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Determinants of Stereospecific Binding of Type I Antiarrhythmic Drugs to Cardiac Sodium Channels

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SUMMARY

The determinants of stereospecific binding of type I antiarrhythmic drugs to specific sites associated with the sodium channel were assessed using rat cardiac myocytes. The asymmetric carbon atoms of stereoisomers may be located at two sites within type I drugs. The structure of these drugs can be schematically illustrated as Aromatic-C_1-link-C_2-Amine, where C_1 and C_2 represent potentially asymmetric carbon atoms. We used enantiomeric pairs with either C_1 or C_2 asymmetric carbon atoms to assess the importance of conformation at these sites to drug binding. The affinities of enantiomers of seven sodium channel blockers were measured with a radioligand binding assay using [^{3}H]batrachotoxinin benzoate ([^{3}H]BTXB) and freshly isolated cardiac myocytes. The enantiomers inhibited [^{3}H]BTXB binding in a dose-dependent manner, with a mean Hill number of 1.0 ± 0.1. The ratios of affinities [IC_{50} of (+)-isomer/IC_{50} of (−)-isomer] were, for the C_1 pairs: quinidine, 0.29; cinchonidine, 0.55; disopyramide, 1.11; RAC 109, 5.33; and for C_2 pairs: flecainide, 1.03; mexiletine, 2.15; tocainide, 3.01. The stereospecific differences in drug binding suggest that the orientations of both the aromatic and the amine groups to the rest of the drug molecule are important determinants of drug binding to the cardiac sodium channel. This also suggests the presence of at least two stereospecific domains within the binding sites for type I antiarrhythmic drugs.

The antiarrhythmic action of the type I antiarrhythmic drugs is thought to be mediated via blockade of the fast sodium channel (1). Models based on electrophysiologic data have been developed to explain the interaction between antiarrhythmic drugs and the cardiac sodium channel (2, 3). According to these models, type I antiarrhythmic drugs bind to specific site(s) or receptor(s) associated with the sodium channels. In addition, we have previously reported biochemical evidence that type I antiarrhythmic drugs bind to a specific receptor site associated with cardiac sodium channels (4). Using a radioligand assay (5), we characterized the binding of type I agents to this receptor as being saturable, reversible, and occurring at pharmacologically relevant concentrations with similar rank order of potency *in vitro* and *in vivo* ($r = 0.95$; $p < 0.01$). This suggests that the binding of type I antiarrhythmic drugs to this site is relevant to their clinical effect.

The interaction of type I drugs with a specific receptor site is further supported by the stereospecific effects of these antiarrhythmic agents on sodium channel blockade (6–9). In particular, we recently reported that the type I drug tocainide, which has stereospecific antiarrhythmic effects (7), has stereospecific effects on two measures of sodium channel blockade, conduction time and inhibition of [^{3}H]BTXB binding to myocytes (9). In each of these experimental models (*R*)-(−)-tocainide was 3-fold more potent than the (*S*)-(+)-isomer. Thus, the stereospecific effect of tocainide on radioligand binding correlates with its stereospecific electrophysiologic and antiarrhythmic effects.

The stereospecific interaction of drugs with the cardiac sodium channel suggests that molecular structure is an important determinant of type I drug binding. Therefore, our objective in the present study was to assess the determinants of stereospecific binding of type I drugs to cardiac sodium channels. Most type I drugs structurally consist of an aromatic lipophilic residue connected via an intermediate group to a hydrophilic amine group. These common structural features are schematically illustrated as aromatic-C_1-link-C_2-amine, where C_1 and C_2 represent potentially asymmetric carbons. To assess the contribution of changes in orientation of the lipophilic aromatic moiety with respect to the rest of the molecule, we assessed the relative affinities of compounds with asymmetric carbon atoms in the C_1 position. Similarly, the relative contribution of the orientation of the hydrophilic amine group was assessed in a series of compounds with asymmetric carbon atoms in the C_2 position. We used an assay that measures the binding of [^{3}H]BTXB, a sodium channel-specific toxin, to isolated rat cardiac

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ABBREVIATION: BTXB, batrachotoxinin benzoate.

myocytes (5, 10, 11). The affinities of enantiomers for the channel were estimated from their ability to inhibit [^{3}H]BTXB binding.

