Identification of a Specific Radioligand for the Cardiac Rapidly Activating Delayed Rectifier K⁺ Channel


Class III antiarrhythmic drugs show promise as effective treatments for the suppression of potentially lethal cardiac arrhythmias. Dofetilide (UK-68,798), is a potent class III antiarrhythmic agent that is presently under clinical investigation. The objective of this study was to determine whether [3H]dofetilide could be used as a specific radioligand for the rapidly activating delayed rectifier K⁺ channel of the heart. We find that [3H]dofetilide binds to high-affinity sites on guinea pig cardiac myocytes. Competition studies using unlabeled dofetilide indicate that binding is characterized by an IC₅₀ of 100±30 nM (mean±SD, n=13). Scatchard analysis of binding indicate a K_d of 70±6 nM and a maximal binding capacity of 0.3±0.02 pmol/mg protein. [3H]Dofetilide is displaced from guinea pig myocytes by dofetilide, clofilium, quinidine, sotalol, and seminalide with a rank order of potency that correlates with functional blockade of the rapidly activating delayed rectifier K⁺ current (correlation coefficient, r=0.95; slope, 0.99±0.19; p=0.014). High-affinity [3H]dofetilide binding is not detected in rat myocytes, which are devoid of delayed rectifier K⁺ current. We conclude that [3H]dofetilide specifically binds to sites associated with the rapidly activating delayed rectifier K⁺ channel of guinea pig myocardium. (Circulation Research 1993;72:707–714)

Key Words: • dofetilide • radioligands • delayed rectifier K⁺ channels • antiarrhythmic agents

Antiarrhythmic therapy has focused on modulation of cardiac electrical activity. Thus, ion channels involved in regulation of the cardiac action potential represent important therapeutic targets for the development of antiarrhythmic agents. Until recently, the in vitro characterization of antiarrhythmic drugs relied primarily on electrophysiological studies of isolated cells and intact cardiac tissue. By such techniques, it has been demonstrated that class I antiarrhythmic agents modulate sarcolemmal Na⁺ currents, class III antiarrhythmics attenuate outward K⁺ currents, and class IV agents block L-type Ca²⁺ channels. With the availability of specific radioligands for cardiac ion channels, it has become possible to characterize drug–channel interaction at the molecular level. Numerous radioligands including [3H]nitrendipine, [3H]verapamil, and [3H]diltiazem have facilitated characterization of drug interaction at the L-type Ca²⁺ channel, and [3H]batrachotoxinin benzoate (BTX-B) has been identified as a radiolabel for the cardiac Na⁺ channel. 1,2

Noticably absent, however, are radioligands for the cardiac K⁺ channels. The development of such K⁺ channel probes is timely, because therapeutic efforts have recently focused on K⁺ channel antagonists as a consequence of the Cardiac Arrhythmia Suppression Trial, which questioned the clinical utility of class I antiarrhythmic agents. 3

A number of methanesulfonamide antiarrhythmic agents that prolong cardiac refractoriness, including dofetilide, sotalol, and E-4031, have been identified. 4–11 Their mechanism of action is attributed to attenuation of the cardiac delayed rectifier K⁺ current (I_K). 11 In guinea pig cardiac myocytes, I_K comprises rapidly and slowly activating components. E-4031 and sotalol specifically block the rapidly activating component of I_K. 11 Dofetilide prolongs cardiac refractoriness in the 5 nM–1 μM concentration range; this enhanced potency relative to other antiarrhythmic agents suggested that dofetilide would be a good candidate for a myocardial K⁺ channel radioligand. Accordingly, the focus of this study was to determine the utility of dofetilide as a specific radioligand for the cardiac rapidly activating delayed rectifier K⁺ channel.

