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 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 Kd 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 Katzung1 and Grant et al2). 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 Hondeghem3) 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 radionabeled neurotoxins has provided a biochemical approach to the structure and function of the nerve sodium channel and its inhibitory ligands, the local anesthetics.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.6,7 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.8,9 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.9 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.10 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.11 Rats were killed by cervical dislocation.
For Readers in the United States

☑ Yes, I want to receive my personal copy of

☐ Circulation $65
☐ Circulation Research 90
☐ Stroke 60
☐ Hypertension 55
☐ Arteriosclerosis 60
☐ Recurring Bibliography of Hypertension 20

In-training rate $32.50 Letter from department chairman stating post held and completion date is required.

☐ 2750 Resident
☐ 30.00 Intern
☐ 10.00 Research Fellow

Institutional rates available upon request.

OFFER ENDS NOVEMBER 1, 1987

PLEASE PRINT

Name ____________________________
Address ____________________________
City ____________________________ State __________ Zip __________

My specialty ____________________________

Please send my subscription beginning with the _____________ issue.

Advance payment required before copies are sent.

☐ Send bill
☐ Check enclosed, payable to the American Heart Association.
☐ This is a renewal. My account number is ____________________________

For Readers Outside the United States, Japan, and Europe

☑ Yes, I want to receive my personal copy of

☐ Circulation $85
☐ Circulation Research 115
☐ Stroke 75
☐ Hypertension 75
☐ Arteriosclerosis 75
☐ Recurring Bibliography of Hypertension 30
☐ Modern Concepts 20

For readers in Japan contact: Nankodo Co., Ltd. 42-6 Hongo 3-chome Bunkyo-ku Tokyo 113, Japan

For readers in Europe contact: Bailliere Tindall 1 St. Anne's Road Eastbourne, East Sussex BN21 3UN, England

Institutional rates available upon request.

OFFER ENDS NOVEMBER 1, 1987

PLEASE PRINT

Name ____________________________
Address ____________________________
City ____________________________ State __________ Zip __________

My specialty ____________________________

Please send my subscription beginning with the _____________ issue.

Advance payment required before copies are sent.

☐ Send bill
☐ (Outside of Europe and Japan) Check enclosed, payable to the American Heart Association.
☐ This is a renewal. My account number is ____________________________

American Heart Association

Please send a sample copy of:

☐ Arteriosclerosis
☐ Circulation
☐ Circulation Research

to the librarian at my institution

☐ Hypertension
☐ Stroke
☐ Recurring Bibliography of Hypertension

with my recommendation to subscribe.
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% O₂-5% CO₂ at 37° C. The solutions were based on Joklik's Minimal Essential Medium supplemented with 1.2 mM MgSO₄, and 1 mM DL-carnitine (MEM). They included a rinse solution (MEM), a digestion solution (MEM with 0.1% vol fatty acid-free bovine albumin and 0.1% collagenase), a calcium solution (MEM with 1 mM CaCl₂, and 1% fatty acid-free bovine albumin), and an incubation solution (MEM with 50 μM CaCl₂, 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 10⁵ cells. 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.¹² 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, albu-

**Results**

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

The effects of 3 type I drugs (O-demethylenicainide, 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
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 that the drugs interact with a single class of sites. There is a striking similarity between IC₅₀ values and 8 type I drugs are listed with their IC₅₀ values and their effective serum concentrations in vivo. Data are from Table 1. 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 analysis⁵ of [³H]BTXB binding in the presence of 1.3 μM ATX II (Figure 4) indicates a single class of binding sites with a Kᵦ 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 Kᵦ for [³H]BTXB. This type of inhibition is typical of allosteric inhibition¹⁴; 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 lidocaine 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.