Methods

Myocyte preparation. Cardiac myocytes were isolated from adult male Sprague-Dawley rats (200–250 g) using the method of Kryski *et al.* (12). Rats were killed by cervical dislocation and hearts were rapidly removed. For each, the aorta was cannulated and the heart was perfused retrograde in a Langendorff perfusion apparatus. The heart was perfused and later incubated with a series of solutions that were equilibrated with 95% O_2/5% CO_2 at 37°. The solutions were based on Joklik's minimal essential medium supplemented with 1.2 mM $MgSO_4$ and 1 mM DL-carnitine (MEM). They included a rinse solution (MEM), a digestion solution (MEM with 0.1%, w/v, fatty acid-free bovine albumin and 0.1% collagenase), a calcium solution (MEM with 1 mM $CaCl_2$ and 1% fatty acid-free albumin), and an incubation solution (MEM with 50 μM $CaCl_2$ and 1% dialyzed bovine serum albumin). The heart first was perfused at 20° and 5 min with rinse solution, then perfused at 37° for 20 min with digestion solution. The ventricles were then removed, minced with scissors, and rinsed at 37° for 15 min with calcium solution. Calcium solution was then removed by aspiration and the tissue pieces were incubated at 37° for 15 min with digestion solution in a shaking water bath. Dispersed cells were decanted into a plastic centrifuge tube and the residual tissue was shaken again with digestion solution. This resulted in almost total dispersion of the heart. The pooled myocytes were then filtered through a 185-μm silkscreen mesh, collected by gentle centrifugation, and rinsed with incubation solution. The cells were again collected by gentle centrifugation, resuspended in incubation solution, and stored at 21° until used. They were used within 30 min. This method routinely yielded about 60 mg (dry weight) of myocytes, which corresponds to 1.2×10^7 cells (12, 13). The cells were 75–85% viable rod-shaped cells that excluded Trypan blue and were tolerant to 1 mM calcium.

Radioligand binding. Myocytes (6×10^5 per assay) in 50 μl of incubation buffer were incubated with 1.3 μM sea anemone toxin II, 13 nM [^{3}H]BTXB (50 Ci/mmol), and 0.13 mM tetrodotoxin for 45–60 min at 37° (see Ref. 13a for a review of sodium channel toxins). Tetrodotoxin was added to prevent depolarization induced by sodium influx; without tetrodotoxin no specific binding is observed (5, 14). Various concentrations of drugs and toxins were included in the incubations. Assays were done in parallel with tubes containing 0.4 mM aconitine to define nonspecific binding. Reactions were terminated by adding 10 ml of KHS buffer (Krebs-Henseleit-bovine serum albumin: 127 mM NaCl, 2.33 mM KCl, 1.30 mM KH_2PO_4, 1.23 mM $MgSO_4$, 25 mM $NaHCO_3$, 10 mM glucose, 50 μM $CaCl_2$, 1% BSA, equilibrated with 95% O_2/5% CO_2, and incubated at 37° for 1 min, then filtered through a Whatman GF-C 24-mm filter and washed four times with 5 ml of rinse buffer (25 mM Tris·HCl, pH 7.4, 130 mM NaCl, 5.5 mM KCl, 0.8 mM $MgSO_4$, 5.5 mM glucose, 50 μM $CaCl_2$). The filters were then dried and counted in Econofluor scintillation fluid. The retained radioactivity represents [^{3}H]BTXB bound to myocytes.

The rationale for the incubation and filtration conditions have previously been described (5). The conditions provide a maximal reduction in background and scatter with a minimal reduction in specific binding. The total wash time is 45 sec. Initial control experiments showed that, under these conditions, less than 10% of the specifically bound [^{3}H]BTXB dissociated from the complex. Under these reaction conditions (13 nM [^{3}H]BTXB, 0.13 mM tetrodotoxin, 1.3 μM sea anemone toxin II), about 60–75% of the total radioactivity retained on the filters is bound specifically to the [^{3}H]BTXB binding site.

Experimental design. To overcome any artifactual differences that might be produced by day to day variability in cell preparations or toxin concentrations, we used a paired experimental design. This involved determining the IC_{50} values of each enantiomeric pair in parallel using common preparations of cells and toxins.

Drug selection. Most type I antiarrhythmic drugs can be schematically represented as Aromatic-C_1-link-C_1-Amine, where C_1 and C_2 represent potentially asymmetric carbon atoms. Stereoisomeric pairs of drugs with asymmetric carbons at C_1 or C_2 sites were chosen for this study. C_1 pairs were quinidine and quinine, cinchonidine and cinchonine, (*S*)-(+)- and (*R*)-(−)-disopyramide, and (*S*)-(+)- and (*R*)-(−)-RAC 109. C_2 pairs were (*S*)-(+)- and (*R*)-(−)-flecainide, (*S*)-(+)- and (*R*)-(−)-mexiletine, and (*S*)-(+)- and (*R*)-(−)-tocainide. The structures of these drugs are shown in Fig. 1. RAC 109 is a local anesthetic drug, which was included because of its structural similarity to type I antiarrhythmic drugs and because electrophysiologic data (8) suggested that RAC 109 has a stereospecific effect on cardiac sodium channel blockade. Cinchonidine is an experimental drug that is structurally very similar to quinidine (desmethoxy-quinidine).

