A Receptor for Type I Antiarrhythmic Drugs Associated With Rat Cardiac Sodium Channels

Robert S. Sheldon, Nancy J. Cannon, and Henry J. Duff

We assessed the effects of type I antiarrhythmic drugs on the binding of ligands to receptors on voltage-sensitive sodium channels of rat cardiac myocytes. The radioligand was [3H]batrachotoxin in A 20α-benzoate ([3H]BTXB), a toxin that binds to the sodium channel. The 8 drugs tested inhibited [3H]BTXB binding in a dose-dependent fashion with IC₅₀ values from 1.34 μM for O-demethylencainide to 811 μM for procainamide. A log-log plot of IC₅₀ versus mean therapeutic serum concentration yielded a regression line with slope of 1.17 and r of 0.95. Scatchard analysis of [3H]BTXB binding showed that lidocaine reduced the maximal binding without altering the Kᵣ for [3H]BTXB binding, indicating allosteric inhibition. The inhibition by lidocaine of [3H]BTXB binding was reversible within 30 minutes when the samples were diluted from 390 to 39 μM lidocaine. In other studies, the stereoisomers of tocainide were shown to have a threefold to fourfold difference in IC₅₀ for inhibition of [3H]BTXB binding. The binding of antiarrhythmic drugs to this site is saturable, reversible, and stereospecific and occurs at pharmacologically relevant concentrations with similar rank order of potency in vivo and in vitro. This suggests that binding at this site relates to pharmacologic activity. (Circulation Research 1987;61:492–497)

The antiarrhythmic effect of type I agents such as lidocaine is most likely related to their ability to slow conduction by effecting sodium channel blockade. The molecular mechanism of this pharmacologic effect is as yet unclear. The data from numerous electrophysiologic experiments have led to the concept that type I drugs bind reversibly to a single site associated with the cardiac sodium channel (e.g., Hondeghem and Katzung and Grant et al). In the modulated receptor hypothesis, there are two proposed mechanisms by which drug binding to the channel could block sodium influx: drugs bound to the activated state might directly block sodium influx, and drugs bound to the inactivated state might slow recovery of the channel from the inactivated state, thereby reducing the number of channels available for activation. Despite the general acceptance of the notion based on electrophysiologic data (e.g., Clarkson and Hondeghem) that type I drugs interact with the sodium channel, there is as yet no biochemical evidence that a specific binding site exists for these drugs associated with the sodium channel.

These theories presuppose an interaction between small ligands (drugs) and a macromolecule (the sodium channel). The development of radiolabeled neurotoxins has provided a biochemical approach to the structure and function of the nerve sodium channel and its inhibitory ligands, the local anesthetics. Alkaloid toxins such as batrachotoxin and aconitine cause persistent activation of the sodium channel by binding preferentially to, and stabilizing, the activated state of the channel. The polypeptide sea anemone toxin (ATX II) enhances persistent activation by alkaloid toxins through an allosteric mechanism that enhances alkaloid toxin binding. A tritiated derivative of batrachotoxin, [3H]batrachotoxinin A 20α-benzoate ([3H]BTXB) has been used to study the interaction of local anesthetics with the nerve channel. The local anesthetics allosterically inhibit alkaloid toxin binding apparently by binding to, and stabilizing, the inactivated state.

In a separate report, we described the binding of [3H]BTXB and ATX II to sodium channels on freshly isolated, adult rat cardiomyocytes. The toxins bind to specific, saturable sites in a manner very similar to their binding to nerve sodium channels. [3H]BTXB binding was stimulated by ATX II and inhibited by other alkaloid toxins (e.g., aconitine). Furthermore, a proportion of the [3H]BTXB binding was voltage-sensitive as would be expected if binding were to a voltage-sensitive sodium channel.

The purpose of this report was to determine whether type I drugs inhibited [3H]BTXB binding in a fashion consistent with their binding to a specific receptor site on cardiac myocytes. In particular, the purpose was to determine whether the drug effect was saturable, reversible, and stereospecific and whether it occurred at pharmacologically relevant concentrations with the same rank order of potency in vitro as in vivo.

