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]batrachotoxinin A 20\alpha\text{-}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 IC_{50} values from 1.34 \mu M for O\text{-}demethylencainide to 811 \mu M for procainamide. A log-log plot of IC_{50} 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 K_{d} 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 \mu M lidocaine. In other studies, the stereoisomers of tocainide were shown to have a threefold to fourfold difference in IC_{50} 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\textsuperscript{1} and Grant et al\textsuperscript{2}). 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\textsuperscript{3}) 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 radiolabelled neurotoxins has provided a biochemical approach to the structure and function of the nerve sodium channel and its inhibitory ligands, the local anesthetics.\textsuperscript{4,5} 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.\textsuperscript{6} 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 20\alpha\text{-}benzoate ([3H]BTXB) has been used to study the interaction of local anesthetics with the nerve channel.\textsuperscript{7} 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.\textsuperscript{8} 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.\textsuperscript{9} 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.\textsuperscript{10} Rats were killed by cervical dislocation...
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Radioligand Binding

Myocytes (6×10^5/assay) in 50 μl incubation buffer were incubated for 45–60 minutes at 37° C with 1.3 μM ATX II, 13 nM [3H]ATX, 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, KH2PO4 1.30 mM, MgSO4 1.23 mM, NaHCO3 25 mM, glucose 10 mM, CaCl2 50 μM, BSA 1%) equilibrated with 95% O2-5% CO2 and incubated at 37° C for 1 minute, then filtered at 37° C for 1 minute with digestion 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 2×10^7 cells.12,13 The cells were 82–92% viable rod-shaped cells that excluded trypan blue. The cells maintained a resting membrane potential of −75 to −80 mV and have been metabolically characterized by Kryski et al.49

Materials

[3H]ATX 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]ATX Binding

The effects of 3 type I drugs (O-demethylencainide, lidocaine, and procainamide) on [3H]ATX 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

![Figure 1. Effect of type I antiarhythmics on [3H]ATX binding to myocytes. Myocytes (6×10^5/point) were incubated with 3 nM [3H]ATX.](https://example.com/figure1.png)
Table 1. Comparison of IC50 Values and Mean Therapeutic Serum Concentrations for 8 Type I Antiarrhythmic Drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50, μM</th>
<th>Therapeutic serum concentration, μM</th>
<th>Hill number</th>
</tr>
</thead>
<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>Procaainamide</td>
<td>811</td>
<td>50</td>
<td>1.14</td>
</tr>
</tbody>
</table>

IC50 values are the means of 3–6 experiments. Serum concentrations are those for the treatment of ventricular tachycardia.

8 type I drugs are listed with their IC50 values and estimated Hill numbers from this assay. The mean Hill number of the 8 drugs is 0.97 ± 0.11 (SD), suggesting again that the drugs interact with a single class of sites. There is a striking similarity between IC50 values and serum concentrations over a one hundredfold range of serum concentrations. The data are plotted in Figure 2 as log(IC50) versus log(serum concentration). Linear regression analysis of the log-log plot yields a slope of 1.17 and a correlation coefficient, r, of 0.95. The IC50 values are about 2–3 times higher than the serum concentrations. Thus, the drugs have the same rank order of potency in vitro as in vivo and have IC50 values at pharmacologically relevant concentrations.

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 Kd 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 Kd. 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 Kd 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 1.3 μM (•) or 26 μM (o) ATX II as well as various concentrations of lidocaine and then incubated for 60 minutes at 37°C.
The concentrations of lidocaine in the two sequential incubations are reviewed in Table 2. If the inhibition by lidocaine of \( ^3H \)BTXB binding is reversible, then the amount of \( ^3H \)BTXB binding should reflect the concentration of lidocaine in the second incubation but be independent of the lidocaine concentration in the first incubation.

The results in Table 2 show that in the absence of lidocaine, 22.85 fmol of \( ^3H \)BTXB bound specifically to the myocytes. Myocytes were first incubated with 39 \( \mu M \) lidocaine, 22.85 fmol of \( ^3H \)BTXB bound specifically. When myocytes were first incubated with 39 \( \mu M \) lidocaine and then diluted to 39 or 390 \( \mu M \) lidocaine, the drug reduced \( ^3H \)BTXB binding to 8.00 and 1.35 fmol, respectively. Thus, there is a similar amount of \( ^3H \)BTXB bound in the presence of a final concentration of 39 \( \mu M \) lidocaine that is independent of whether the myocytes were first incubated with 39 \( \mu M \) lidocaine (6.34 fmol) or 390 \( \mu M \) lidocaine (8.00 fmol).