Materials and Methods

Animal Care and Use

All procedures conformed to the National Institutes of Health “Guide for the Care and Use of Laboratory Animals” (NIH publication No. 86-23, 1985) and the Animal Welfare Act (P.L. 89-544, as amended) and were approved by the Institutional Animal Care and
Myocyte Isolation

Ventricular myocytes were isolated from adult male guinea pigs (400–500 g) and rats (450–550 g). For guinea pigs, anesthesia was achieved by intraperitoneal injection of ketamine hydrochloride (150 mg) combined with xylazine base (10 mg). Animals were then heparinized (400 units) via the abdominal vena cava. Rats were euthanized by CO₂ asphyxiation. Hearts were rapidly excised, attached to a Langendorff apparatus, and perfused (30 ml/min) at 37°C with oxygenated buffer A containing (mM) NaCl 136, KCl 5.4, MgCl₂ 1.0, glucose 10, NaH₂PO₄ 0.33, and HEPES 10, adjusted to pH 7.48 (23°C) with NaOH. When the perfused solution appeared colorless, the hearts were perfused with buffer A (50 ml per heart) supplemented with collagenase B (0.625 mg/ml, Boehringer Mannheim Corp., Indianapolis, Ind.) and protease (0.04 mg/ml, No. P-5147, Sigma Chemical Co., St. Louis, Mo.). The solution used for perfusion was oxygenated and recycled until digestion was complete (20–40 minutes), as judged by softening of the myocardium. Canine cardiac myocytes were similarly isolated from adult male mongrel dogs after pentobarbital anesthesia (860 mg/kg). A section of the left ventricle (approximately 10 g) was perfused through the left anterior descending coronary artery. Minor arteries were occluded to ensure adequate perfusion of the myocardium.

For electrophysiological studies, myocytes were isolated in buffer A and filtered through nylon mesh (250 μm, PGC Scientific). Cells were resuspended and stored in minimum essential medium (No. 410-2000EB, Gibco, Grand Island, N.Y.). For [¹²⁵I]dofetilide binding studies, hearts and cardiac sections were perfused with an additional 50 ml of buffer B containing (mM) KCl 40, KH₂PO₄ 20, MgCl₂ 5, KHCO₃ 0.5, glucose 10, potassium glutamate 50, potassium aspartate 20, heptanoic acid 14, EGTA 1, and HEPES 10, adjusted to pH 7.40 (23°C) with KOH, and myocytes were isolated in the same buffer. Heptanoic acid was included in buffer B because it has been reported to maintain primary cell cultures in aerobic metabolism.12 Myocytes were allowed to sediment without centrifugation, and buffers were exchanged at least three times. As estimated by the percentage of rod-shaped cells, 36–9% of myocytes were viable (mean±SD, nine preparations). Cells were used within 6 hours of isolation.

[¹²⁵I]Dofetilide Binding

Myocytes (5–12 mg protein/ml) were incubated with [¹²⁵I]dofetilide in 0.10 ml buffer B containing 0.1% bovine serum albumin (BSA). Incubation was for 1 hour at 34°C. Myocytes were filtered on GF/C filters (Whatman Inc., Clifton, N.J.) with a Brandel 48 harvester. Filters were pretreated for at least 1 hour with wash buffer (25 mM Tris-HCl, 130 mM NaCl, 5.5 mM KCl, 5 mM glucose, 0.8 mM MgCl₂, and 50 μM CaCl₂ [pH 7.4]) containing 1.0% BSA and washed at 25°C with approximately 1,500 ml of the same buffer containing 0.1% BSA. Bound [¹²⁵I]dofetilide was determined by liquid scintillation counting after equilibration of filters with Beckman Ready Protein.

In competitive binding studies, the concentration of [¹²⁵I]dofetilide was 10–15 nM. In Scatchard analyses, the specific activity of dofetilide was maintained constant at 44.2 Ci/mmol. All incubations were performed in triplicate, and nonspecific binding was determined by incubation with 10 μM unlabeled dofetilide, except where indicated otherwise in the figure legends.