Materials and Methods

Myocyte Preparation

Cardiac myocytes were isolated from adult male Sprague-Dawley rats (200–250 g) using the method of Kryski et al. Rats were killed by cervical dislocation.
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and the heart rapidly removed. 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<sub>2</sub>-5% CO<sub>2</sub> at 37° C. The solutions were based on Joklik’s Minimal Essential Medium supplemented with 1.2 mM MgSO<sub>4</sub> and 1 mM CaCl<sub>2</sub> (MEM). They included a rinse solution (MEM), a digestion solution (MEM with 0.1% wt:vol fatty acid-free bovine albumin and 0.1% collagenase), a calcium solution (MEM with 1 mM CaCl<sub>2</sub> and 1% fatty acid-free bovine albumin), and an incubation solution (MEM with 50 μM CaCl<sub>2</sub> and 1% dialyzed bovine serum albumin). The heart first was perfused at 20° C for 5 minutes with rinse solution, then perfused at 37° C for 20 minutes with digestion solution. The ventricles were then removed, minced with scissors, and rinsed at 37° C for 15 minutes with calcium solution. Calcium solution was then removed by aspiration, and the tissue pieces were incubated at 37° C for 15 minutes with digestion solution in a shaking water bath. Dispersed cells were decanted into a plastic centrifuge tube, and the residual tissue 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, were collected by gentle centrifugation, and were rinsed with incubation solution. The cells were again collected by gentle centrifugation and resuspended in incubation solution.

This method routinely yielded about 100 mg (dry weight) of myocytes, which corresponds to 2 x 10<sup>7</sup> cells. The cells were 82–92% viable rod-shaped cells. Myocytes (6 x 10<sup>9</sup>/assay) in 50 μl incubation buffer were incubated for 45–60 minutes at 37° C with 1.3 μM ATX II, 13 nM [3H]BTXB (50 Ci/mmol), and 0.13 mM tetrodotoxin. Tetrodotoxin was added to prevent depolarization induced by sodium influx. 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-BSA; NaCl 127 mM, KCl 2.33 mM, KH<sub>2</sub>PO<sub>4</sub> 1.30 mM, MgSO<sub>4</sub> 1.23 mM, NaHCO<sub>3</sub> 25 mM, glucose 10 mM, CaCl<sub>2</sub> 50 μM, BSA 1%) equilibrated with 95% O<sub>2</sub>-5% CO<sub>2</sub> and incubated at 37° C for 1 minute, then filtered through a 24-mm filter (model GF-C, Whatman Bio Systems, Inc., Clifton, N.J.) and washed 4 times with 5 ml rinse buffer (Tris Cl 25 mM, pH 7.4; NaCl 130 mM, KCl 5.5 mM, MgSO<sub>4</sub> 0.8 mM, glucose 5.5 mM, CaCl<sub>2</sub> 50 μM). The filters were then dried and counted in Econofluor scintillation fluid. The retained radioactivity represents [3H]BTXB bound to myocytes.

The rationale for the incubation and filtration conditions were described by Sheldon et al. The conditions provide a maximal reduction in background and scatter with a minimal reduction in specific binding. The total wash time is 45 seconds. Initial control experiments showed that under these conditions less than 10% of the specifically bound [3H]BTXB dissociated from the complex. Under these reaction conditions (13 nM [3H]BTXB and 1.3 μM ATX) about 60–75% of the total radioactivity retained on the filters is bound specifically to the receptor.

**Drug Selection**

Eight representative class I antiarrhythmic drugs were selected for study. They were selected if they were thought to be effective in treating ventricular tachycardia, if they were active themselves without necessarily invoking active metabolites, and if they possessed known therapeutic serum concentrations. (Procainamide is thought to be active even in the absence of its metabolite N-acetylprocainamide).

**Materials**

[3H]BTXB was purchased from New England Nuclear, Boston, Mass.; tetrodotoxin, aconitine, albumin, and sea anemone toxin from Sigma Chemical Co., St. Louis, Mo., and collagenase from Cooper Biomedical, Mississauga, Ontario, Canada. Antiarrhythmic drugs were provided by their manufacturers.

**Results**

**Type I Antiarrhythmic Drugs Inhibit [3H]BTXB Binding**

The effects of 3 type I drugs (O-demethylencainide, lidocaine, and procainamide) on [3H]BTXB binding to myocytes is shown in Figure 1. These results are representative of single experiments; each drug was studied 3–6 times. The inhibition by the drugs is dose dependent and follows a sigmoid curve characteristic of ligand binding to a single class of saturable sites.