Statistical analysis. The statistical significance of the difference between the IC_{50} values for each pair of enantiomers for inhibition of [^{3}H]BTXB binding was assessed using a paired Student *t* test.

Materials. [^{3}H]BTXB and econoflow were purchased from New England Nuclear (Boston, MA), collagenase from Cooper Biomedical (Malvern, PA), and tetrodotoxin, aconitine, albumin, and sea anemone toxin from Sigma Chemical Co. (St. Louis, MO). The following drugs were kindly provided by their manufacturers: disopyramide (Roussel, Paris); RAC 109 and tocainide (Astra, Westboro, MA); mexiletine (Boehringer Ingelheim, Ridgefield, CT); and flecainide (Riker, St. Paul, MN).

Results

Employing a paired experimental design (see Methods), enantiomeric pairs of seven drugs with asymmetric carbon atoms

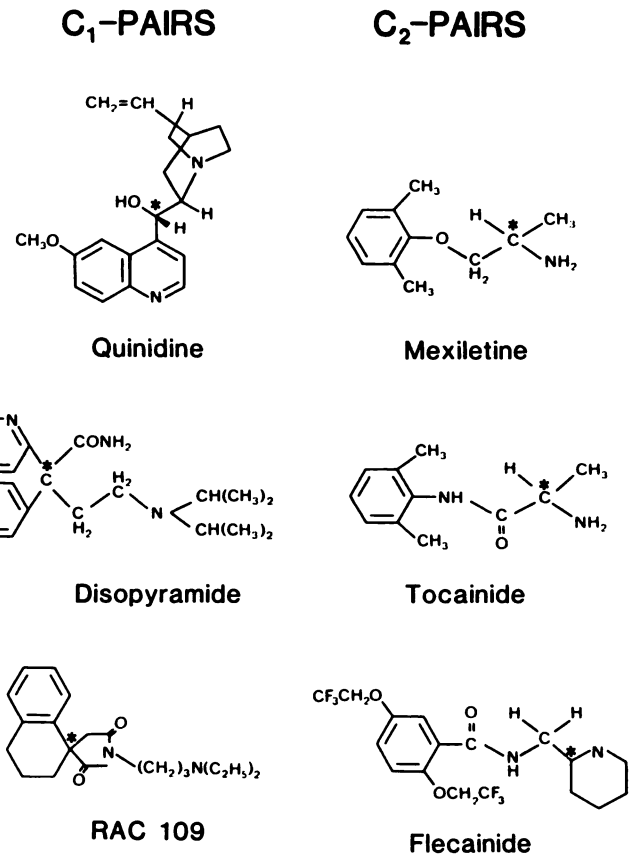

Fig. 1. Chemical structures of antiarrhythmic drugs with an asymmetric carbon at the C_1- or C_2-position. The enantiomers of C_1 pairs differ only in the orientation of the aromatic group with respect to the rest of the drug molecule, whereas the enantiomers of C_2 pairs differ only in the orientation of the terminal amine group with respect to the rest of the drug molecule.

at sites C_1 or C_2 were used to determine the stereospecific requirements for antiarrhythmic drug binding to specific sites associated with the cardiac sodium channel. The IC_{50} for drug binding is defined as the concentration of drug that inhibited [^{3}H]BTXB binding by 50%. All enantiomers tested inhibited [^{3}H]BTXB binding in a dose-dependent manner with mean Hill numbers ranging from 0.89 to 1.11. The mean Hill number of all drugs tested was 1.0 ± 0.1, suggesting that their interaction is with a single class of saturable sites. There was no significant difference between the Hill numbers for any of the drug pairs evaluated. A typical experiment is shown in Fig. 2. In this example, the IC_{50} values of quinidine and quinine were 45 and 127 μM, respectively, and the ratio of the IC_{50} values was 0.35 for this experiment. The mean IC_{50} values (± standard deviation) for three experiments were 41 ± 15 μM for quinidine and 140 + 43 μM for quinine ($p < 0.05$). The mean ratio of (IC_{50} quinidine)/IC_{50} quinine) was 0.29 ± 0.05. These data indicate that quinidine inhibits [^{3}H]BTXB binding at 3-fold lower concentrations than its stereoisomer quinine. This suggests a stereospecific interaction of quinidine with specific sites associated with the cardiac sodium channel.