Preparation of Canine Cardiac Sarcolemma

Canine cardiac sarcolemma vesicles were isolated by sucrose flotation.13 This procedure gives a sarcolemma preparation characterized by specific [³H]quinuclidinyl benzilate binding of 8.1±1.3 pmol/mg protein (mean±SD, n=7), corresponding to a >60-fold enrichment.13 [³H]Quinuclidinyl benzilate (87 Ci/mmol) was from New England Nuclear, Boston, and binding was determined as described by Jones.13

[¹²⁵I]Desmethoxyverapamil Binding

Canine cardiac sarcolemma (50 μg protein) was incubated with [¹²⁵I]desmethoxyverapamil (D888, New England Nuclear; specific activity, 87 Ci/mmO) in buffer containing 50 mM Tris-HCl (pH 7.4) and 0.1% BSA for 90 minutes at 25°C in a volume of 0.25 ml. All incubations were performed in triplicate, and nonspecific binding was determined in the presence of 30 μM S-­­verapamil (Research Biochemicals Inc., Natick, Mass.). In competitive binding studies, the incubations contained 5 nM [³H]D888 and 0–300 μM dofetilide. For Scatchard determinations, [¹²⁵I]D888 concentrations of 1–32 nM were used. Bound radioactivity was determined after filtration on Whatman GF/C filters using a Brandel 48 harvester. Before use, filters were blocked by incubation for 60 minutes at room temperature in 20 mM Tris-HCl (pH 7.4) containing 0.5% polyethyleneimine. Filters were washed with 2,000 ml of 10 mM HEPES-Tris (pH 7.4) containing 10% ethanol (vol/vol). [¹²⁵I]D888 binding to purified canine cardiac sarcolemma was characterized by a Bₘₐₓ of 0.74±0.09 pmol/mg and Kᵦ of 4.5±0.3 nM (mean±SD, n=3).

[¹²⁵I]Batrachotoxinin Benzate Binding

Canine cardiac sarcolemma (50 μg protein) was incubated with [¹²⁵I]BTX-B (New England Nuclear; specific activity, 52.7 Ci/mmol) in 0.1 ml buffer containing 130 mM choline chloride, 50 mM HEPES-Tris (pH 7.4), 5.4 mM KCl, 0.5 mM EDTA, 0.4 mM MgCl₂, 0.1% BSA, and 0.12 mg/ml Leuira quinquestriatus venom (V 5251, Sigma). All incubations were performed in triplicate for 60 minutes at 37°C. Nonspecific binding was determined in the presence of 0.3 mM veratridine-HCl (Sigma). In competitive binding studies, the incubations contained 5 nM [¹²⁵I]BTX-B and 0–300 μM dofetilide. In Scatchard analyses, [¹²⁵I]BTX-B concentrations of 1–32 nM were used. Filtration was performed as described for [¹²⁵I]D888 binding, except that filters were presoaked for 60 minutes in 20 mM Tris-HCl (pH 7.4) containing 0.25% polyethyleneimine.

[¹²⁵I]BTX-B binding to purified canine cardiac sarcolemma was characterized by a Bₘₐₓ of 0.7±0.2 pmol/mg protein and a Kᵦ of 5.1±2.6 nM (mean±SD, n=3).

Electrophysiology

Isolated cells in solution were placed on a glass coverslip (bath volume, approximately 300 μl) and
viewed using a Zeiss inverted microscope. Rod-shaped quiescent cells were used. Cells were perfused at a rate of 5 ml/min with buffer C containing (mM) NaCl 145, KCl 4.5, MgCl$_2$ 1, CaCl$_2$ 0.1, HEPES 10, glucose 12, and NiCl$_2$ 2, (pH 7.4). Bath temperature was maintained at 33–35°C. Membrane currents were obtained by standard whole-cell patch-clamp techniques with electrodes pulled from thin-walled glass (World Precision Instruments, New Haven, Conn.). Electrodes were filled with buffer D containing (mM) NaCl 10, KCl 112, EGTA 10, HEPES 10, K$_2$-ATP 5, and Mg-ATP 5, (pH 7.2) and had resistances of approximately 3 MΩ. Voltage-clamp recording was performed on an Axopatch 1C amplifier (Axon Instruments, Foster City, Calif.), and data were digitized with a LabMaster 125 kHz DMA board and a Compaq Deskpro 386 computer. All data acquisition and analyses were performed with the pClamp software package (Axon Instruments). Recordings were digitized at 0.25–1 kHz and filtered at 0.2 kHz. Series resistance compensation was used in all experiments.