In Table 1, the therapeutic serum concentrations of...
Table 1. Comparison of IC$_{50}$ Values and Mean Therapeutic Serum Concentrations for 8 Type I Antiarrhythmic Drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC$_{50}$, µM</th>
<th>Therapeutic serum concentration, µM</th>
<th>Hill number</th>
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<tbody>
<tr>
<td>O-demethylencainide</td>
<td>1.34</td>
<td>0.44</td>
<td>0.93</td>
</tr>
<tr>
<td>Propafenone</td>
<td>11.5</td>
<td>2.5</td>
<td>1.00</td>
</tr>
<tr>
<td>Quinidine</td>
<td>25</td>
<td>11</td>
<td>0.88</td>
</tr>
<tr>
<td>Mexiletine</td>
<td>26</td>
<td>10</td>
<td>1.10</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>52</td>
<td>18</td>
<td>0.79</td>
</tr>
<tr>
<td>Disopyramide</td>
<td>79</td>
<td>12</td>
<td>1.02</td>
</tr>
<tr>
<td>Tocainide</td>
<td>160</td>
<td>40</td>
<td>0.88</td>
</tr>
<tr>
<td>Procainamide</td>
<td>811</td>
<td>50</td>
<td>1.14</td>
</tr>
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IC$_{50}$ values are the means of 3–6 experiments. Serum concentrations are those for the treatment of ventricular tachycardia.

Type I Drugs Indirectly Inhibit [3H]BTXB Binding

We showed previously that the binding of ATX II and [3H]BTXB are allosterically coupled. Thus, the inhibition by type I drugs of [3H]BTXB binding could be due to a primary inhibition of either ATX II binding or [3H]BTXB binding. To first assess the effect of drugs on ATX II binding, we determined the inhibitory effect of procainamide on [3H]BTXB binding in the presence of 1.3 µM or 26 µM ATX II. The results in Figure 3 show that procainamide has the same effect on [3H]BTXB binding in the presence of either concentration of ATX II. This suggests that type I drugs do not directly inhibit ATX II binding to its receptor site.

Scatchard analysis of [3H]BTXB binding in the presence of 1.3 µM ATX II (Figure 4) indicates a single class of binding sites with a K$_D$ of 21 nM, similar to that reported previously. Lidocaine (39 µM) reduced the maximal binding capacity from 23.5 to 12.6 fmol [3H]BTXB but had little effect on the K$_D$. These results indicate that the inhibition by lidocaine of [3H]BTXB binding is noncompetitive, resulting in a reduction in binding capacity with little or no change in K$_D$ for [3H]BTXB. This type of inhibition is typical of allosteric inhibition, that is, binding of lidocaine to a site distinct from the toxin binding site alters the conformation of the toxin site rendering it unavailable for [3H]BTXB binding.

Drug Inhibition of [3H]BTXB Binding is Reversible

The reversibility of lidocaine inhibition of [3H]BTXB binding was determined by incubating the myocytes sequentially in two different concentrations of lidocaine and assessing whether the degree of inhibition of [3H]BTXB binding was determined by the first or the final concentration of lidocaine. Myocytes were incubated first with ATX, TTX and either 39 µM or 390 µM ligand for 30 minutes. The myocytes were then diluted tenfold into a solution containing the ATX, TTX, [3H]BTXB, and various concentrations of lidocaine and then incubated for 60 minutes at 37°C.

![Figure 2](image2.png)

**Figure 2.** Correlation of IC$_{50}$ values for inhibition of [3H]BTXB binding by type I antiarrhythmic agents in vitro with their effective serum concentrations in vivo. Data are from Table 1. •, O-demethylencainide; ○, propafenone; □, quinidine; ■, mexiletine; △, lidocaine; ▲, disopyramide; ▽, tocainide; and ▼, procainamide.

![Figure 3](image3.png)

**Figure 3.** Effect of ATX II concentrations on the inhibition by procainamide on [3H]BTXB binding to myocytes. Myocytes (6 x 10⁶) were incubated with 13 nM [3H]BTXB, 0.13 mM tetrodotoxin, and 1.3 µM (▲) or 26 µM (○) ATX II as well as various concentrations of procainamide. The specifically bound [3H]BTXB was measured as described in "Materials and Methods."
**Table 2. Reversibility of Lidocaine Inhibition**

<table>
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<tr>
<th>Incubation 1</th>
<th>Incubation 2</th>
<th>fmol [3H]BTXB bound</th>
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<tr>
<td>39 μM</td>
<td>39 μM</td>
<td>6.34 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>390 μM</td>
<td>1.90 ± 0.85</td>
</tr>
<tr>
<td>390 μM</td>
<td>39 μM</td>
<td>8.00 ± 0.19</td>
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<td></td>
<td>390 μM</td>
<td>1.35 ± 0.39</td>
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<tr>
<td>None</td>
<td>None</td>
<td>22.25 ± 0.82</td>
</tr>
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Myocytes (6 × 10^6/point) were first incubated for 30 minutes at 37°C with 5 μM ATX, 0.13 mM TTX, and either 39 μM or 390 μM lidocaine (Incubation 1), then diluted tenfold into 5 μM ATX, 0.13 mM TTX, and the indicated concentrations of lidocaine (Incubation 2). After a further 60 minutes incubation at 37°C, the myocytes were filtered and the specifically bound [3H]BTXB measured.
that antiarrhythmic drugs bind to a specific receptor site associated with the cardiac sodium channel.