The data for all C_1 and C_2 stereoisomeric pairs are shown in Table 1. Stereospecific differences were seen in most pairs with either C_1 or C_2 asymmetric carbon atoms. For C_1 pairs, the mean ratio of [IC_{50} of (+)-isomer/IC_{50} of (−)-isomer] ranged from 0.29 ± 0.05 for quinidine to 5.33 ± 1.38 for RAC 109; whereas, for C_2 pairs, the mean IC_{50} ratios ranged from 1.03 ± 0.02 to 3.01 ± 0.20 for flecainide and tocainide, respectively. The implications of stereospecific differences in drug binding are discussed below.

TABLE 1

IC_{50} values for stereoisomeric pairs of seven drugs with their associated potency ratios

Values are the means ± standard deviation of three experiments per pair.

	IC_{50} μM	[IC_{50}(+)-isomer]/[IC_{50}(−)-isomer]
C_1 asymmetric carbon		
Quinidine		
(*S*)-(+)	41 ± 15[a]	0.29 ± 0.05
(*R*)-(−)	140 ± 43	
Cinchonidine		
(*S*)-(+)	50 ± 17[a]	0.55 ± 0.20
(*R*)-(−)	79 ± 5	
Disopyramide		
(*S*)-(+)	122 ± 45	1.11 ± 0.29
(*R*)-(−)	109 ± 6	
RAC 109		
(*S*)-(+)	87 ± 14[b]	5.33 ± 1.38
(*R*)-(−)	17 ± 5	
C_2 asymmetric carbon		
Flecainide		
(*S*)-(+)	57 ± 15	1.03 ± 0.02
(*R*)-(−)	56 ± 16	
Mexiletine		
(*S*)-(+)	145 ± 22[a]	2.15 ± 0.71
(*R*)-(−)	78 ± 27	
Tocainide		
(*S*)-(+)	546 ± 64[b]	3.01 ± 0.20
(*R*)-(−)	184 ± 13	

[a] Statistically significant difference between IC_{50}-(+)-isomer and IC_{50}-(−)-isomer, $p < 0.05$.
[b] Significant difference at $p < 0.01$.

Discussion

Electrophysiologic and biochemical data indicate that type I antiarrhythmic drugs bind to a receptor associated with the cardiac sodium channel (2–4). The stereospecific electrophysiologic and biochemical interactions of type I drugs with the cardiac channel (6–9) suggested that molecular structure is a determinant of type I drug binding to cardiac sodium channels. In this study we assessed whether the orientation of the aromatic (C_1-position) and amine (C_2-position) components of the type I drug molecule are determinants of drug binding. Using established biochemical techniques (4, 5, 9), we compared the relative affinities of stereoisomeric drug pairs for specific sites associated with the cardiac sodium channel. Stereospecific differences in binding were observed, which suggest that the orientations of both aromatic and amine groups to the rest of the molecule are important determinants of drug binding to cardiac sodium channels.

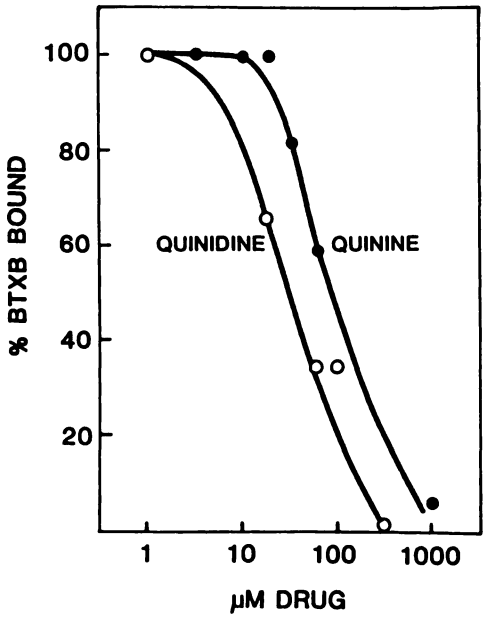

Fig. 2. Effect of quinidine stereoisomers on the binding of BTXB to myocytes. Myocytes (5 × 10^5/point) were incubated with 13 nM [^{3}H] BTXB, 0.13 mM tetrodotoxin, 1.3 μM sea anemone toxin, and various concentrations of quinidine or quinine for 60 min. Specifically bound [^{3}H] BTXB was measured as described in Methods.