Sodium currents were inactivated by using membrane holding potentials of -40 mV. Calcium currents and sodium-calcium exchange current were eliminated by reducing extracellular calcium to 0.1 mM and inclusion of 2 mM NiCl$_2$ in the bath solution. ATP (10 mM) was included in the pipette solutions to eliminate ATP-gated potassium currents. Under our recording conditions, ATP concentrations <10 mM were not sufficient to completely eliminate a contribution from ATP-gated potassium channels. $I_\text{Ca}$ was defined as the amplitude of the deactivating tail currents at -40 mV after voltage steps to potentials > -10 mV, as previously described.

After establishment of the whole-cell recording mode, cells were stabilized for several minutes before acquiring control data. Cells were then exposed to drug for 2 minutes, followed by acquisition of test data. No run-down of time-dependent or tail currents was observed during temporal controls.

**Curve Fitting**

The equation $I = A[1 + ([\text{drug}]IC_{50})^n]^{-1} + B$ was fit to the electrophysiological data using commercially available software (GraphPad Software, Inc., San Diego, Calif.), where I is normalized current, [drug] is the test concentration of a particular drug. IC$_{50}$ is the concentration at which the current is reduced by 50% of the maximum reduction, n is the Hill coefficient, A is the fraction of current that was blocked at saturating drug concentrations, and B is 1 - A (residual current). For quinidine and clofibrate, A was fixed at 1. Binding data were fit to a single-component competitive binding model using a four-parameter logistic equation (GRAPHPAD). In the binding studies, IC$_{50}$ is defined as the concentration of test agent that displaces 50% of the specific binding. Unless otherwise indicated, the control and nonspecific binding levels were normalized to 100% and 0%, respectively, and held constant.

**Protein Determination**

Protein was determined according to the method of Lowry using BSA as a standard.

**Materials**

[1H]Dofetilide (44.2 Ci/mmol) was prepared from the dibromo precursor 1-(3-bromo-4-methanesulfonyl-diphenyloxy)-2-[(N-(3-bromo-4-methanesulfonylmethyl)-N-methylamino)-ethane by 2H exchange (New England Nuclear). Dofetilide, dibromodofetilide, and sematilide were synthesized at Sterling Winthrop Pharmaceuticals Research Division at Rensselaer, N.Y., and Alnwick, UK. Quinidine was purchased from Sigma. Clofibrate and sotalol were gifts from Eli Lilly and Co., Indianapolis, Ind., and Bristol-Myers Squibb Co., Evansville, Ind., respectively.

**Results**

As illustrated in Figure 1, unlabeled dofetilide displaced [1H]dofetilide from guinea pig cardiac myocytes with an IC$_{50}$ of 100±30 nM (mean±SD, n=13, Table 1), indicating high-affinity binding. Scatchard analyses (inset, Figure 1) were consistent with high-affinity binding to a site with a $K_a$ of 70±6 nM (mean±SD, n=3) and a $B_{max}$ of 0.30±0.02 pmol/mg protein (mean±SD, n=3). Myocytes from canine heart also bound [1H]dofetilide with high affinity (IC$_{50}$ 54 nM; Table 1), although the binding capacity was 17% of that in guinea pig myocytes (Table 1). Rat cardiac myocytes were devoid of high-affinity [1H]dofetilide binding (Figure 1, Table 1).