**Lidocaine Allosterically Inhibits \([^{3}H]BTXB\) Binding**

Lidocaine reduced the \(B_{\text{max}}\) for \([^{3}H]BTXB\) binding without significantly altering the \(K_{D}\). This pattern of inhibition is typical of allosteric inhibition and occurs with other receptors including the \(\beta_{2}\)-adrenoceptor, \(\alpha_{2}\)-adrenoceptor, and the dopamine receptor. One of the implications of allosteric inhibition is that the \(K_{D}\) and \(IC_{50}\) of a ligand are identical. Thus, we estimate that the \(K_{D}\) of lidocaine for the binding site identified under the conditions in this study is 52 \(\mu\)M.

This allosteric inhibition by a type I antiarrhythmic drug of \([^{3}H]BTXB\) binding bears directly on electrophysiologic models of the mechanism of drug action. Numerous electrophysiologic experiments have shown that the effect of antiarrhythmic drugs on sodium channel blockade is both voltage dependent and frequency dependent. These data have led to models in which the drugs bind preferentially to certain states of the sodium channel. One of the ways in which the drugs are thought to block the sodium channel is by binding tightly to the inactivated state, thereby stabilizing it and by so doing decrease the number of sodium channels available for activation. Our data bear on this idea. From extensive work on the nerve sodium channel, alkaloid toxins are known to bind with high affinity to activated state(s) of the channel. This binding is synergistically enhanced by ATX II. Thus, any channels that are labelled with \([^{3}H]BTXB\) must be in an activated state. That lidocaine is an allosteric inhibitor of \([^{3}H]BTXB\) binding implies that it binds to sites associated with states other than the activated state. Thus, our data are consistent with the notion that at least lidocaine can cause sodium channel blockade by binding to a site associated with the channel and by stabilizing a nonconducting state of the channel.

Postma and Catterall showed that local anesthetics and certain anticonvulsant agents inhibited \([^{3}H]BTXB\) binding in a somewhat similar fashion to nerve sodium channels. Scatchard analysis indicated that these drugs competitively inhibited \([^{3}H]BTXB\) binding in contradistinction to our findings. However, subsequent kinetic analysis showed that the drugs did not reduce the rate of association of \([^{3}H]BTXB\) with the channel (as would be expected with competitive inhibition) but rather increased the rate of dissociation of \([^{3}H]BTXB\) from the channel (as would be expected with allosteric inhibition). From these data, Willow, Postma, and Catterall concluded that local anesthetics and anticonvulsants were indirect competitive allosteric inhibitors of \([^{3}H]BTXB\) binding. They elaborated a model in which these drugs allosterically inhibited \([^{3}H]BTXB\) binding by stabilizing a nonconducting state of the channel.

Our data describe an example more characteristic of allosteric inhibition: lidocaine reduces the \(B_{\text{max}}\) but does not alter the \(K_{D}\) for \([^{3}H]BTXB\). Thus, our data can be explained by Catterall's model of allosteric inhibition. The nature of the differences between the nerve and cardiac sodium channels, not only the mechanism of allosteric inhibition but also the relative affinities for ligands at the various sites, is an interesting and unresolved problem that merits further attention. Nonetheless, sodium channels of both neural and cardiac tissue share a common characteristic of blockade by drugs that allosterically inhibit the binding of \([^{3}H]BTXB\) to the activated state.

In review, type I antiarrhythmic drugs bind to specific sites related to cardiac sodium channels. The characteristics of their binding, i.e., being saturable, reversible, and stereospecific, having rank order of potency similar to the drugs' pharmacologic potency, and occurring at pharmacologically relevant concentrations, suggest that drug binding to this site is involved in their pharmacologic effect.

**Acknowledgment**

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KEY WORDS • sodium channel • antiarrhythmic drug receptor • [3H]batrachotoxinin benzoate
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