A wide variety of chemical structures produce conduction block in both nerve and cardiac tissues, thereby making identification of the chemical features responsible for the biological activity of these compounds a difficult task. There are a number of approaches that have been adopted to examine the structural requirements for local anesthetic-type antiarrhythmic drug binding to both nerve and cardiac sodium channels. For example, many studies have screened a wide variety of structurally unrelated drugs and have correlated their potency of sodium channel blockade with lipid solubility, pK_a, and molecular weight (15–18). However, because these properties depend on a variety of structural features, it is difficult to relate specific portions of the molecule to its pharmacological activity. A second approach is to correlate the potencies of closely related structural homologs of a single compound with their structural and physicochemical properties. Using this approach one can selectively alter the structure of the drug and assess the effect on its potency (19–23). The results of these studies also indicate that lipid solubility, pK_a, and molecular weight are determi-

nants of the potency with which these agents block sodium channels.

Furthermore, this approach has also been used to show that there are additional structural features that influence the potency of sodium channel blockade (22–24). This is supported by electrophysiologic and biochemical studies, which have demonstrated that local anesthetic-type antiarrhythmic agents exhibit significant stereospecificity in their ability to block sodium channels (5–8, 14, 25). The use of stereoisomers of antiarrhythmic drugs in these studies represents a third approach that can be adopted to examine the structural requirements for drug binding to the cardiac sodium channel.

It was this approach that was used in the present study. We have examined the effects of conformational alterations within a single compound on the ability of the compound to interact with specific sites associated with the cardiac sodium channel. This approach has the advantage that any differences in potency that are observed between (*S*)-(+)- and (*R*)-(−)-enantiomers cannot be attributed to differences in the lipid solubility, pK_a, or molecular weight because these parameters are identical for each of the enantiomers. Furthermore, we chose a series of compounds that enabled us to selectively alter the conformation of two domains of the drug molecule. The orientation of the aromatic and amine groups with respect to the rest of the molecule were altered for C_1 and C_2 pairs, respectively.

In the present study, conformational alterations at both these domains were determinants of drug binding to cardiac sodium channels. For example, quinidine, cinchonidine, and RAC 109 (C_1 pairs) stereospecifically inhibited [^{3}H]BTXB binding, which suggests that the orientation of the aromatic domain with respect to the rest of the drug molecule is a determinant of drug binding. The stereospecific effect of quinidine on [^{3}H]BTXB binding is compatible with the findings of Mirro *et al.* (26) in dog Purkinje fibers. Although Mirro *et al.* (26) did not observe stereospecific differences between quinidine and quinine at all concentrations, these authors (Ref. 26, Table 3) showed that quinidine at 10 μM depressed V_{max} whereas quinine at the same concentration did not. In the same study, the stereoisomers of disopyramide had a nonstereospecific effect on sodium channel blockade, which is also in keeping with the present findings. This may result from the structural similarity between the benzene and pyridine rings of disopyramide (see Fig. 1). The 5-fold difference in the IC_{50} for inhibition of [^{3}H]BTXB binding between the enantiomers of RAC 109 compares favorably with the 7-fold difference reported by Postma and Catterall (14) for the nerve sodium channel. Furthermore, Clarkson (8) has reported a 3-fold difference between the enantiomers of RAC 109 for cardiac sodium channel blockade in voltage-clamped guinea pig myocytes. The stereospecific effect of RAC 109 in the present study can be attributed to the markedly different orientation of the aromatic group of its (*S*)-(+)- and (*R*)-(−)-isomers (see Fig. 1).

Drug binding to cardiac sodium channels was also affected by conformational alterations at the amine domain. Both tocainide and mexiletine (C_2 pairs) stereospecifically inhibited [^{3}H]BTXB binding, suggesting that the orientation of the amine domain is also a determinant of drug binding to cardiac sodium channels (Fig. 1). This is supported by Bokesch *et al.* (24) who showed that lidocaine homologs that differed only in the structure of their respective amine groups produced markedly different effects on sodium channel blockade. The nonstereospecific effect of the C_2 pair flecainide may be due to the incorporation of its amine group within piperidine (see Fig. 1).

In conclusion, the stereospecific differences in drug binding suggest that the orientations of both the aromatic and the amine groups to the rest of the drug molecule are important determinants of drug binding to cardiac sodium channels. This also suggests the presence of at least two stereospecific domains within the binding sites for type I antiarrhythmic drugs.

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