As shown in Figure 2, the protein dependence of specific [1H]dofetilide binding to guinea pig myocytes deviates slightly from linearity. However, the IC$_{50}$ values for competitive displacement of [1H]dofetilide by dofetilide, clofibrate, and dibromo-sotalol were identical when determined at myocyte protein concentrations of 5 and 11 mg/ml (results not shown). The binding studies herein reported used myocyte protein concentrations <12 mg/ml. At a myocyte protein concentration of 6.8 mg/ml and a [1H]dofetilide concentration of 12 nM, the total and nonspecific binding values were 2.7% and 0.5% of the total radiolabel in the incubation, respectively. The guinea pig myocyte preparations used in these studies consisted of 36±9% rod-shaped cells, indicating a majority of nonviable cells. However, we find similar [1H]dofetilide binding characteristics for myocyte preparations having 25–55% rod-shaped cells (results not shown). Most likely, therefore, [1H]dofetilide binds to both viable and nonviable cells.

The time course of [1H]dofetilide binding to guinea pig myocytes at 34°C is shown in Figure 3A. Specific binding of [1H]dofetilide reaches equilibrium within 20 minutes and is stable for an additional 45 minutes. The observed association rate constant was 0.172 min$^{-1}$. Nonspecific [1H]dofetilide binding was not time dependent. In competitive binding experiments and Scatchard analyses, incubation was for 60 minutes at 34°C. Figure 3B illustrates the dissociation of specifically bound [1H]dofetilide from guinea pig myocytes in the presence of excess unlabeled dofetilide at 34°C. The data fit to a single exponential decay model, indicating dissociation from a single class of sites. The dissociation rate constant was 0.158±0.007 min$^{-1}$. The calculated association rate constant was 9.8×10$^{-10}$ M$^{-1}$ min$^{-1}$. The $K_d$ calculated from the kinetic data was 161 nM, which is in agreement with an IC$_{50}$ value of 100 nM determined from competition studies and the $K_d$ of 70 nM determined from Scatchard analyses. We estimate that approximately...
16% of the specifically bound [3H]dofetilide is lost during the 1-minute filtration time used in these studies.

To investigate the effects of dofetilide on delayed rectifier tail current amplitude, voltage-clamp experiments were performed on isolated guinea pig, rat, and canine ventricular myocytes. Figure 4 illustrates the membrane current elicited by a depolarizing voltage pulse to +20 mV in guinea pig and rat myocytes and the effect of 1 μM dofetilide on these currents. In guinea pig myocytes, time-dependent outward currents were observed during the test pulse, and deactivating tail currents were observed on repolarization. Dofetilide (1 μM) blocked both the current during the pulse and the tail current (Figure 4B). In rat myocytes, the same voltage-clamp protocol elicited a transient outward current during depolarization19 (Figure 4C). However, no significant tail current was observed after repolarization, and dofetilide (1 μM) was without effect (Figure 4C). Canine myocytes gave results similar to those in the guinea pig (Table 1), although the total potassium tail current was 17% of that observed in the guinea pig (Table 1).

The current–voltage relation for potassium current in guinea pig ventricular myocytes in the absence and presence of dofetilide (30 nM and 300 nM) is illustrated.

**Table 1.** [3H]Dofetilide Binding and Tail Current Density in Cardiac Myocytes

<table>
<thead>
<tr>
<th>Species</th>
<th>[3H]Dofetilide binding (fmol/mg)</th>
<th>Tail current density (pA/pF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig</td>
<td>38.5±5.3 (n=3)</td>
<td>0.71</td>
</tr>
<tr>
<td>Dog</td>
<td>6.6 (n=2)</td>
<td>0.12</td>
</tr>
<tr>
<td>Rat</td>
<td>ND (n=2)</td>
<td>ND</td>
</tr>
</tbody>
</table>

n, Number of preparations; ND, not detectable.