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>Procainamide</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.
Table 2. Reversibility of Lidocaine Inhibition

<table>
<thead>
<tr>
<th>Lidocaine concentrations</th>
<th>Incubation 1</th>
<th>Incubation 2</th>
<th>fmol [3H]BTXB bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>39 µM</td>
<td>39 µM</td>
<td>6.34±0.44</td>
<td></td>
</tr>
<tr>
<td>390 µM</td>
<td>390 µM</td>
<td>1.90±0.85</td>
<td></td>
</tr>
</tbody>
</table>

Myocytes (6 x 10⁶/point) were first incubated with 5 µM ATX, 0.13 mM TTX, and either 39 or 390 µM lidocaine (Incubation 1), then diluted tenfold into 5 µM ATX, 0.13 mM TTX, and 13 nM [3H]BTXB, 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.

Figure 4. Scatchard analysis of the effect of lidocaine on [3H]BTXB binding. Myocytes (6 x 10⁶/point) were incubated with 1.3 µM ATX in the absence (○) or presence (●) of 39 µM lidocaine and various concentrations of [3H]BTXB. Specifically bound [3H]BTXB was measured as described in “Materials and Methods” and subjected to Scatchard analysis. Linear regression best-fit values were B∞ of 23.5 fmol, Kd of 21 nM in the absence of lidocaine; and B∞ of 12.6 fmol, Kd of 24 nM in the presence of lidocaine.

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 µM lidocaine. At a final incubation concentration of 39 or 390 µM, lidocaine reduced [3H]BTXB binding to 6.34 and 1.90 fmol, respectively. In comparison, when myocytes were first incubated with 390 µM lidocaine and then diluted to 39 or 390 µM 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 µM lidocaine that is independent of whether the myocytes were first incubated with 39 µM lidocaine (6.34 fmol) or 390 µM 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 IC₅₀ values (± SD) for the R-(−) and S-(+) forms were 184 ± 8 and 346 ± 37 µM, respectively (p < 0.003).

In contrast, the stereoisomers of disopyramide have very similar concentration-dependent effects on the upstroke of the action potential in isolated dog Purkinje fibers. When tested in the radioligand binding assay, the n and l optical isomers had IC₅₀ values of 122 ± 45 and 109 ± 6 µM, respectively. Thus, the stereoisomers of 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. 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. This is the first biochemical evidence...
that antiarrhythmic drugs bind to a specific receptor site associated with the cardiac sodium channel.

**Lidocaine Allosterically Inhibits [3H]BTXB Binding**

Lidocaine reduced the B∞ for [3H]BTXB binding without significantly altering the Kᵦ. This pattern of inhibition is typical of allosteric inhibition and occurs with other receptors including the β₂-adrenoeceptor, the dopamine receptor, and the calcium channel. One of the implications of allosteric inhibition is that the Kᵦ and IC₅₀ of a ligand are identical. Thus, we estimate that the Kᵦ of lidocaine for the binding site identified under the conditions in this study is 52 μM.

This allosteric inhibition by a type I antiarrhythmic drug of [3H]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, alkaid 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 [3H]BTXB must be in an activated state. That lidocaine is an allosteric inhibitor of [3H]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 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 [3H]BTXB binding in a somewhat similar fashion to nerve sodium channels. Scatchard analysis indicated that these drugs competitively inhibited [3H]BTXB binding in contradistinction to our findings. However, subsequent kinetic analysis showed that the drugs did not reduce the rate of association of [3H]BTXB with the channel (as would be expected with competitive inhibition) but rather increased the rate of dissociation of [3H]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 [3H]BTXB binding. They elaborated a model in which these drugs allosterically inhibited [3H]BTXB binding by stabilizing a nonconducting state of the channel.

Our data describe an example more characteristic of allosteric inhibition: lidocaine reduces the B∞ but does not alter the Kᵦ for [3H]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 [3H]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**

The authors wish to thank Mr. Gregory Douglas for his assistance in preparing his manuscript.

**References**


Key Words • sodium channel • antiarrhythmic drug receptor • [3H]batrachotoxinin benzoate
A receptor for type I antiarrhythmic drugs associated with rat cardiac sodium channels.
R S Sheldon, N J Cannon and H J Duff

_Circ Res._ 1987;61:492-497
doi: 10.1161/01.RES.61.4.492
_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/61/4/492

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/