This demonstrates that the inhibition of \( ^3H \)BTXB binding by lidocaine is reversible and, therefore, that lidocaine does not irreversibly alter the \( ^3H \)BTXB receptor.

Antiarrhythmic Drug Binding is Stereospecific

The effect of the stereoisomers of tocainide and disopyramide on \( ^3H \)BTXB binding was determined. R-\((-\))-tocainide is significantly more potent than S-\((+\))-tocainide in prolonging conduction time in an ex vivo rabbit myocardium model (Sheldon et al, submitted). This suggests that R-\((-\))-tocainide would bind more tightly to the sodium channel than would S-\((+\))-tocainide, and the R-\((-\))-tocainide would more potently inhibit \( ^3H \)BTXB binding than would S-\((+\))-tocainide. In radioligand studies, the IC\(_{50}\) values (± SD) for the R-\((-\)) and S-\((+\)) forms were 184 ± 8 and 346 ± 37 \( \mu M \), respectively (\( p < 0.003 \)).

In contrast, the stereoisomers of disopyramide have very similar concentration-dependent effects on theupstroke of the action potential in isolated dog Purkinje fibers.\(^{11}\) When tested in the radioligand binding assay, the \( \beta \) and \( \alpha \) optical isomers had IC\(_{50}\) values of 122 ± 45 and 109 ± 6 \( \mu M \), respectively. This suggests that tocainide had stereospecific effects on both prolonging conduction time and inhibiting \( ^3H \)BTXB binding, while the stereoisomers of disopyramide exhibited stereospecific effects on neither conduction time nor inhibition of \( ^3H \)BTXB binding. Therefore, the stereospecific effects on both conduction time and \( ^3H \)BTXB binding seem to correlate.

Discussion

Antiarrhythmic Drugs Bind to a Specific Site on Myocytes

Type I antiarrhythmic agents are thought to be effective because they block the sodium channel and prolong conduction time. How they do so is still unclear. Our understanding of this mechanism has evolved from the concept that these drugs function as general membrane depressants by interacting with the lipid bilayer and from more recent models invoking binding of the drugs to specific sites associated with sodium channels.\(^{12}\) These models have not yet been assessed biochemically.

Using a radioligand binding technique, we have shown that type I antiarrhythmic drugs bind to specific sites on myocytes and that this binding inhibits the binding of toxins to the cardiac sodium channel. This strongly suggests that these drugs bind to a specific site associated with the sodium channel. Furthermore, the characteristics of this binding, i.e., it is saturable, reversible, and stereospecific, has rank order of potency similar to the drugs' pharmacologic potency, and occurs at pharmacologically relevant concentrations, suggest that this binding is involved in their pharmacologic effect.\(^{11}\) This is the first biochemical evidence...
that antiarrhythmic drugs bind to a specific receptor site associated with the cardiac sodium channel.

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

Lidocaine reduced the \(B_{\text{max}}\) for \[^3\text{H}\]BTXB binding without significantly altering the \(K_p\). This pattern of inhibition is typical of allosteric inhibition and occurs with other receptors including the \(\beta_2\)-adrenoreceptor, the dopamine receptor, and the calcium channel. One of the implications of allosteric inhibition is that the \(K_p\) and \(IC_50\) of a ligand are identical. Thus, we estimate that the \(K_p\) 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 \[^3\text{H}\]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, alkalioid 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 \[^3\text{H}\]BTXB must be in an activated state. That lidocaine is an allosteric inhibitor of \[^3\text{H}\]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 and Willow and Catterall showed that local anesthetics and certain anticonvulsant agents inhibited \[^3\text{H}\]BTXB binding in a somewhat similar fashion to nerve sodium channels. Scatchard analysis indicated that these drugs competitively inhibited \[^3\text{H}\]BTXB binding in contradistinction to our findings. However, subsequent kinetic analysis showed that the drugs did not reduce the rate of association of \[^3\text{H}\]BTXB with the channel (as would be expected with competitive inhibition) but rather increased the rate of dissociation of \[^3\text{H}\]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 \[^3\text{H}\]BTXB binding. They elaborated a model in which these drugs allosterically inhibited \[^3\text{H}\]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 \(K_p\) for \[^3\text{H}\]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 \[^3\text{H}\]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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