± 6</td>
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<td>Loratadine</td>
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<td>Diphenhydramine</td>
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<td>Fexofenadine</td>
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<td>Ca$^{2+}$ antagonist</td>
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<td>601 ± 110</td>
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<td></td>
<td>Verapamil</td>
<td>3,902 ± 529</td>
<td>1.11 ± 0.13</td>
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<tr>
<td>Antipsychotic</td>
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<td>14 ± 4</td>
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<td>Haloperidol</td>
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<td>Thoridiazine</td>
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<td>Ziprasidone</td>
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<td></td>
<td>Imipramine</td>
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<td>Amitriptyline</td>
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<tr>
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<td>Glibenclamide</td>
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<td>1.57 ± 0.31</td>
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<td>Sumatriptan</td>
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<td>0.95 ± 0.14</td>
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<tr>
<td>Vasodilator</td>
<td>Sildenafil</td>
<td>&gt;1,000,000</td>
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All values represent the mean ± S.E.M. for 5 observations.
The present study demonstrated that drugs known to block HERG/I\textsubscript{K}\textsubscript{r} inhibit \[^{3}H\]astemizole binding for HERG binding sites in a competitive manner and do so with a rank order of affinity closely parallel to that of inhibitory potency against HERG in electrophysiological studies in HEK293. Furthermore, the assay is robust and has adequate sensitivity. Consequently, the \[^{3}H\]astemizole binding assay is pharmacologically relevant in detecting compounds that have affinity for the HERG/I\textsubscript{K}\textsubscript{r} channels.

The voltage-gated HERG/I\textsubscript{K}\textsubscript{r} K\textsuperscript{+} channel contains six transmembrane regions with a single pore. The internal vestibule of the pore composed of residues from S5 and S6 segments facing the intracellular side contributes
to key binding sites (Tyr652 and Phe656) for methanesulfonanilide compounds such as dofetilide, E4031, and MK499; the external entry to the channel pore has binding sites for toxins and blockers as well (4). Chadwick et al. (14) first reported the use of [3H]dofetilide binding in guinea pig myocytes, which was followed by Duff and associates (15, 17). Fiset et al. (17) reported that three Class III antiarrhythmic agents including dofetilide, E-4031, and sotalol inhibited [3H]dofetilide binding to the high affinity sites (related to I_K) in guinea pig myocytes at concentrations similar to those required to inhibit 50% of I_K in electrophysiological studies. The characterization of HERG channel binding sites in HEK293 was most recently reported by Finlayson et al. (13) using [3H]dofetilide, which showed E-4031, pimozone, haloperidol and terfenadine with K_i values (nM) of 241, 71, 549, and 381, respectively, and a K_i value of 58 nM for dofetilide. Our [3H]astemizole binding assay seems to compare favorably with the [3H]dofetilide binding, as K_i/K_d values for the same five compounds evaluated by Finlayson et al. (13) were within 2- to 5-fold of the K_i values in the present study. The higher affinity to HERG inhibitors in this [3H]astemizole binding may be accounted for by differences in labeled ligand and assay conditions (e.g., amounts of HERG protein, ligand concentrations, assay temperature, etc.). In the present study, the inclusion of 0.1% BSA in both assay and wash buffers reduced non-specific binding, which raised the signal-to-background ratio an average of 80% for 10 μg membrane protein (data not shown). The negligible effects of BSA in the assay buffer reported by Finlayson et al. (18) were not observed. DMSO was used as a universal solvent in a final assay concentration of 1% and had negligible effects on competitive binding data.

Abbott et al. (19) reported that HERG channels co-assembled with MiRP1 resemble native cardiac I_K channels. However, there is not yet evidence indicating that absence of MiRP1 affects [3H]dofetilide or [3H]astemizole binding assays for HERG channels. This may not be unexpected as the primary binding sites are known to be associated with HERG (4).

It is of note that this radioligand binding assay compares favorably with functional assays using voltage-sensitive dyes or compares favorably with functional assays using sites are known to be associated with HERG (4). This may not be unexpected as the primary binding

Acknowledgments

The authors are indebted to Ms. Phuong Nguyen, Mr. Glenn Soja, and Dr. David Kirk for expert preparation of cell membranes. Dofetilide was a generous gift of Pfizer, Inc., Sandwich, UK.

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