The levels of [3H]dofetilide binding were determined at [3H]dofetilide concentrations of 12 nM. [3H]Dofetilide binding to canine myocytes was characterized by an IC50 of 54 nM.
in Figure 5A. At test depolarizations ≤+20 mV, dofetilide (300 nM) reduced current amplitude by >80%. At more positive potentials, however, proportionally less K⁺ current was blocked by dofetilide. Dofetilide concentrations up to 30 μM produced no additional block. Concentration–response relations for dofetilide inhibition of potassium current after test depolarizations to voltages of +20 and +60 mV are illustrated in Figure 5B. Dofetilide maximally blocks 86% of potassium current after voltage steps to +20 mV, whereas tail currents after depolarizations to +60 mV are blocked no more than 55%. The respective IC₅₀ values for tail current block were 29 nM (95% confidence interval, 20–43 nM) and 44 nM (95% confidence interval, 35–56 nM) for +60 and +20 mV data, respectively. These estimates are comparable to the IC₅₀ for [³H]dofetilide binding to guinea pig myocytes (100±30 nM, Figure 1).

Competitive inhibition of [³H]dofetilide from guinea pig myocytes by dofetilide, clofilium, quinidine, DL-sotalol, and sematilide is shown in Figure 6. The respective IC₅₀ values and Hill coefficients are listed in Table 2. Figure 7 shows the effect of the same antiarrhythmic agents on potassium tail currents recorded in guinea pig ventricular myocytes after test depolarizations to +20 mV. Clofilium and quinidine show similar inhibition profiles in that, over the concentration range tested, they completely block potassium tail current. In contrast, dofetilide, DL-sotalol, and sematilide maximally inhibit current amplitude by approximately 85%. The IC₅₀ values and Hill coefficients for inhibition of tail currents are listed in Table 2. The correlation between antiarrhythmic displacement of [³H]dofetilide and inhibition of potassium tail current (test potential, +20 mV) is illustrated in Figure 8. A correlation coefficient of 0.951 (p<0.014) with a slope of 0.99±0.19 was obtained.

Dofetilide displaced [³H]D888 from the L-type Ca²⁺ channel and [³H]BTX-B from the Na⁺ channel of enriched canine cardiac sarcolemma with respective IC₅₀ values of 60 μM (n=2) and >300 μM (n=2), demonstrating a lack of high-affinity interaction at the respective channels.

Discussion

The primary focus of this study was to assess the utility of dofetilide as a radioligand for cardiac K⁺ channels. Dofetilide is a potent methanesulfonamide...
FIGURE 5. Panel A: Current–voltage relation for delayed rectifier tail current in guinea myocytes. Tail current density (pA/pF) at –40 mV is plotted vs. test potential. Control values (□) were normalized to the maximum tail currents that were elicited by voltage steps to +60 mV. Tail current amplitudes after exposure to 30 nM (●) or 300 nM (Δ) dofetilide were also normalized to control values after test pulses to +60 mV. Each point is the mean ± SEM of n=12 values in the control condition and n=6 values for 30 and 300 nM dofetilide data. Panel B: Concentration–response relations for dofetilide block of delayed rectifier currents. Tail currents were measured in the control condition (I_{control}) and in varying concentrations of dofetilide (I_{drug}); relative tail current amplitude (I_{drug}/I_{control}) is plotted vs. concentration after voltage steps to either +20 mV (●) or +60 mV (○). Values are mean ± SEM with n=3–7 values per concentration. Smooth curves are fits to the data (see “Materials and Methods” for equation and Table 1 for IC_{50} values and Hill coefficients).

An antiarrhythmic agent that attenuates I_{Ks}\textsuperscript{a,19} Consistent with these pharmacological observations, we detect high-affinity binding of [3H]dofetilide in guinea pig (Figures 1 and 4B) and canine myocytes (Table 1). Rat myocytes, however, which are devoid of I_{Ks} (Figure 2C, Reference 18), show no high-affinity dofetilide binding (Figure 1). [3H]Dofetilide binding to guinea pig myocytes is characterized by an IC_{50} value of 100 nM (Figure 1). Scatchard analyses (Figure 1, inset) give a K_{d} of 70 nM and are consistent with the existence of specific high-affinity [3H]dofetilide binding sites.

Previously, it has been demonstrated that I_{Ks} can be pharmacologically separated into a rapidly activating component (I_{Ks}^{\text{r}}) and a slowly activating component (I_{Ks}^{\text{s}}).\textsuperscript{11} Therefore, we sought to determine the K\textsuperscript{+} channel specificity of dofetilide. In addition to more rapid activation kinetics, I_{Ks} activates at more negative potentials than I_{Ks}.\textsuperscript{11} Thus, relatively short (700-msec) voltage steps to +20 mV elicit membrane currents composed primarily of I_{Ks}. Voltage steps to +60 mV, however, activate both I_{Ks} and I_{Ks}^{\text{s}}, allowing one to determine the specificity of a blocker for the different components of I_{Ks}. As illustrated in Figure 5A, the degree of attenuation of I_{Ks} by dofetilide is dependent on test potential. After voltage steps to +20 mV, dofetilide maximally inhibits approximately 85% of I_{Ks}, whereas after steps to +60 mV, approximately 55% of I_{Ks} is blocked (Figure 5B). The greater attenuation of I_{Ks} by dofetilide after steps to +20 mV (Figure 5B) is consistent with specific block of I_{Ks}. Conversely, the increase in drug-insensitive current at more positive test potentials demonstrates that dofetilide does not block I_{Ks}.

An initial report of I_{Ks} block by dofetilide has previously appeared.\textsuperscript{9} This earlier study did not investigate the specificity of I_{Ks} versus I_{Ks} block, however, and only two concentrations of dofetilide were examined in voltage-clamp experiments (50 nM and 2 μM). Comparable to the IC_{50} values determined in our complete concentration–response studies (Figures 5B and 7, Table 2), 50 nM dofetilide blocked approximately 60% of I_{Ks} and consistent with our findings, 2 μM dofetilide reduced current amplitudes by no more than 85%, suggesting a residual drug-insensitive component of I_{Ks}.

Analogous to dofetilide, sotalol and sematilide maximally inhibit approximately 85% of I_{Ks} after voltage steps to +20 mV (Figure 7), indicating that these agents also specifically block I_{Ks}. In contrast, as indicated by complete block of I_{Ks} at voltage steps of +20 mV (Figure 7), clofilium and quinidine inhibit both I_{Ks} and I_{Ks}. Accurate IC_{50} values for block of I_{Ks} by agents such as clofilium and quinidine can only be obtained at low
Depolarization voltages, where the contribution of $I_{Kr}$ to total K⁺ tail current is small. As discussed above, $I_{Kr}$ constitutes approximately 85% of the total current when depolarization steps to +20 mV are used. Unacceptable signal-to-noise ratios precluded $I_{Kr}$ measurement at lower depolarization voltages. Therefore, we consider that the +20 mV IC₅₀ values listed in Table 2 provide the most accurate estimates for specific block of $I_{Kr}$. Notably, the IC₅₀ values for block of $I_{Kr}$ by clofilium and quinidine are significantly higher when determined at voltage steps of +60 mV (Table 2), indicating that these agents block $I_{Kr}$ less potently than $I_{Kr}$.

The IC₅₀ determined for dofetilide block of $I_{Kr}$ (44 nM, Table 2) is similar to the IC₅₀ value and $K_d$ values (100 nM and 70 nM, respectively; Figure 1, Table 2) characteristic of [³H]dofetilide binding to guinea pig myocytes. Also, [³H]dofetilide is displaced from guinea pig myocytes by other antiarrhythmic agents including clofilium, quinidine, sotalol, and sematilide (Figure 6, Table 2), with a rank order of potency that correlates with block of $I_{Kr}$ (correlation coefficient, 0.951; slope, 0.99; Table 2, Figure 8). These data indicate that dofetilide binds to high-affinity sites on guinea pig myocytes that are associated with the rapidly activating delayed rectifier K⁺ channel. In ancillary receptor screens, we find that dofetilide displaces [³H]D8888 from the L-type Ca²⁺ channel and [³H]BTX-B from the Na⁺ channel with IC₅₀ values of 60 μM and >300 μM, respectively. These findings demonstrate a lack of high-affinity interaction at the cardiac Ca²⁺ and Na⁺ channels and further illustrate the channel specificity of dofetilide.

This study provides the first quantitative estimates of $I_{Kr}$ channel density within cardiac membranes. A $B_{max}$ of 0.3 pmol/mg is estimated for dofetilide binding to the high-affinity site in guinea pig myocytes (Figure 1, inset). As determined by [³H]BTX-B binding, the Na⁺ channel density in rat myocytes is approximately 0.025 pmol/mg protein. Assuming that similar levels of [³H]BTX-B binding are observed in guinea pig myocytes and a stoichiometric interaction of [³H]dofetilide and [³H]BTX-B with the respective channels, these data suggest a 10-fold greater density of the rapidly activating delayed rectifier K⁺ channel relative to the Na⁺ channel. These biochemical data are consistent with the predicted $I_{Kr}$ channel densities calculated from our data and other published values. If the single channel conductance of $I_{Kr}$ channels is similar to those in rabbit pacemaker cells, the conductance in 4.5 mM K⁺ would be 1.4 pS. Assuming an $E_K$ value of −90 mV and an open channel probability near 1, the average tail current amplitude at −40 mV (194±11 pA [n=41], test potential = +20 mV) that we observed would be equivalent to approximately 2,700 channels per cell. Sodium channel densities have been estimated to be approximately 670 per cell. Since it is unlikely that the open channel probability of $I_{Kr}$ channels is 1, the electrophysiological data are consistent with a 10-fold greater $B_{max}$ value for $I_{Kr}$ channels compared with Na⁺ channels. The high $I_{Kr}$ channel density in guinea pig ventricular myocytes may facilitate further studies of this membrane protein at the biochemical level.

[³H]Dofetilide binding to guinea pig myocytes was determined in a “depolarizing” buffer containing 110 mM K⁺ and zero Na⁺. Under these conditions, myocytes...
are not expected to possess a negative membrane potential. Thus, \[^{[3]H}\]dofetilide binds to the \(I_{Kr}\) channel in the absence of a polarized membrane. The limited viability of the myocyte preparations used in these studies (36±9%) precluded investigation of the effect of resting membrane potential on \[^{[3]H}\]dofetilide binding. In addition, we cannot exclude the possibility that binding characteristics may have been affected by the limited viability of the myocyte preparations used in these studies. Although \[^{[3]H}\]dofetilide specifically binds to collagenase-dispersed myocytes from both dog and guinea pig, we have not been able to detect \[^{[3]H}\]dofetilide binding to preparations of canine cardiac sarcolemma which are significantly enriched with regard to the sarcolemmal marker \[^{[3]H}\]quinuclidinyl benzilate. Such findings suggest that a component necessary for the interaction of \[^{[3]H}\]dofetilide with the \(I_{Kr}\) channel may be lost during membrane isolation, which involves extraction in high–ionic strength media (0.75 M NaCl). Alternatively, the \(I_{Kr}\) channel may denature during the sarcolemma isolation.

In summary, our results provide the first characterization of the receptor biochemistry of \(I_{Kr}\) channels. Heretofore, binding assays have been available for the cardiac \(Na^+\) and \(Ca^{2+}\) channels, targets for current antiarrhythmic agents. Thus, a \[^{[3]H}\]BX-T binding assay has been described for the cardiac \(Na^+\) channel,\(^ {2,6}\) and ligands including \[^{[3]H}\]nitrendipine, \[^{[3]H}\]verapamil, and \[^{[3]H}\]diltiazem can be used to characterize L-type \(Ca^{2+}\) channel interaction.\(^ {4,5}\) \[^{[3]H}\]Dofetilide expands this list and facilitates characterization of drug interaction at the \(I_{Kr}\) channel.
Identification of a specific radioligand for the cardiac rapidly activating delayed rectifier K+ channel.


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